THE CUMULATIVE EFFECTS OF SEVEN DAYS OF IMPOSED EXERCISE ON ENERGY BALANCE AND APPETITE REGULATION

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By

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A thesis submitted to the University of Bedfordshire, in fulfilment of the requirements for the degree of MSc by Research

April 2016

Declaration

I declare that this thesis is my own unaided work. It is being submitted for the degree of Master of Science by Research at the University of Bedfordshire.

It has not been submitted before for any degree or examination in any other University.

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Date: 06/04/2016

Abstract

Increasing energy expenditure (EE) through regular exercise is a promising strategy to prevent body fat gain. However, imposed exercise interventions often produce weight loss that is less than theoretically expected, possibility due to compensatory mechanisms in energy intake (EI) and EE. Study one was designed to determine whether a combined written and photographic food diary was a reliable measure of EI within a free-living environment across seven days. The results suggested this method was reliable at the group level. However, 95% limits of agreement (LoA) showed large variability (-1258 to 1545 kcal/day) at the individual level. Study two investigated acylated ghrelin, PYY and energy balance in response to 7-days of imposed exercise and a control condition. EI increased by 511 kcal/day in the exercise condition (P=0.005). Late-postprandial acylated ghrelin concentrations were higher in the exercise condition (P=0.072), but did not change from pre- to post intervention. There was a larger, but non-significant, increase in EI at the postprandial *ad libitum* pasta meal in the exercise condition (P=0.285). In conclusion, 7-days exercise resulted in increased EI under free-living conditions; similar results were found when assessed in a controlled laboratory environment. A larger sample size would allow confirmation of the findings.

Acknowledgements

Firstly I would like to thank both of my supervisors Dr John Hough and Dr Julia Zakrzewski-Fruer for their constant support and guidance throughout this degree. Their expertise, knowledge and skills they have taught me throughout will be invaluable for when I move onto my next stages in my academic or otherwise career. Their time and patience has been greatly appreciated throughout.

Secondly to Paul Mackie, who I have worked closely from the beginning, without his friendship, time and hard work we have both put in through this project it would not have been possible to have completed this project. And lastly to Rachael Champion who assisted with data collection as much as she could; this was a great help and her meticulous approach to the tasks she carried out is greatly appreciated and made data collection easier.

Abbreviation List

AEE	Activity energy expenditure
BM	Body mass
CCK	cholecystokinin
CV	Coefficient of variation
DLW	Doubly labelled water
EARS	Electronic appetite rating scales
EB	Energy balance
EE	Energy expenditure
EI	Energy intake
ELISA	Enzyme linked immunosorbent assay
EX	Imposed Exercise
g	Grams
GLP-1	Glucagon-like peptide-1
HCL	Hydrochloric acid
HR	Heart rate
kcal	Kilocalories
kg	kilogram
LMM	Linear mixed model
LoA	Limits of agreement
Min	MinuteMJ Mega joule
NaOH	Sodium hydroxide
N-EX	No exercise
NexEE	Non-exercise energy expenditure
PBS	Potassium phosphate buffer solution
PHMB	P-hydroxymercuribenzoic acid
PP	Pancreatic polypeptide
PYY	Peptide Tyrosine Tyrosine
Q-Q	Quantile-quantile
RPE	Rate of perceived exertion
SD	Standard deviation
tAUC	Total area under the curve

TEE	Total energy expenditure
VAS	Visual analogue scales
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake

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1.0 Introduction

Currently, a large proportion of adults (62%) in England are classified as overweight or obese (HealthandSocialCareInformationCentre, 2015). The issue of overweight and obesity is, therefore, often described as an 'epidemic' and there is a need for strategies to prevent body fat gain. Indeed, higher levels of body fat are associated with increased risk of serious chronic diseases, including type 2 diabetes, hypertension, and hyperlipidaemia; these associated outcomes of excess body fat are major risk factors of cardiovascular disease and related mortality (HealthandSocialCareInformationCentre, 2015). Overweight and obesity has also been linked with cancer, disability, reducing quality of life and can lead to premature death (Health and Social Care Information Centre, 2015).

Energy intake (EI) is a fundamental component of energy balance (EB) (Rennie et al., 2007) and one of the significant issues with finding preventative strategies for overweight and obesity is the ability to accurately measure the EI of individuals, especially in free-living environments. Common methods by which EI is measured are self-report questionnaires and weighed food diaries. Both of these methods have their weaknesses that have been widely raised within the literature, including limited accuracy and participant burden (Carlsen et al., 2010, Adams, 1998, Livingstone et al., 1990). A method of EI measurement that has received less attention within the literature is that of using digital photography. So far, this method has been found to be accurate and reliable when compared to a weighed food diary, which is often considered to be the 'gold standard' of EI measurement (Small et al., 2009). However, the use of digital photography to measure EI is used in a limited number of studies to date (Williamson et al., 2004, Williamson et al., 2003, Ngo et al., 2009). Although the assessment of free-living EI may be more representative of habitual EI when compared with controlled laboratory or even cafeteria settings, the reliability of a photographic food diary within free-living conditions is yet to be investigated.

Weight gain is a result of an extended period where EI exceeds EE (positive EB). Increasing EE would therefore, appear to be an appropriate strategy to prevent weight gain, overweight and obesity. Contrary to this, weight loss from imposed exercise interventions has not been to the degree that would be expected from such increases in EE (Thomas et al., 2012); this indicates that there are mechanisms compensating for the increase in EE over the long term, potentially an increase in EI or a decrease of 'non-exercise energy expenditure' (NexEE; the energy expended outside of imposed exercise, including rest and all other physical activities) (Church et al., 2009, Schneider et al., 2009). Consequently, the strategy of increasing EE to prevent weight gain appears not to be as simple as expected. Evidence within the literature has shown that a single bout of aerobic exercise does not cause a compensatory response through either perceptions of appetite or EI, thus the exercise-induced energy deficit remains (Donnelly et al., 2014). Further to this, a high-intensity bout of exercise, 60-90 minutes at ~70% maximal oxygen uptake ($\dot{V}O_{2max}$), may actually induce transient declines in hunger (Broom et

al., 2009). Research has observed that an acute bout of exercise produces an energy deficit that can last at least 1-2 days (King et al., 2015), it is therefore, contradictory that longer term exercise interventions result in modest weight loss. It may be proposed that 'compensation' must negate the exercise energy expenditure (ExEE) in the period after an acute exercise bout, which is not typically detected in the first 24 h after exercise. Few acute or chronic studies have examined the role of NexEE as well as EI, in opposing the negative energy balance induced by imposed exercise. The limited studies that have been completed examining the effect of seven days of imposed exercise on energy balance, in summary, have indicated that partial compensation in EI and NexEE may oppose imposed ExEE, and that these compensatory changes can be observed during the first seven days of an exercise regimen, at least in non-overweight adults (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b, Whybrow et al., 2008). However, these studies to date have not investigated the influence of a period of imposed exercise on the responses of appetite-regulating hormones to provide a potential objective explanation of the mechanisms responsible for the apparent partial compensatory increase in EI.

Possible hormonal mechanisms controlling acute exercise-induced declines in hunger that have been documented are a suppression of acylated ghrelin and increase in Peptide YY (PYY) during and immediately after a single session of aerobic exercise (60-90 minutes of treadmill running at ~70% $\dot{V}O_{2max}$) (Broom et al., 2009, King et al., 2010a). Furthermore, the energy deficit seen from a single aerobic bout of exercise has been shown to correlate with the suppression in acylated ghrelin and increase in PYY, with appetite rating scores following the same pattern and correlating with the appetite-regulating hormone responses (Broom et al., 2009, King et al., 2010a). The response of these hormones to a longer exercise intervention has yet to be investigated to aid in the understanding of EI compensation in response to 5 to 7 days of aerobic exercise (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b, Whybrow et al., 2008) and potentially answer questions relating to why weight loss is not as significant as theoretically expected following long term exercise interventions. Acutely, various modalities of exercise (e.g., treadmill running, rowing, cycling) have induced suppressions in acylated ghrelin and increases in PYY (Broom et al., 2009, Jurimae et al., 2007, King et al., 2010a, King et al., 2010b, Ramson et al., 2012, Ueda et al., 2009); however, the most consistent changes seen from these hormones is in response to treadmill running at ~70% $\dot{V}O_{2max,}$ inducing an energy deficit over 1-2 days. Determining the possible change in acylated ghrelin and PYY pre- to post- a 7-day period of running at ~70% $\dot{V}O_{2max}$ could be vital to understanding the mechanistic basis of compensatory responses to an increase in EE over this period of time, as well as investigating free-living EI and EE to provide an overall view of the degree to which compensation occurs.

Despite the importance of regular exercise in the maintenance of healthy body composition, well-controlled studies investigating the effects of periods of sustained exercise on EI, EE and appetite are limited. Moreover, changes in appetite-regulating hormones have typically not been assessed in

response to a meal and following periods of sustained exercise, which could explain compensatory changes in habitual EI.

The aims of this project are:

- To examine the reliability of a 7-day combined photographic and written food diary for the measurement of EI in free-living men.

- To examine the effect of seven consecutive days of imposed exercise compared with a control condition on free-living EI and EE in men.

- To examine the cumulative effect of seven consecutive days of imposed exercise compared with a control condition on perceived appetite and appetite-regulating hormones in response to a meal in men.

2.0 Review of Literature

The purpose of this chapter is to review and critically examine the literature that investigates the validity, reliability and feasibility of EI measurements and the effects of exercise on EB and appetite-regulating hormones. The first section reviews the current most frequently used methods used to measure EI. This is followed by a section that looks at the measurement of EE methods. The third section examines the relationship between exercise and energy balance, specifically detailing the effect acute and long term exercise has on EI. This chapter then moves on to focus on the response of appetite-regulating hormones to both an acute bout of exercise and longer term exercise interventions with an in-depth discussion of potential compensatory mechanisms. Finally, the chapter will end with a brief summary of the literature that has been reviewed.

2.1 Energy Intake Measurement

At present, there is a requirement for more valid, reliable and feasible methods of measuring free-living EI (Gemming et al., 2015), as the available methods, which include self-report estimated and weighed food diaries, continue to provide substantial error and bias (Pikholz et al., 2004, Rennie et al., 2007).

2.1.1 Self-report Estimated Food Diary

Estimated food diaries rely on participants to estimate the weight or portion size of food that they have consumed and recall what they have eaten immediately, later in the day or even the following day. Evidence suggests that there is a tendency for participants to underreport and over report food intake when using self-report estimated food diaries (Barrett-Connor, 1991). This method is generally low cost and attempts to reduce participant burden by asking participants to estimate the weight or portion size rather than physically weigh each food item (although this may not always be the case in reality), but can lead to indifferent findings and reduce the power to identify associations (Kirkpatrick et al., 2014). Limitations of estimated food diaries include inaccuracies from the subjective nature of the method, errors in estimates of portion sizes and the decreasing accuracy seen the more days the intake diary is used (‘participant fatigue’) (Higgins et al., 2009). The most prominent problem with this method is asking participants to estimate portion sizes, which has been known for some time. For example, Lansky and Brownell (1982) completed three self-report estimated food diaries and weight loss predictions in an obese population and also examined the impact of standard training on food weights and calories contained in each food. The mean error, prior to basic training, for the amount of calories (kcal) in food was an average of 64% overestimation, and when estimating weight of the food 42% of the estimates were in error. Following basic training and a calorie guide, errors were still made on the calorie content and weights of food, with both under- and over- estimates of 371 kcal per person.

These studies begin to highlight the inaccuracies of self-report estimated food diaries and the need for a further improved method of measuring EI.

Underreporting is another major limitation of estimated food diaries (Thompson et al., 2008), particularly in certain populations such as resistant dieters (Adams, 1998, Lichtman et al., 1992). When compared with Doubly Labelled Water (DLW) (a gold standard method for measuring EI that however, cannot be used over shorter measurement periods or provide an indication of dietary composition) studies have seen underreporting across many populations and up to 50% of actual EI (Bandini et al., 1990, Livingstone et al., 1990, Mertz et al., 1991, Prentice et al., 1986, Haraldsdottir and Hermansen, 1995, Kaczkowski et al., 2000, Kroke et al., 1999, Livingstone and Robson, 2000). Although underreporting has been observed with this method of measuring EI, Candilo et al. (2007) reported that nutrient intake estimated in a three day food diary significantly increased after follow up phone calls in 13 year old girls; thus it may be possible to reduce some of the inaccuracies associated with self-report estimated food diaries. The reasons for this apparent underreporting can be due to many factors; for example, omissions of certain foods and foods that are perceived to be 'unhealthy' (Adams et al., 1998). Indeed, many individuals may not want to be perceived as eating unhealthily and social desirability can lead to changes in dietary habits or even purposefully missing certain foods out of the diary (Adams, 1998).

Overall, the current research generally shows that self-report estimated food diaries are an inaccurate and unreliable measure of EI; even with training, participants are unable to estimate the weight or calorie content of food (Lansky and Brownwell, 1982). Moreover, there is a large element of underreporting associated with self-report methods (Adams et al. 1998). Despite this, estimated food diaries are an easily implemented and less burdensome tool to use for assessing dietary intake compared with other methods, such as weighed food diaries.

2.1.2 Weighed Food Diary

Weighed food diaries provide a more accurate measure of EI when compared with estimations; thus, they have become the 'gold standard' method of measuring free-living dietary intakes (Lee-Han et al., 1989, Small et al., 2009). This method involves weighing each item of food and beverage consumed pre- and post- meal, with the latter accounting for leftovers to allow the exact amount consumed to be calculated. However, over an extended period this type of food diary can be very demanding on participants ('participant burden') and potentially lead to a change in a participants natural eating habits (Carlsen et al., 2010). For example, weighed food diaries can lead to reduced EI, a more monotonous diet, and eating foods that are easy to weigh rather than food that would be eaten under normal circumstances (Barrett-Connor (1991). Furthermore, the longer the recording period, the less likely a

complete and correct diary is kept (Gersovitz et al., 1978). A review of EI assessment concluded that measurement periods of seven days or more saw more discrepancies, underestimations and incomplete records compared to periods lasting 3-5 days (Trabulsi and Schoeller, 2001). These findings further add evidence to the increased burden placed on participants when EI is being measured via a weighed food diary. Thus, despite the accuracy gained from weighing each item of food, the effect it has on a participant's free-living diet may not make the method an accurate measure of habitual, free-living EI.

2.1.3 Photographic Food Diary

The use of photographs to analyse EI was first used in 1983 (Bird and Elwood, 1983, Elwood and Bird, 1983); since then, this has become established as a reliable and valid method of EI measurement (Williamson et al., 2004, Williamson et al., 2003, Ngo et al., 2009). Potential advantages of using digital photography include the reduction in disruption to participants eating patterns (Ngo et al, 2009) and that it is more convenient for participants due to rapid data collection (Williamson et al., 2003). Photographic food diaries also attenuate the potential skewing of macronutrient intakes in children and adults by reducing the misreporting of fat calories (Higgins et al., 2009). Although each individual item is not weighed directly, research conducted in cafeteria settings shows that estimated portion sizes through digital photography are valid in measuring plate waste and food selections, revealing a high correlation (Pearson correlation: 0.89 - 0.94) with weighed foods (Williamson et al., 2003) with no evidence of systematic bias and a mean difference of only 5.2 g between estimates and weighed portions (Williamson et al., 2003). However, there are variances in the range of difference between food categories; Williamson et al. (2003) reported the mean overestimation for beverage intake was 7.6 g, but was only 4.9 g for condiments. During a weighed diet consumed for 3 days under free-living conditions, Higgins et al. (2009) found that there were no significant differences in the accuracy of EI estimates assessed via a weighed food diary and a combined photographic and written food diary; furthermore, feedback from participants indicated a preference for the photographic diary due to the ease of using this method. In agreement, Small et al. (2009) reported that families preferred the photographic diary over the weighed diary and that full completion of the food diary using photographs rose by up to 90% (Small et al., 2009). In further support, Martin et al. (2007) revealed that digital photography is a valid and reliable method of measuring food intake over a 5 day period. However, these studies have mainly been confined to samples of children and adolescents (Higgins et al., 2009; Martin et al., 2007). Therefore, future research needs to be conducted to determine the reliability of a photographic food diary for the measurement of free-living EI, particularly in adults, to confirm whether the use of this method can be applied to the daily routines of individuals.

Overall, these findings suggest that the use of digital photographs is a reliable and accurate measure of EI within a cafeteria and laboratory setting. Not only this, but participants, given the choice, prefer the use of a camera to weighing out each item they are to consume and are more likely to provide a complete food diary (Small et al., 2009). Furthermore, this method may lead to a more accurate measure of free-living habitual EI, as weighed food diaries are likely to restrict natural EI and lead to

the omission of some foods (Carlsen et al., 2010, Barrett-Connor, 1991). However, within free-living conditions this method's reliability is yet to be confirmed.

2.2 Measuring Energy Expenditure

The 'gold standard' method for measuring EE in free-living individuals is DLW (Livingstone and Black, 2003). This technique requires participants to consume a dose of stable isotopes (deuterium and oxygen 18 are commonly used in water). Urine samples are collected pre, post and daily during the free-living period. The samples are analysed by isotope ratio mass spectrometry to ascertain the rates of disappearance of the isotopes consumed which measures total body water and water-plus-carbon dioxide turnover rates. Total EE is then calculated by the production of carbon dioxide using indirect calorimetric equations (Livingstone and Black, 2003). This technique has been validated against respiratory gas exchange measures of EE in an array of participants with varying characteristics and circumstances (i.e., exercise and rest), and has been shown to be accurate within 8% in a review of 14 studies (Schoeller and Hnilicka, 1996). Although an accurate measure of EE, the equipment and the costs associated with employing this method mean that it is not often used as a routine measure of EE (Livingstone and Black, 2003). Other measures of EE such as heart rate monitoring, accelerometers or physical activity questionnaires have been used as alternatives, but their validity and accuracy of EE are distinctly lower than that of DLW (Livingstone and Black, 2003). Another measure of free-living EE is the use of combined heart rate-accelerometry; current available devices include the Actiheart (CamNtech, Cambridge, UK), which simultaneously measures heart rate and movement to calculate predicted total and activity EE (Barreira et al., 2009). This device has been shown to be valid, reliable and accurate in measuring HR; in the laboratory setting it was seen to accurately reflect walking and running intensity when compared to ECG (Brage et al., 2005), and in a range of field set activities there was little variation in EE when compared against indirect calorimetry (Crouter et al. 2008). When comparing free-living activities with the Actiheart, the AEI Moxus Metabolic Cart, Actigraph accelerometer, Polar heart rate monitor, and ECG, the Actiheart minimally underestimated EE at the highest workload and there was a high correlation in EE between the Actiheart and all other measures, suggesting that the use of the Actiheart device provides a suitable measurement of free-living EE (Barreira et al., 2009).

Although the most accurate measure of free-living EE is DLW, the complex and expensive nature of this method limits its feasibility (Livingstone and Black, 2003). Importantly, DLW cannot distinguish between different exercise intensities to quantify time spent sedentary and in light, moderate and vigorous physical activity. Combined heart rate-accelerometry has been shown to be an accurate, valid and reliable measure of EE in a variety of settings and would appear to be a suitable method for measuring EE and to distinguish between different activity intensities (Barreira et al., 2009, Brage et al., 2005, Crouter et al., 2008).

2.3 Energy Balance

The relationship between EE and EI was first notably investigated by Edholm et al. (1955), who found no correlation between EE and EI over a day in army cadets. However, over the two week period EI and EE began to correlate and a clear relationship between the two was seen; Edholm et al. (1955) then postulated that changes in EE may instigate changes in EI. Similar findings were reported by Mayer et al. (1956), who observed an increase in EI in jute mill workers as physical exertion levels increased above a certain level of EE (see Figure 1). Further observations showed that in jobs, which could now be classed as 'sedentary', the relationship between EE and EI appeared to fade and EI increased to levels that did not correlate with EE (Figure 1), a finding that would appear to have current applications within society when considering the high levels of sedentary behaviour and levels of obesity (HealthandSocialCareInformationCentre, 2015).

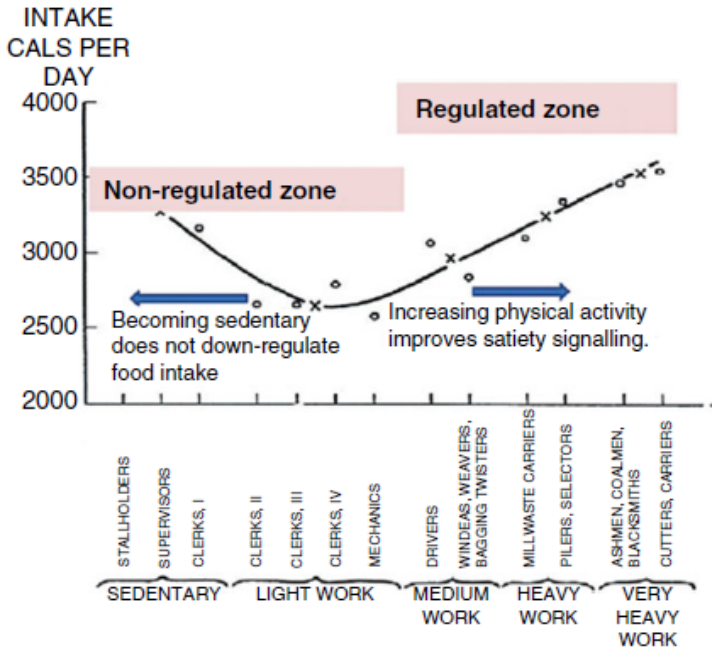

Figure 1. A modified version of the original graphic from the article by Mayer et al. (1956) highlighting how the level of EE affects EI, only above a certain level of EE (Blundell et al., 2015).

Mayer et al. (1956) also highlighted higher body weights in workers deemed more sedentary and suggested that these higher body weights were a direct consequence of the sedentary behaviour, as well as the observation that a reduction in activity did not induce a reduction in EI. Closer to the present day, the correlation between activity and EI and the effects on body weight and composition have been investigated more extensively. It is now known that a sustained period of positive EB, where EI exceeds EE, results in weight gain; increasing EE through regular exercise would, therefore, be a promising strategy for the prevention of overweight and obesity.

2.3.1 Acute Exercise and Energy Balance

The relationship between EE and EI is of particular interest when considering the regulation of body weight; even assessing the impact of acute exercise can provide essential information. It may be logical to suggest that an increase in EE through exercise would result in increases in feelings of hunger and consequently, increases in EI to restore the energy deficit created by exercise. Studies that have investigated the effects of an acute bout of exercise on energy balance, however, do not show this to be true (Harris and George, 2008, King et al., 1996, Jokisch et al., 2012, Tremblay et al., 1994). Rather than levels of hunger increasing post-exercise, a large amount of literature has shown that exercise results in an immediate suppression in hunger that persists for 30 to 60 minutes after exercise with no change in EI post-exercise for up to two days (Donnelly et al., 2014). This post-exercise suppression in hunger has been termed 'exercise-induced anorexia' (Blundell et al., 2003) and is most apparent in response to exercise that is completed at intensities greater than 70% $\dot{V}O_{2max}$. This phenomenon is short lived as hunger returns to basal levels after 60 minutes (King et al., 1994).

Studies investigating the effect of acute exercise on EB have typically included a period of EI observation for several hours post exercise to determine whether compensation occurs. A protocol used by King et al., (1997), where participants ran on a treadmill at 70% of maximum heart rate for 50 minutes twice in one day and post exercise EI was measured using a weighed self-record diary for the two days following, showed that the exercise induced a significant short term (one day) energy deficit . Not only did exercise induce an energy deficit, but also produced transient reductions in feelings of hunger, measured through electronic appetite rating scales (EARS), in the exercise condition compared with the control (King et al., 1997). Similarly, Broom et al. (2009) reported that both aerobic treadmill exercise and resistance exercise reduced hunger ratings when compared to a rest condition, thus it is not only aerobic exercise that induces transient declines in hunger (Broom et al., 2009). There is some evidence that the compensatory response to exercise may depend on habitual activity level. Jokisch et al. (2012) investigated whether habitually active and sedentary individuals responded differently to exercise at 65-75% of age predicted maximum heart rate, with EI measured 60 minute post-exercise using an *ad libitum* buffet meal, and with EI for the rest of the day assessed using a self-report food diary (Jokisch et al., 2012). Interestingly, the inactive group negatively compensated by -35.4% in the exercise compared the control condition *ad libitum* meal, whereas the active group increased EI by 22.7% during the exercise condition *ad libitum* meal. Notwithstanding the limited accuracy of measuring EI using food diaries (e.g. underestimation of portion size), this difference was also seen between the two groups for EI during the rest of the day, with the active group showing a greater level of compensation (35.6±135.2%) compared to the inactive group (3.0±135.0%). The authors suggest that this difference in compensation was due to the active group's regular activity resulting in them being able to somewhat acutely compensate for the extra EE, perhaps indicating a more 'sensitive' appetite control system (Jokisch et al., 2012). Since higher intensity exercise reduces appetite further than lower intensity exercise (Deighton et al., 2013a), an alternative explanation for the lack of compensation in the inactive group could be that the exercise was perceived as higher intensity when

compared with the active group (Jokisch et al., 2012). Although, it is important to note that neither group fully compensated for the energy deficit induced by the exercise over the course of the day, confirming the negative EB induced by acute exercise.

The majority of studies mentioned so far have examined the impact of high intensity exercise (at least 70% $\dot{V}O_{2max}$) on EB. A study by Harris and George (2008) showed that it is not only high intensity exercise that produces an energy deficit that is not then compensated for by an increase in EI, with a 60 minute treadmill walk (at 60-65% of age-predicted maximum heart rate) not producing any compensatory effects through EI. However, 12 hour post-exercise EI was measured through a dietary recall the day after, a method that has been shown to lack accuracy and is prone to under estimations in EI (Adams 1998). Despite this, the fact that this response to the exercise was seen in a sample size of 80 individuals (male, average age 30±8 years) adds strength to the findings. Not only has exercise been shown to induce an energy deficit that is not compensated for on the day of the exercise, but also the day following exercise. When assessing EI in the 48 hours post-exercise at 55-60% $\dot{V}O_{2max}$ for 60 minutes, there was still no compensatory increase in EI (Tremblay et al. 1994). Furthermore, King et al. (2015) found that perceptions of hunger did not significantly change the day after an exercise bout when compared with a control condition, again suggesting that no compensatory effects occurred in the day following exercise. Therefore, the energy deficit produced by the exercise was not compensated for even when followed up over 48 hours. However, the accuracy of EI measures in this study may have been limited, as participants did not feed *ad libitum* in a controlled laboratory environment at any point during the trials.

In summary, there is overwhelming evidence that an acute bout of exercise creates a short term energy deficit that individuals do not compensate for through increased EI for up to 48 hours, and can even acutely reduce EI and feelings of hunger (King et al., 2015, Jokisch et al., 2012, Broom et al., 2009). The suppression of appetite post-exercise is mostly seen at exercise intensities of at least 70% $\dot{V}O_{2max}$, although lower intensities still produce an energy deficit (Harris and George, 2008, King et al., 1996, Jokisch et al., 2012, Kelly et al., 2012, Tremblay et al., 1994). However, it is clear that there is still more research that is required to confirm and extend these findings that involves more valid and reliable analysis of EI post-exercise, as some studies continue to use a self-report methods to measure EI (Jokisch et al., 2012, Harris and George, 2008). The effects of multiple bouts of exercise also require greater examination, as the effects of a single bout of exercise are now well-documented.

2.3.2 Chronic Exercise and Energy Balance

Exercise has been shown to result in an energy deficit for up to 48 hours. It is, therefore, somewhat paradoxical that longer term exercise interventions do not elicit the weight loss that would theoretically be expected (Thomas et al., 2012). It has been suggested there are compensatory mechanisms, including an increase in EI and a decrease in 'non-exercise energy expenditure' (NexEE; the energy expended outside of imposed exercise, including rest and all other physical activities), that may

compromise exercise-induced weight loss (Church et al., 2009, Schneider et al., 2009). However, few studies have investigated the effects of more than a single session of imposed exercise on EB.

Stubbs et al. (2002a) and Stubbs et al. (2002b) conducted similar studies on the effect of seven days of imposed exercise on EB in men (Stubbs et al., 2002b) and in women (Stubbs et al., 2002a). In a within-subjects repeated measures design, EE and EI were measured throughout seven days of no exercise, moderate level exercise (two 40 minute sessions per day, 21.4 kJ/kg/day, equating to 1.6 MJ/day for a 70 kg subject) and high level exercise (three 40 minute sessions per day, 42.8 kJ/kg/day, equating to 3.2 MJ/day for a 70 kg subject). Only the women showed any type of compensation in EI, and this was only a partial compensation of ~30% of the ExEE during the high exercise compared with no exercise condition (Stubbs et al., 2002a). The study investigating men saw no EI compensation at all in any of the conditions, leaving a substantial negative EB over the exercise period; the authors highlighted the remarkable nature of these results given the large energy deficit that was tolerated and the fact the participants were able to eat *ad libitum* without markedly increasing EI (Stubbs et al., 2002b). There were, however, some indications of EE compensation in the men. Indeed, another interesting finding from both studies was that both men and women demonstrated a decrease in total daily EE as the exercise accumulated over the 7-day period. This finding suggests partial compensation through a reduction in NexEE (Stubbs et al., 2002a, Stubbs et al., 2002b), potentially due to fatigue by the end of the 7-day period. In a later study, Stubbs et al. (2004) compared a sedentary period against a moderate activity level period with EE measured via indirect calorimetry and EI measured by the researchers when participants requested food throughout the two 7-day periods. During this study there were no increases or decreases in EE outside of the imposed exercise, thus there was no compensation for exercise through NexEE (Stubbs et al., 2004), unlike indications from previous studies (Stubbs et al., 2002b). The only changes in the amount of intake from the participants was the increased fluid consumption (Stubbs et al., 2004). These results suggested, again, that the body is able to cope with a significant negative EB. Furthermore, the change to a more sedentary lifestyle also did not influence EI, suggesting that a period of positive EB can occur without the body changing its EI habits to 'compensate' for a reduction in EE (Stubbs et al., 2004). In longer term training interventions (4-40 weeks), spontaneous physical activity has not been reduced (Westerterp, 1998). It is possible that the reduction in NexEE seen in the men in Stubbs et al. (2002b) study was due to the exercise being every day and inducing higher levels of fatigue compared to many 4-40 week interventions where fatigue may not be such a factor, resulting in less prominent reductions in NexEE. This further highlights the differences in the study designs and findings of shorter and longer term exercise intervention, making it difficult to directly compare between studies.

Whybrow et al. (2008) extended the investigations of Stubbs et al. (2002a) and Stubbs et al. (2002b) to a 14 day period and reported that EE was markedly increased in a moderate level exercise and high level exercise condition compared to a no exercise condition. In contrast to Stubbs et al. (2002b), the study in men, there was an average of a 30% compensation through EI. This finding had only been seen in women previously (Stubbs et al., 2002a); however, Whybrow et al. (2008) did not directly

compare women and men to determine differences between sexes. The inclusion of a 14-day rather than 7-day exercise intervention and more sedentary participants in this study compared to previous studies (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b) may explain the increased EI over the exercise period that had only previously been seen in women (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b). The subjects were noted as finding the exercise bouts "arduous", possibly indicating high levels of physiological stress (Whybrow et al., 2008). Jokisch et al. (2012) suggest that an increase in perceived stress from exercise could alter the EI post exercise, potentially creating a larger energy deficit to begin with, and then increased EI in the latter stages of the test period. However, it is important to note that complete compensation still did not occur, leaving a substantial energy deficit. A limitation of this study was that only total EE was measured for each participant; therefore, any potential decreases in NexEE were unable to be determined (Whybrow et al., 2008).

It is apparent that the use of exercise in weight control may be more complicated than expected. In the short term, exercise does not produce compensatory responses in EI, whereas, longer term interventions show some evidence of partial compensation through increased EI and reduced NexEE (Stubbs et al., 2002a, Whybrow et al., 2008). Further research is required to investigate EI and EE in an attempt to understand potential compensatory mechanisms to imposed exercise; currently only a small number of studies have investigated exercise and EB and the results are inconsistent. Furthermore, although the studies discussed indicate that partial compensation may oppose ExEE, the potential mechanisms contributing to this partial compensation, including appetite-regulating hormones, have not been examined.

2.4 Appetite-regulatory Hormones

Various hormones have a role in the regulation of appetite, including glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), pancreatic polypeptide (PP), ghrelin and peptide YY (PYY) (Suzuki et al., 2010). In particular, ghrelin and PYY have become intriguing research targets due to their responsiveness to nutrient intake and acute exercise (King et al., 2011).

2.4.1 Ghrelin

Ghrelin is the only known orexigenic (appetite-stimulating) peptide hormone (Kojima et al., 1999) and is produced primarily by cells in the oxyntic glands of the stomach. Ghrelin circulates in two forms, deacylated and acylated. Deacylated ghrelin forms more than 90% of total ghrelin and acylated ghrelin less than 10% (Delporte, 2013). Acylation of ghrelin is essential for ghrelin to bind to growth hormone secretagogue receptors and to be able to cross the blood-brain barrier, whereas deacylated ghrelin is unable to perform this type of activity and is thus considered unimportant for appetite regulation (Broom et al., 2007, Broglio et al., 2003). Ghrelin is unusual among peptide hormones because serine 3 must be n-octanoylated for ghrelin to perform its actions (Sato et al. 2012); this modification is the first

known case among mammals and begins the acylation process (Kojima et al., 1999). The process of octanoylation, is the modification of a protein amino acid by an octanoic acid. An eight carbon-fatty octanoic acid modifies serine 3 and acylated ghrelin is produced post-translationally from this process (Lim et al., 2011). This octanoylation is required for the acylated ghrelin to be able to bind to the growth hormone secretagogue receptor 1a (GHS-R1a) and be able to perform its biological functions, such as stimulating food intake (Lim et al. 2011). Indeed, the acylation of ghrelin (addition of an acyl group) allows it to bind to a particular gene (GHS-R1a) that regulates the orexigenic effects of acylated ghrelin. After secretion from the stomach, ghrelin binds to growth hormone secretagogue receptors (GHS-R) on the vagus nerve and hypothalamic nuclei to stimulate food intake (Delporte, 2013, Sato et al., 2012).

Research into the pre- and post-prandial concentrations of total ghrelin have established its role as a hormone that stimulates hunger and initiates feeding (Cummings et al., 2001). Concentrations of acylated ghrelin are at their highest in the 1-2 hours pre-prandial and drop to nadir in the hour postprandial (Al Awar et al., 2005). Some have questioned whether the rise in pre-prandial total ghrelin concentrations are in expectation of a meal as peaks in total ghrelin concentrations are in line with meal patterns (Frecka and Mattes, 2008), although this investigation measured total rather than acylated ghrelin.

2.4.2 Peptide Tyrosine Tyrosine

Peptide tyrosine tyrosine (PYY) is a 36-amino acid peptide first isolated from the porcine upper small intestine (Tatemoto and Mutt, 1980) and is released following meals, being secreted from the endocrine L cells within the small and large bowel (Neary et al., 2004). There are two circulating forms of PYY: PYY_{1-36} acts on all Y receptors whereas the more prominent form PYY_{3-36} only binds to the Y2 receptor (Y2R) within the hypothalamus, suppressing food intake (Suzuki et al., 2012, Suzuki et al., 2010). The mechanism by which PYY_{3-36} suppresses appetite is not confirmed, as conflicting results have been seen regarding the vagal-brainstems role in appetite suppression. It is, however, known that PYY_{3-36} exerts action directly into the arcuate nucleus within the hypothalamus (Suzuki et al., 2012), where it binds to the Y2R and subsequently suppresses food intake (Suzuki et al. 2010). In support, Batterham et al. (2002) found that peripheral injection of PYY_{3-36} inhibits food intake in rats and mice, but not in mice that are Y2R null. As the central nervous system contains Y2R throughout, there is potential for PYY_{3-36} to exert its action via vagal activation as well as the Y2R in the hypothalamus, however, this is yet to be confirmed (Karra et al. (2009).

Despite PYY_{3-36} being the most prominent form (~65%) of the peptide PYY, there is evidence that total PYY when measured reflects PYY_{3-36} (Suzuki et al., 2012, Broom et al., 2009). The pre-prandial decrease and post prandial increase in PYY have caused this peptide to become a research target in attempts to understand the objective mechanisms of appetite control.

2.5 Appetite-regulatory Hormones and Exercise

From the literature investigating exercise and EB, it is evident that acutely, exercise suppresses appetite and EI, and chronically, a partial compensation through EI may be seen. It is therefore, interesting to determine the responses of acylated ghrelin and PYY to exercise to detect the potential physiological mechanisms underpinning the relationship between exercise and appetite or EI.

2.5.1 Appetite-regulatory Hormones and Acute Exercise

The energy deficit and suppression in appetite produced by an acute bout of exercise strongly correlates with a post-exercise suppression in acylated ghrelin and simultaneous increase in PYY (Broom et al., 2007, King et al., 2010a). From the literature it is clear that the modalities (running, cycling, rowing) and intensities (~70% $\dot{V}O_{2max}$) of exercise that have so far been investigated cause a suppression of acylated ghrelin and increase in PYY (Broom et al., 2007, Holmstrup et al., 2013, Deighton et al., 2013b). The most significant changes are seen from these hormones when exercise intensity reaches ~70% $\dot{V}O_{2max,}$ inducing an energy deficit over 1-2 days and no compensatory response from acylated ghrelin or PYY (King et al., 2015). Broom et al. (2007) was one of the first to investigate the effects of acute exercise on acylated ghrelin, using fasted exercise at 75% $\dot{V}O_{2max}$ for 1 hour followed by an 8 hour rest. Interestingly, the responses of acylated ghrelin to the bout of exercise differed from those reported of total ghrelin in other studies: total ghrelin has been shown to remain unaffected in response to a single bout of exercise (Kraemer et al., 2004, Schmidt et al., 2004, Burns et al., 2007), whereas acylated ghrelin decreases during and post-exercise (Broom et al., 2009, Broom et al., 2007, King et al., 2010a). Acylated ghrelin responds quicker than total ghrelin to glucose ingestion (Hosoda et al. (2004), which could partly explain the lack of a response in total ghrelin to exercise (Broom et al., 2007). Further to this, as stated in section 2.4.1, only acylated ghrelin can perform hunger-stimulating actions, but only forms ~10% of total ghrelin, thus changes in acylated may have been masked when measuring total ghrelin. Indeed, Broom et al. (2007) also reported a positive correlation between feelings of hunger and acylated ghrelin concentrations, further supporting the notion that acylated ghrelin stimulates feeding. Further findings from this study include a suppression of acylated ghrelin during and immediately post-exercise and lower concentrations of acylated ghrelin at the 9 hour time point in the exercise condition compared to the control (Broom et al., 2007). However, more frequent blood sampling has since been used (Broom et al., 2009, King et al., 2010a) to provide a more detailed indication of the response to exercise and EI; also, the analysis of PYY as well as acylated ghrelin would have provided a greater understanding of the mechanistic responses of appetite to exercise.

Studies that have investigated PYY have found the opposite response (i.e., increased concentrations) to that of acylated ghrelin. Deighton et al. (2013b) reported that both steady state (60% $\dot{V}O_{2max}$) and high

intensity (85-90% $\dot{V}O_{2max}$) intermittent exercise increased PYY concentrations post-exercise when compared with a control condition. However, this response was only statistically significant in the steady state condition, despite greater appetite suppression during the high intensity intermittent exercise condition compared with steady state exercise. In addition, exercise did not affect EI at the *ad libitum* buffet meal provided 7 hours after exercise, a finding that has been seen in previous literature and indicates a lack of any compensatory mechanism to increase EI post-exercise (Deighton et al., 2013b). Holmstrup et al. (2013) investigated the effects of continuous and intermittent exercise (60–65% $\dot{V}O_{2peak}$) on PYY and appetite, intermittent exercise was broken up into 5 minute bouts every hour and the continuous bout was in the morning of the test day. Immediately following the continuous bout of exercise, PYY concentrations were elevated; these elevations were only transient, lasting no longer than 2 hours post exercise. The intermittent exercise bouts did not elevate PYY concentrations; however, feelings of satiation and reduced hunger were greater in this condition compared to rest and continuous suggesting that intermittent exercise could further reduce EI compared to continuous exercise and that effects of exercise on appetite may depend on the type of exercise performed (Holmstrup et al., 2013). A limitation of the discussed studies is that they did not measure both acylated ghrelin and PYY simultaneously.

Studies have investigated both acylated ghrelin and PYY and have seen similar results to Broom et al. (2007), Deighton et al. (2013b) and Holmstrup et al. (2013). Similar protocols with various modalities of exercise (i.e., cycling, rowing, and resistance exercise) have been used when investigating these hormones. Broom et al. (2009) compared the difference between treadmill running and resistance exercise. Within this study, hormone concentrations were measured every half hour until hour 6 and then every hour until hour 8 to provide a more detailed examination on these responses than previous work (Broom et al., 2007). Once again, exercise suppressed acylated ghrelin and hunger during both aerobic and resistance exercise and for up to 1 hour post exercise when compared to the resting control condition. Although PYY concentrations increased in both exercise trials, a greater suppression was seen in the running condition, perhaps due to the increased EE and the higher levels of stress placed upon the gut during the running exercise (Broom et al., 2009). Other studies have reported similar findings, with less strenuous exercise not resulting in increased PYY concentrations while more strenuous exercise (~70% $\dot{V}O_{2max}$) sees a transient increase in PYY. Deighton et al. (2013a) compared the effect of sprint interval exercise (six 30 second Wingate tests), prolonged endurance exercise (68% $\dot{V}O_{2max}$) or no exercise (control), performed on an electronically braked cycle ergometer, to determine whether exercise-induced reductions in appetite were dependent on exercise intensity. Although the sprint interval exercise induced a greater suppression of acylated ghrelin and increase in PYY compared to endurance exercise, the prolonged endurance exercise induced a greater 24 hour energy deficit due to the higher EE during the exercise coupled with the similar EI between conditions. In line with previous work, acylated ghrelin was suppressed until 45 minutes post exercise in the endurance condition and until 2 hours and 45 minutes post exercise in the sprint interval exercise condition when compared with a non-exercise control. PYY was elevated in both exercise conditions until 45 minutes post exercise buffet meal, compared to a non-exercise control. Perceptions of appetite in all conditions

reached similar values preceding the 45 minutes post-exercise buffet meal, after which levels of hunger remained similar in all conditions. Unexpectedly, there was a significant suppression of acylated ghrelin that remained in the sprint interval exercise condition post the 45 minute post exercise buffet meal; this brings into question the role of acylated ghrelin as a regulator of appetite, as a decrease in EI compared to other conditions did not occur. Further to this, EI did not alter between the three conditions at any of the meal opportunities, adding further support to the finding that acute exercise does not induce a compensatory response through EI (Deighton et al., 2013a). This study not only further adds to the evidence that an acute bout of exercise suppresses hunger and acylated ghrelin and elevates PYY without inducing any compensatory increase in EI, but also that a differing intensity (maximal sprint exercise) and an additional modality (cycling) to running and resistance training have similar effects. In contrast, another study reported that brisk walking, exercise defined in this study as 'intensity yielding a mild shortening of breath yet still enabling the individual to converse', had no effects on acylated ghrelin (King et al., 2010b), possibly due to the exercise intensity being too low. Although acylated ghrelin was not actually suppressed following brisk walking during this study, there was still no change in EI or appetite in the hours following the brisk walk and, therefore, an energy deficit remained (King et al., 2010b). Although PYY was not measured during this study, it could be assumed that no change in PYY concentrations would have occurred post exercise due to the lack of acylated ghrelin response. However, further research would be required to determine this. Overall, this suggests that the exercise-induced response of these hormones appears to be intensity-dependent (Hazell et al., 2015).

The evidence discussed suggests that a suppression in acylated ghrelin and an elevation in PYY during and for up to 1 hour post exercise offers a potential mechanism to explain why there is a transient suppression in appetite and no compensatory changes in EI following an acute bout of exercise. This suppression of acylated ghrelin and increase in PYY is generally seen in intensities of exercise of 70% $\dot{V}O_{2max}$ and above (Broom et al., 2007, King et al., 2010a), while lower intensities still induce and uncompensated for energy deficit.

2.5.2 Appetite-regulatory Hormones and Chronic Exercise

Longer term exercise interventions (more than one day) that have been investigated to determine how exercise affects EB have seen that there is a partial compensation through EI (~30% of the exercise EE) over 7-14 days (see section 2.3.2), whereas there are no short term compensatory increases in EI following acute exercise for at least 1-2 days (see section 2.5.1). It is possible that a rise in circulating levels of acylated ghrelin and suppression of PYY may offer an explanation into the partial compensation seen in the longer term EB studies. However, few studies have investigated the long term effects of exercise and the concentrations of ghrelin, and those that have, do not typically assess acylated ghrelin. PYY has also received little attention in response to longer term exercise interventions. Nevertheless, unlike acute exercise, it has been suggested that longer term exercise interventions may provide enough time for total ghrelin concentrations to be affected (Hosoda et al.,

2004). Despite this, there is a need for the responses of acylated ghrelin and PYY to longer term exercise to be investigated.

Studies measuring total ghrelin over longer term exercise interventions lasting more than one day have observed conflicting results. Ramson et al. (2008) investigated four weeks of exercise where the relative amounts of training were kept similar: 80% of training volume was low-intensity endurance rowing training, 10% was low-intensity running or cycling and 10% was strength endurance training. Within the first 3 weeks, the training load was increased each week from a baseline of 10 hours in week one, increasing by 50% in week two and a further 10-15% in week three; in the fourth week training returned to baseline levels (Ramson et al., 2008). The exercise programme did not affect fasting ghrelin concentration at any point, but did result in increased total ghrelin concentrations 30 minutes post-exercise at all assessment times when compared to pre-exercise concentrations, with significance being reached for the 24 hours post week 4 test day. This finding suggests that four weeks of exercise increases post-exercise total ghrelin concentrations. Although this increased total ghrelin could contribute to higher EI, acylated ghrelin was not measured, and previous research has shown that a rise in total ghrelin does not necessarily increase EI (Christ et al., 2006). Therefore, there is a need for similar research assessing the impact of long term exercise on acylated ghrelin specifically. One interesting finding from this study was the increase in EI, assessed through a diet diary, from week one to week two (when training load increased by 50%), yet no significant change at any other times was observed. However, in line with previous findings (Stubbs et al., 2002a, Stubbs et al., 2002b, Whybrow et al., 2008), this partial compensation in EI was not sufficient to account for the ExEE, thus participants remained in negative EB throughout the exercise intervention (Ramson et al., 2008). It is possible that the less marked (10%) increase in training load in week three was not sufficient to induce further compensation. In a follow-up study with a similar study design, Ramson et al. (2012), (Ramson et al., 2008) used lower intensity exercise, 50% being endurance training and 50% resistance training, with the load increasing by 50% each week. In contrast to the previous study (Ramson et al., 2008), total ghrelin concentrations were reduced from pre- to post-exercise in week two, with no other changes observed, suggesting that that long term exercise may not affect, or may even lower, total ghrelin concentrations. However, the lower exercise intensity used in this study could explain this finding and the disparity with findings of the previous study (Ramson et al., 2008). Since the participants within these studies were highly trained, the results cannot be generalised to other populations, as previous literature suggests that a more active lifestyle potentially improves appetite control and the stress of exercise may have a less marked effect on these 'active' individuals (Jokisch et al., 2012). Furthermore, total ghrelin, rather than acylated ghrelin was measured, which has been shown to be unaffected by acute exercise (Kraemer et al., 2004, Burns et al., 2007), and subjective measures of hunger were not taken, meaning the effect exercise had on feelings of hunger could not be compared to total ghrelin concentrations. Added to this, no postprandial assessment of ghrelin was carried out to investigate the response to a meal, as has been used in acute studies (Broom et al., 2009, King et al., 2015, King et al., 2010a). The measurement of average daily EI was only made over the final three days of each training week via an estimated food diary, a method found to be unreliable and

likely to yield underreported levels of EI (Adams, 1998). It would have been interesting to measure the full week to ascertain whether EI increased over time across the week as the exercise training accumulated. Since these studies did not measure PYY, it was also not possible to determine the potential effects on this hormone as a compensatory mechanism to the exercise intervention.

Most of the research that has measured acylated ghrelin, rather than total ghrelin, in response to longer term exercise has been in samples of children and adolescents. Therefore, it should be noted from the outset that the findings cannot be applied directly to the adult population and that the use of different populations could partly explain discrepancies in findings across literature investigating ghrelin (Stensel, 2010). Nevertheless, this literature has provided comparable results with the work assessing total ghrelin in adults, with no changes in fasting acylated ghrelin concentrations reported in response to longer term exercise (Kim et al., 2008). However, the response to a meal was not measured, a limitation also seen in the studies measuring total ghrelin in adults (Jones et al., 2009). In overweight children, Kim et al. (2008) reported differences in the response of acylated ghrelin, unacylated ghrelin and total ghrelin concentrations following a 12 week programme that induced significant weight loss. Fasting total and unacylated ghrelin concentrations significantly increased from before to after the exercise programme, whereas acylated ghrelin did not change. This could have potentially contributed to the weight loss during this programme; the lack of change in acylated ghrelin may have meant that hunger did not increase, resulting in no EI compensation. Thus, the exercise-induced energy deficit may be more likely to be maintained. However, once again, the response to a meal was not measured to assess ghrelin concentrations, an important variable to measure given that individuals spend the majority of the day in the postprandial state. Also, PYY was not measured to allow for further understanding of any potential objective mechanisms in the regulation of appetite. Jones et al. (2009), however, did measure PYY and saw increases in post-exercise intervention (3 sessions of 45 minutes aerobic exercise per week for 32 weeks) concentrations, potentially indicating greater feelings of fullness. However, only fasting PYY concentrations were measured, meaning that it is not possible to ascertain whether this would lead further compensatory responses from PYY throughout a day where the majority of time is spent in the postprandial state (Williams, 1997). Another study, Kanaley et al. (2014), importantly, measured the PYY response to a test meal. Following a 15 day exercise intervention carried out over 3 weeks, no changes in PYY concentrations were found in response to six meals of 250 kcal throughout a 12 hour measurement day. No weight loss occurred during this intervention which could suggest the exercise was not strenuous enough to induce a significant energy deficit, or that some form of compensation occurred. However, no EI analysis was carried out during this study, so the possibility of EI compensation cannot be confirmed. Acylated ghrelin was also not measured during this study to determine possible compensatory mechanisms that can affect appetite. Therefore, it is difficult to gain a detailed insight in the compensatory response to this exercise programme (Kanaley et al., 2014).

Only a very small number of studies have investigated both acylated ghrelin and PYY in response to a longer term exercise intervention (more than one day of imposed exercise) in adults (Martins et al.,

2010). In a sample of healthy sedentary overweight and obese individuals, Martins et al. (2010) examined the effect of a supervised 12 week exercise intervention (five days per weeks) designed to induce an energy deficit of 500 kcal per day while maintaining normal dietary intakes, as assessed via 3 day estimated food diaries with the use of photographic booklets. Following the exercise intervention, there was an average weight loss of 3.5 kg, an increase in fasting acylated ghrelin and an increase in fasting subjective hunger, but no change in acylated ghrelin in response to the test meal. This increase in fasting acylated ghrelin may be viewed as a compensatory mechanism to induce an increase EI. In contrast, no changes in fasting PYY concentrations occurred, which may be surprising given the changes in acylated ghrelin. It is possible that the changes in acylated ghrelin concentrations would not reflect the true effects of the exercise intervention under 'real life' conditions, as participants in this study were asked 'not to alter their food intake', which may have resulted in them eliciting some dietary restraint (Martins et al., 2010). Moreover, there is evidence that energy deficits induced by diet and exercise induce differing responses. Compared with an exercise-induced energy deficit, an acute energy deficit induced by dietary restraint has been shown to increase acylated ghrelin concentrations, hunger and desire to eat, whereas exercise suppresses acylated ghrelin and appetite (King et al., 2011). Furthermore, dietary energy deficit attenuated the PYY response to a meal and increased appetite following a meal when compared with the exercise-induced energy deficit, which did not change appetite or PYY responses to a meal (King et al. 2011). Further research is required to determine the response of PYY to long term exercise.

Overall, the small body of evidence on the effects of long term exercise on acylated ghrelin and PYY has produced inconclusive findings, perhaps due to between-study differences in participants and study design, and the use of methods with significant limitations. It is, therefore, clear that more extensive and sound research is required to understand the responses of acylated ghrelin and PYY to exercise interventions lasting more than one day.

2.6 Summary

Overweight and obesity has reached epidemic levels, bringing with it a need for research to identify factors that have an impact on energy balance to prevent body fat gain. One major issue in providing these strategies is the accurate and feasible measurement of EI during free-living studies; self-report estimated food diaries are inaccurate and unreliable and weighed food diaries increase the burden placed on participants, leading to incomplete diaries and changes in habitual dietary intakes. A promising strategy for the measurement of free-living EI with reduced participant burden is that of a combined photographic and written food diary, with studies showing that this method may provide an accurate measure when compared to weighed food diaries. However, the reliability of this method is yet to be tested within a free-living environment across seven days.

Energy balance and appetite are key factors in determining strategies to prevent body fat gain. Specifically, investigating the effects of exercise on EB and appetite could be key to providing

strategies that will prevent overweight and obesity, and understanding potential mechanisms that underpin compensatory behaviours is crucial. In response to acute exercise, evidence has clearly shown that there is an uncompensated for energy deficit that can last 1-2 days. At intensities of ~70% $\dot{V}O_{2max}$ and above there is a suppression of appetite that lasts for up to one hour post exercise; potential mechanisms for this relate to the response of appetite-regulating hormones. Indeed, there is ample research that shows a suppression of acylated ghrelin and an increase in PYY in response to an exercise bout of ~70%$\dot{V}O_{2max}$ and above. In response to longer term exercise interventions (more than one day of exercise) the research is not consistent, with many conflicting results. Over the long term, exercise interventions often do not elicit as much weight loss as would theoretically expected given the high degree of negative EB created by an acute bout of exercise, suggesting compensation occurs at some point. Paradoxically, studies have observed minimal compensatory changes in EI to account for the deficit produced by the exercise (only 30% partial compensation through EI). This has led to suggestions that other compensatory mechanisms, such as a reduction in NexEE, compromise the weight loss effects of exercise. However, this notion has not been researched thoroughly to date. Thus, there is a need for further research to fully determine the effects of more than a single bout of exercise on energy balance and appetite-regulating hormones.

3.0 General Methods

The purpose of this chapter is to describe the methods used in the experimental studies that follow. These methods were common between studies 1 and 2; methods that specifically relate to individual studies are described within the experimental chapters (Sections 4.2 and 5.2).

3.1 Participants

Following ethical approval, active males aged 18-30 years were invited to participate in study one and study two. All participants were provided with an information sheet (Appendix A) detailing the nature and purpose of the study. A consent form (Appendix B), Physical Activity Readiness Questionnaire (PAR-Q), a Pre-test Medical Questionnaire and blood, analysis form (for study 2) (Appendix C) were completed and signed by each participant before testing commenced.

3.2 Preliminary Measurements and Familiarisation Trial

3.2.1 Anthropometry

Anthropometric characteristics were assessed and recorded during preliminary visits. Stature was measured using a stadiometer (Holtain Ltd, Crymych, Dyfed, UK) to the nearest cm and body mass (BM) was measured using TANITA scales (Hoogoorddreef 56E, Amsterdam, Netherlands) to the nearest 0.1 kg.

3.2.2 Resting Metabolic Rate

Participants arrived to the laboratories at 09:00 in a fasted state (participants were asked to not eat or drink anything except water after 21:00 the day before) having been instructed not to consume any alcohol or caffeine and not to have taken part in any strenuous physical activity for of the previous 24 hours. Participants then lay supine for 20 min; expired air was collected through online gas analysis (Metalyzer 3b, Leipzeg, Germany) for the final 10 min to ensure that they reached a true resting steady state. Resting metabolic rate (RMR) was then calculated by inserting the averaged oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) values from the final five minutes of the RMR test into the Weir equation: $[(3.941)(\dot{V}O_2)+(1.106)(\dot{V}CO_2)]*1440$ adopted to calculate human energy requirements (kcal/day) (Weir, 1948). Measured RMR values were subsequently used for individual calibration of the device used to measure free-living EE (Actiheart, CamNtech, Cambridge, UK).

3.2.3 Preliminary Exercise Tests

Participants completed two exercise tests on a treadmill (Woodway, PPS55 Med-i, D-79576 Weil am Rhein, Germany) to determine the relationship between heart rate and energy expenditure (for studies 1 and 2) and to estimate the speed corresponding to 70% peak oxygen uptake ($\dot{V}O_{2peak}$) (for study 2 only). For the submaximal test, the treadmill was set at a gradient of 1% as this has been shown to most accurately reflect the energy cost of outdoor running (Jones and Doust, 1996). The protocol used consisted of 4 x 4 min stages; the first two stages remained consistent between participants and were kept at 4.5 and 5.8 km/h; the last two stages were set at speeds between 7 km/h and 11 km/h depending on the individual heart rate (HR) and respiratory exchange ratio (RER) of the participant, to ensure an RER of <1.00 during the final stage (Zhang et al., 2012). Online gas analysis (Metalyzer 3b, Leipzeg, Germany) was used to continuously measure expired air throughout the treadmill test and rate of perceived exertion (RPE) was taken in the last 15 seconds of each 4 min stage.

Following the submaximal test, the participants were given a 10 min rest period before being asked to complete a maximal exercise test. Participants ran at a set pace which was determined by their submaximal test (the speed corresponding to a HR of 150 bpm or an RPE of 12). The test started at a gradient of 1% and increased every minute by 1% until the participant reached volitional exhaustion. RPE was taken in the last 15 s of each stage. The criteria used to determine whether the participants had reached their true $\dot{V}O_{2max}$ was a plateau in $\dot{V}O_2$ values (less than 2.1 ml/kg/min during the final stages) (Taylor et al., 1955); if a plateau was not observed, the following secondary subjective criteria had to be attainment for the confirmation of $\dot{V}O_{2peak}$: an RER of equal to or greater than 1.15, heart rate within 10 bpm of the subject's age predicted heart rate maximum and an RPE of 18 or above.

3.2.4 Assessment of Free-living Energy Intake

Throughout each 7-day measurement period, participants were asked to complete a combined written and photographic food diary (Appendix D). All participants were provided with a digital camera (Vivitar, ViviCam 46, China) and instructed to photograph all foods and beverages consumed, alongside completing an estimated food diary, where they were asked to report the day and time of all foods and drink consumed, brand of the food, description of the food including preparation method, portion size (an estimate, e.g. a bowl, a handful) and any leftovers. The participants were asked to use photographs as a recall method when completing their food diaries. During the preliminary testing, the participants received a tutorial on using the digital cameras, completing their food diaries, and were given written instructions to accompany the tutorial (Appendix E). In brief, each participant took a photograph diagonally down (65-75 degrees) of their food before and after their meals including a standard knife, fork or spoon to confirm the size of the plate or bowl.

Amounts (grams) of each food item were estimated by comparing the digital photographs taken by the participants' with the Young Person's Food Atlas (Foster et al., 2010). The Young Person's Food Atlas allowed the researcher to convert estimations of portion size from the photographs into grams consumed. The use of food photographs to estimate food portion size has been shown to increase the accuracy of food portion size estimation compared with unaided estimates (Lucas et al., 1995 & Robinson et al., 1997). In addition, the use of digital photography to estimate food portion sizes, compared directly to weighed food intake, has been shown to have a high validity (Williamson et al., 2003; for review see Martin et al., 2013). Subsequently, the 7-day food diaries were analysed using Dietplan 6.70 (Forestfield Software, Horsham, UK) to estimate energy intake (kcal/day) during each measurement period.

3.2.5 Assessment of free-living energy expenditure

Participants were fitted with a combined heart-rate/accelerometer (Actiheart, CamNtech, Cambridge, UK) to measure free-living physical activity EE and assess daily total EE (TEE) and activity EE (AEE) for the duration of each 7-day measurement period. The primary purpose of the Actiheart is to predict physical activity energy expenditure (PAEE) and it has been shown to be a valid and reliable measure in adults (Brage et al., 2005). The monitor was fitted on day 0 and removed on day 8 of each condition. Following the completion of a signal test to determine the clarity of the HR signal, the Actiheart was attached to participants via two ECG electrodes; the medial electrode was attached on the skin at the base of the sternum, with the lateral electrode horizontally to the left side. The Actiheart wire was straight but not taut. At the point when the monitor is fitted, participants were provided the following message both verbally and in writing to ensure that only genuinely meaningful behavioural responses are recorded: "Your lifestyle choices during this free-living monitoring period are central to this study. We are interested in any natural changes in your diet and/or physical activity habits, which you may or may not make in response to the intervention. This monitoring period has been carefully scheduled to avoid any pre-planned changes in these habits, such as a holiday or diet/exercise plan. You should inform us immediately if unforeseen factors external to the study may influence your lifestyle."

The Actiheart was then set to record HR and movement in the 'Advanced Energy Expenditure' recording mode continuously over 15-s epochs. Individual calibration was performed by inserting the EE values (calculated using the Weir equation) corresponding to the four different exercise intensities (expressed as heart rate) performed during the preliminary submaximal treadmill test, and the measured RMR and $\dot{V}O_{2peak}$ values calculated from preliminary testing (detailed in sections 3.2.1 and 3.2.2). Data was downloaded after each measurement period using a reader interface unit and analysed using Actiheart software (Version 2.132, Cambridge Neurotechnology Ltd, Cambridge, UK).

4.0 Study 1

4.1 Introduction

The accurate measurement of free-living EI is difficult. Weighed food diaries have been criticised for being burdensome for the participant and changing a participant's normal eating habits, which may affect the free-living element of studies employing this method (Carlsen et al., 2010, Adams, 1998, Livingstone et al., 1990). One method that has recently been shown to be an effective alternative way to measure free-living EI is the use of a photographic food diary; this method reduces participant burden compared with a weighed food diary, and shows similar accuracy for EI estimations when studied in laboratory and cafeteria environments (Williamson et al., 2004, Williamson et al., 2003). These findings make the photographic food diary a potentially viable option to measure free-living EI. One of the main advantages of using digital photography is that the reduced burden placed on participants can reduce missing data from food diaries when this method is used over periods longer than 3-5 days (Small et al., 2009). However, the use of a photographic food diary over a 7-day period in a free-living environment is yet to be investigated. Despite the need for effective methods to assess the free-living EI of participants, studies to date have not asked the participants to take photographs of their meals, but have used a video camera in a cafeteria setting to capture portion sizes and have them independently estimated (Williamson et al., 2004, Williamson et al., 2003). It would therefore, be of interest to investigate the reliability of a photographic food diary over a 7-day period under free-living conditions. Further to this, EE is a fundamental measurement in the assessment of EB, and, although already deemed to be a valid and reliable measure of EE (Barreira et al., 2009, Brage et al., 2005, Crouter et al., 2008, Spierer et al., 2011), it would be valuable to confirm the reliability of combined HR-accelerometry for assessing free-living EE.

The primary aim of this study was:

- To examine the reliability of a combined photographic and written food diary to assess free-living energy intake across seven days in men.

The secondary aim of this study was:

- To examine the reliability of a combined heart-rate accelerometry device to assess free-living energy expenditure across seven days in men.

4.2 Methods

4.2.1 Participants

Thirteen males aged 18-30 years participated in the present study. Prior to the main trials, participant's anthropometric data was collected (detailed in section 3.2.1 and preliminary RMR, submaximal and maximal exercise tests were completed (detailed in sections 3.2.2 and 3.2.3). The participant characteristics are shown in Table 1.

Table 1. Participant characteristics

Variables	*n* = 13
Age (years)	23 ± 1
Body mass (kg)	82.1 ± 15.1
Height (cm)	176.5 ± 5.9
RMR (kcal/day)	1681.61 ± 270.98
$\dot{V}O_{2peak}$ (ml/kg/min)	44.3 ± 6.9

Values are expressed as means ± SD

4.2.2 Study Design

Using a repeated measures design, participants were required to complete 2 x 7 day trials separated by a 7 day washout period (Figure 2). During each trial, participants completed a combined photographic and written food diary for the assessment of daily EI, and wore an Actiheart monitor (CamNtech, Cambridge, UK) to assess daily TEE and AEE (detailed in section 3.2.5). Participants were provided with a paper food diary and a digital camera (Vivitar, ViviCam 46, China) during the 7-day trials to manually record and photograph all consumed food and fluids throughout each day (detailed in section 3.2.4). The participants were asked to follow their normal daily routine, to eat and drink naturally and were specifically told they were not restricted as to what they could or could not eat or drink.

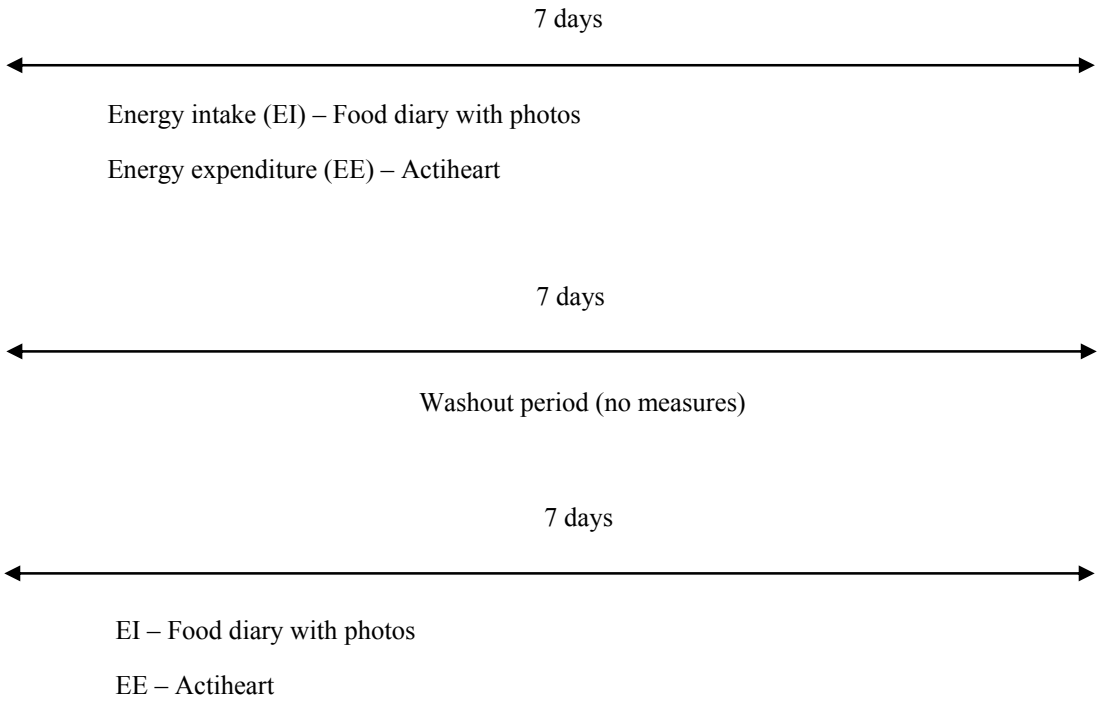

Figure 2. Schematic of main trials

4.2.4 Statistical Analyses

Statistical analyses were completed using Statistical Package Social Sciences software version 22. Due to some missing data points, linear mixed models (LMM) were used to compare the two, 7-day test weeks. Quantile-Quantile (Q-Q) plots were used to test whether the data was normally distributed. The smallest Hurvich and Tsai's criterion was used to select the most suitable model. Normality and homogeneity of variance were measured on all residuals data for all variables using Q-Q plots. Statistical significance was accepted at $P \leq 0.05$.

Limits of agreement (LoA) were used to compare EI and EE at the individual level (Bland and Altman, 1986). Systematic error (SE), or bias, was determined by calculating the mean difference between each test week and the random error (RE) was the standard deviation (SD) of the paired differences, as outlined by Bland and Altman (1986). The LoA were then calculated by determining a 95% limit above and below the mean difference (bias ± (1.96 × RE)). Physical activity level (PAL) and EI:RMR ratio were calculated to determine the plausibility of the results obtained.

4.3 Results

No main effect of week was observed for EI (F=2.505, P=0.116) or day (F=1.127, P=0.349). There was no week by day interaction (F=1.763, P=0.110) (Figure 3). Individual paired data provided a systematic bias ± random error of 143 ± 715 kcal/day, resulting in 95% LoA of -1258 to 1545 kcal/day (see Figure 4).

There was no main effect of week observed for TEE (F=0.17, P=0.897) or day (F=0.341, P=0.914) (see Figure 5) and no week by day interaction (F=0.753, P=0.608). Individual paired data provided a systematic bias ± random error of -15 ± 455 kcal/day, resulting in 95% limits of agreement (LoA) of -907 to 876 kcal/day (see Figure 6).

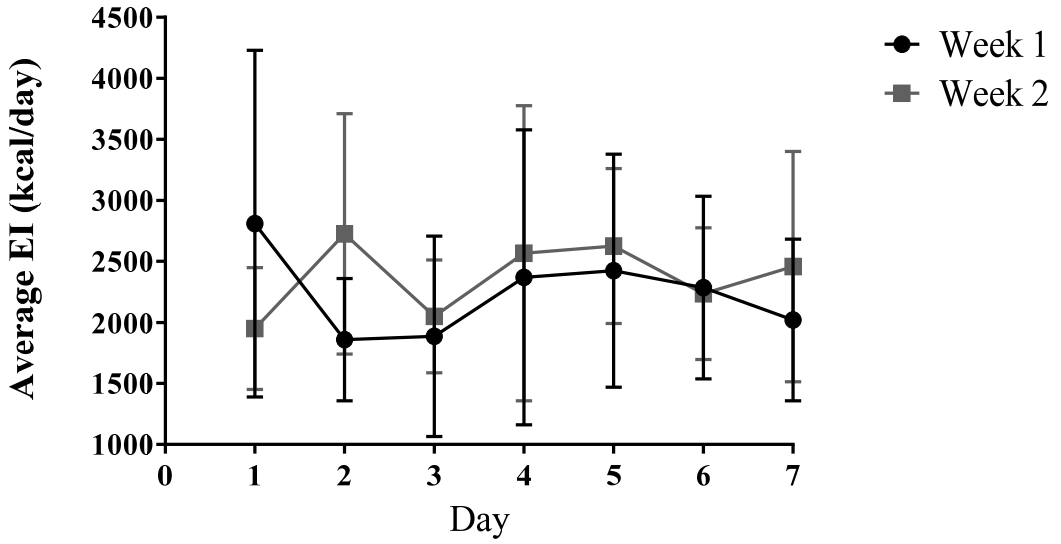

Figure 3. Average EI (kcal/day) for week one and week two.

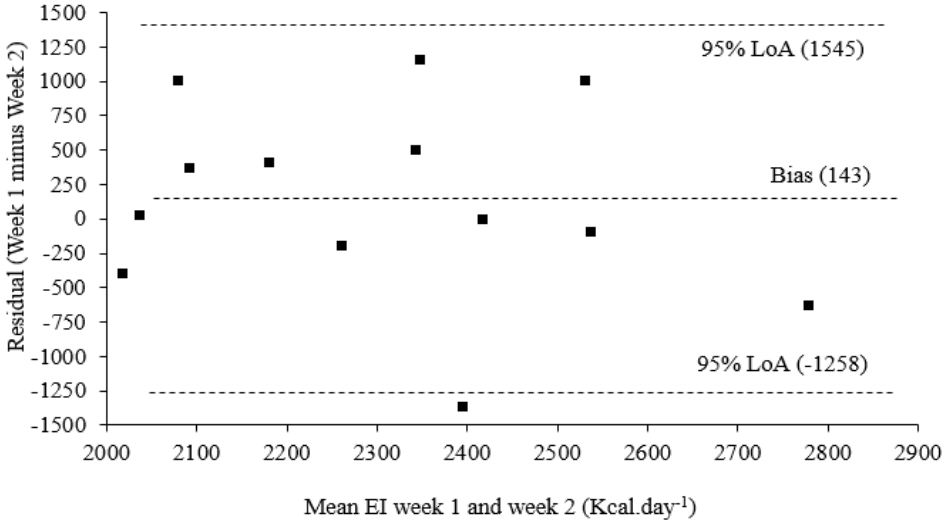

Figure 4. Bland-Altman plot of mean EI for week one and week 2.

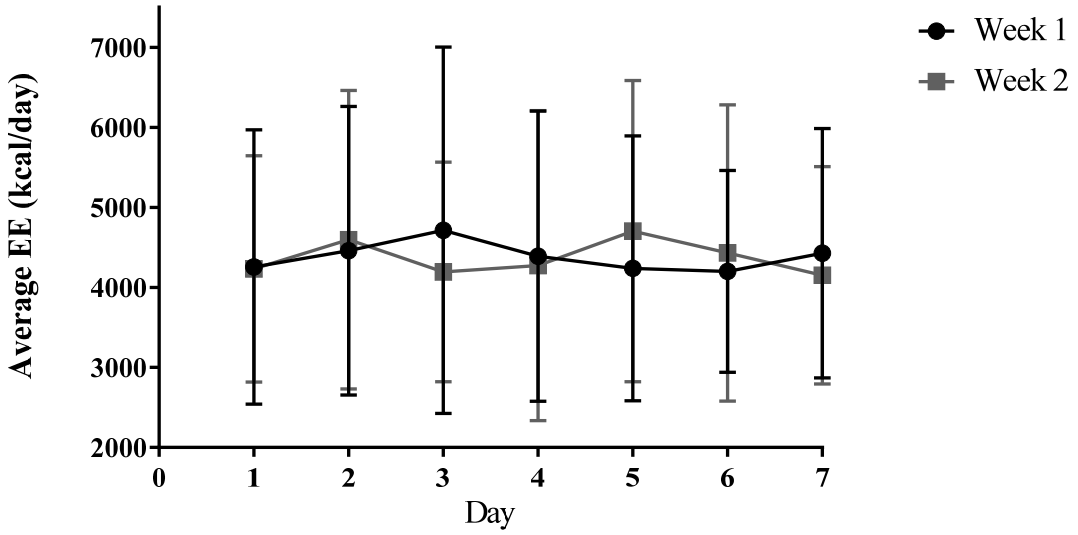

Figure 5. Average total energy expenditure (TEE) (kcal/day) for week one and week two.

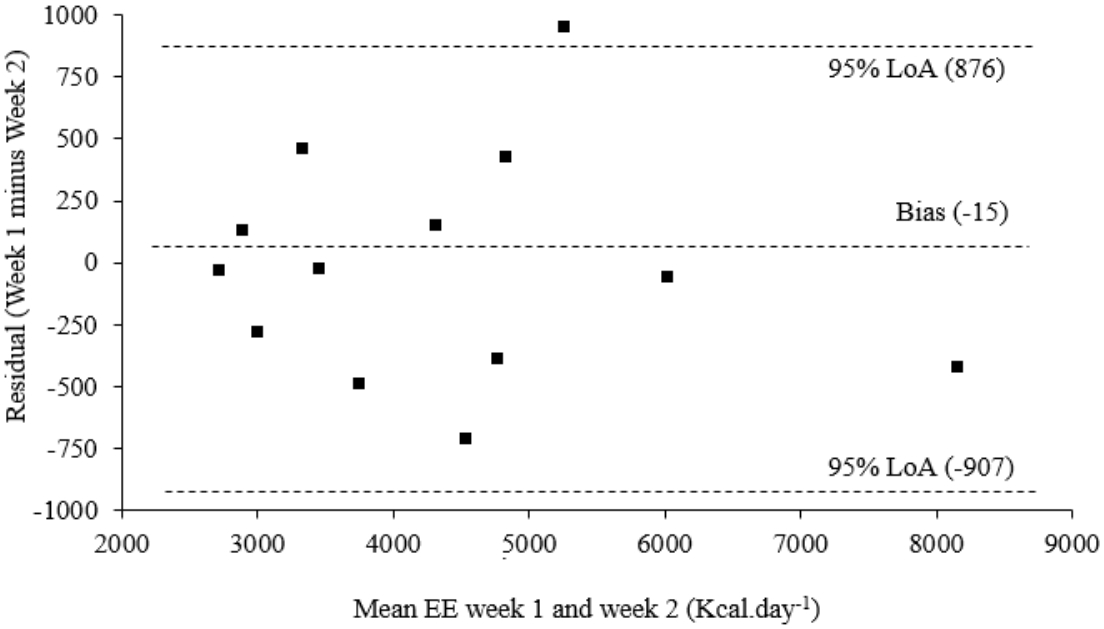

Figure 6. Bland-Altman plot of average total energy expenditure (TEE) for week one and week two.

Table 2. Mean values for Physical activity levels and Energy intake:Resting metabolic rate ratio.

	Week 1	**Week 2**
Physical activity level	2.62 ± 0.79	2.6 ± 0.72
Energy intake:Resting metabolic rate ratio	1.36 ± 0.33	1.43 ± 0.25

Values are expressed as means ± SD.

4.4 Discussion

The aim of the current study was to determine the reliability of a 7-day combined written and photographic food diary to assess free-living EI however, it was not possible to determine the differences between measurement variability and natural variations in participant's intakes. This study therefore, provides an estimate of the variation that could be expected across two weeks with no intervention. Furthermore, without the use of a control measure within this study with which the method could be compared it is not possible to suggest any potential improvements brought about via this method of EI assessment, the same also applies for the Actiheart device used to measure EE within the current study.

The plausibility of the results obtained within this current study can be questioned, PAL over the assessment periods were 2.62 and 2.60 a level deemed too high according to Goldberg et al. (1991) cut off points for the participants within this study, with an appropriate PAL of 1.7, suggesting an overestimation of EE by the actiheart device. Not only this the EI:RMR ratio was also calculated to provide a measure of the potential levels of underreporting and with results of 1.36 and 1.43 it would appear that there was some element of underreporting using this method of assessment a level too low to be seen as a valid recording of EI, an acceptable level would be to have reached 1.7.

The results however, do indicate that this method is a suitable measure of EI at a group level, with an average difference in EI of 136 kcal/day between the two weeks. Furthermore, there was no interaction with day of the week, adding evidence that the photographic food diary method is an appropriate for the measurement of free-living EI throughout a 7-day measurement period on a group level. However, individual-level analysis revealed wide LoA of -1258 to 1545 kcal/day. Therefore, this method may not be sensitive enough to detect small intervention effects. These findings can be used to inform further research using this method, which includes the experimental study in Chapter five of this thesis. Specifically, the LoA will be used to gauge whether potential changes in EI due to the exercise intervention used in study two can be considered to be meaningful and not within short-term variation.

To date, research has not examined the reliability of a combined written and photographic food diary. Although previous studies have measured the validity of the photographic method of measuring EI, the methods used differ from those used in this current study. Specifically, other studies have not used a free-living environment nor have they analysed their photographs using a food atlas (Higgins et al., 2009, Williamson et al., 2004, Williamson et al., 2003). Rather, these studies compared weighed food diary records and photographic portion estimations within laboratory and cafeteria environments to determine the validity of EI measures. Martin et al. (2007) measured the reliability of this method however, not in free-living conditions or in an adult population. This difference in study methods highlights the novelty of the current study, but means that is it not possible to compare our results directly with previous research examining the reliability, rather than the validity, of this method.

One of the major weaknesses that has been highlighted of the weighed food diary is the high burden placed on participants during the measurement period. Moreover, a review of previous research has shown that the longer the period of measurement using a weighed food diary record, the higher the risk of incomplete and inaccurate records (Trabulsi and Schoeller 2001). Of the 13 participants who completed the present study over two measurement periods, there were only three days that were not complete due to participants leaving their camera and written record at home and not completing their diaries. This means that of the 13 participants there was a total of 182 days measured and a successful completion of food diaries of ~98%; this finding adds further support to the finding of Small et al. (2009) that the use of a photographic food diary may aid in the full completion of food diaries. Thus, it is possible to suggest that the replacement of weighing scales with a camera may help to reduce the problem of participant burden and incomplete data that is commonly highlighted as a limitation of weighed food diaries (Trabulsi and Schoeller, 2001). However, it should be noted that the present study did not include a weighed food diary for a direct comparison with this method. Another weakness associated with weighed food diaries is the restricting of natural eating habits; this may have been minimised with the use of cameras in the current study. Indeed, all participants provided photos of food eaten away from home or takeaway meals that had been ordered, and did not appear to follow a monotonous diet during the measurement periods. Small et al. (2009) reported that families preferred to use photographs over weighing each item of food when completing food diaries, something that participants within the current study also stated, although we did not provide a direct measure of preferences for weighed compared with photographed food diaries; adding this measure would add further strength to the current study.

Well documented issues with the self-report method of EI analysis include underreporting, inaccurate participant estimations and the inter-individual estimation differences between participants (Lansky and Brownell, 1982, Adams, 1998, Higgins et al., 2009). The use of the photographic food diary method may reduce inter-individual difference in estimations. Indeed, researchers are not reliant on the participants to estimate their own portion sizes and can use a consistent, standardised approach, with the same researchers estimating portion sizes for all participants. Further to this, unlike paper food diaries, the photographic method used in the current study provides direct evidence of each item of food that the participants consumed, which may also reduce the chances of underreporting or misreporting food items. Furthermore, the photographs can be taken easily and quickly during the day and used as a valuable recall aid when completing the written record of the food diary, thus the participants themselves do not need to rely on memory to recall their daily intakes.

The Actiheart device has been shown to be a reliable and accurate measure of TEE in a variety of settings (Barreira et al., 2009, Brage et al., 2005, Crouter et al., 2008). This current study adds further evidence to the current knowledge by establishing the expected variation, of this device, between two weeks assessment with no intervention. No significant differences were found in average daily TEE

between the two measurement periods, something that would be expected as the participants were free living, taking part in no interventions. At the individual level, however, the LoA were wide (-907 to 876 kcal/day). Therefore, similar to our EI findings, this measurement of TEE may not be sensitive enough to assess small intervention effects. This finding will be taken into account when determining whether differences between the interventions used in study 2 within this thesis can be considered to be meaningful. No other studies have used the LoA to determine the reliability of this measure, so it is not possible to make direct comparisons with other literature to determine if this is a consistent finding (Barreira et al., 2009, Brage et al., 2005, Crouter et al., 2008) and further research to confirm the findings reported here would be valuable.

A limitation of the current study is that we did not include a reference measure to compare the results of the photographic food diary to in order to assess the validity of the EI estimate. To add strength to the results of this current study, the addition of a comparison of a weighed diary and photographic diary would have been beneficial. However, the additional burden placed on the participants may have made this approach unfeasible and it would be difficult to guarantee valid EI values even when using the weighed food diary due to issues such as underreporting. Although ~98% of the food diaries were completed, there were issues with participants failing to take photographs or taking photos but not including the photographed foods within the written element of the diary. This caused some difficulties with the analysis and lead to some potentially less accurate estimations or assumptions that an "average serving" was consumed. Therefore, the validity of using this method to quantify free-living EI requires examination.

To conclude, the current study has shown that a combined written and photographic food diary is a reliable measure of free-living EI over a seven day period on a group level. However, the LoA were -1258 to 1545 kcal/day which may not be sensitive enough to detect small differences at an individual level. Nevertheless, the limited participant burden and higher rates of completeness of using this method may make it a recommendable measure. The second experimental chapter within this thesis will take into account these findings when using this method to assess potential changes in EI and EE in response to a 7-day exercise intervention.

5.0 Study 2

5.1 Introduction

To be able to attempt to understand the possible compensatory mechanisms that could oppose the energy deficit created by an exercise intervention, both EB and appetite-regulatory hormones require investigation. It is currently well known that acute exercise, especially at an intensity of at least 70% $\dot{V}O_{2max}$, induces an immediate suppression of hunger and an energy deficit for 1-2 days (Broom et al., 2009, King et al., 2015). There is also some, albeit limited, evidence that over a longer period of imposed exercise, seven days, women appear to compensate through EI by ~30% of the exercise-induced energy deficit and men through a reduction in NexEE (Stubbs et al., 2002a, Stubbs et al., 2002b, Whybrow et al., 2008). What, however, is somewhat paradoxical is that despite these apparent large energy deficits that are induced by exercise, weight loss over longer exercise interventions is not as much as would theoretically be expected (Thomas et al., 2012). However, the effects of more than just a single session of exercise on appetite-regulatory hormones have only been assessed in a small number of studies. Moreover, these longer term studies have typically not been well-controlled and have not measured the form of ghrelin responsible for appetite-stimulation (i.e., acylated ghrelin), PYY and EB simultaneously (Ramson et al., 2008, Ramson et al., 2012). Yet to be investigated is the effect of imposed exercise over several days on the responses of appetite-regulating hormones, specifically acylated ghrelin and PYY, as well as EB. Various modalities of acute exercise (e.g., treadmill running, rowing, cycling) that have been investigated have seen suppressions in acylated ghrelin and increases in PYY (Broom et al., 2009, Jurimae et al., 2007, King et al., 2010a, King et al., 2010b, Ramson et al., 2012, Ueda et al., 2009); however, the most consistent changes seen from these hormones is treadmill running at ~70% $\dot{V}O_{2max,}$ inducing an energy deficit in the day after an acute exercise bout. Determining the possible change in acylated ghrelin and PYY pre- to post- a 7-day period of running at ~70% $\dot{V}O_{2max}$ could be vital to understanding the mechanistic basis of compensatory responses to an increase in EE over the longer term, as well as investigating EI and NexEE to provide an overall view of the degree to which compensation occurs.

The aims of this study are:

- To examine the effect of seven days of imposed exercise compared with a control condition on energy balance in men

- To examine the effect of seven days of imposed exercise compared with a control condition on appetite-regulating hormones in men

5.2 Methods

5.2.1 Participants

Following ethical approval, seven physically active, healthy males aged 18-30 years were recruited to participate in this study. Prior to participation within the study, participant's anthropometric data was collected (detailed in section 3.2.1) and preliminary RMR, submaximal and maximal exercise tests were completed (detailed in sections 3.2.2 and 3.2.3). Participant characteristics are show in Table 2. Unfortunately, due to illness and injury, only five were able to fully complete the protocol; their characteristics are shown in Table 2. All testing was completed in the Sport and Exercise Science Laboratories at the University of Bedfordshire. Preliminary testing and assessment of habitual training was used to confirm whether prospective participants would be able to complete the seven days of imposed exercise the exercise condition. In order to confirm that the imposed exercise intervention (i.e., 800 kcal/day exercise energy expenditure) was in excess of the participant's normal training load, it was ensured that the participants did not exceed ~40 min of running on every day for a normal 7-day period. Participants who exceeded this amount of habitual exercise were not eligible to participate in the study. All participants were provided with an information sheet (Appendix A) detailing the nature and purpose of the study. A consent form (Appendix B), PAR-Q form and Pre-test Medical Questionnaire (Appendix C) and a blood screening form (Appendix C) were all completed before testing. This ensured that no participants completed this study with underlying health concerns or issues that would put the participant or the experiment at risk (e.g. blood borne diseases).

Table 3. Participant characteristics

Variables	*n* = 5
Age (Years)	23 ± 1
Height (cm)	179 ± 7
RMR (kcal/day)	1950 ± 684.49
$\dot{V}O_{2peak}$ (ml/kg/min)	48.9 ± 4.4

Values are expresses as means ± SD

5.2.2 Study Design

Using a repeated measures design, participants were required to complete 2 x 7 day conditions separated by a 7-day washout period: imposed exercise (EX) and no exercise (CON) (Figure 7). The conditions were performed at random. The imposed exercise condition involved completing 800 kcal·day^{-1} of exercise at a treadmill speed eliciting 70% $\dot{V}O_{2peak.}$ The exercise was completed over two 400 kcal exercise sessions per day separated by a maximum 20 minute resting recovery; all exercise

sessions were supervised to ensure compliance to the intervention. For weekdays, expired air during the final five minutes of the first ten minutes and final five minutes of each exercise session was analysed to confirm the % $\dot{V}O_{2peak}$ attained and to ensure that participants had expended 800 kcal during the exercise session (predicted exercise duration was changed accordingly). For weekend days, participants exercised at the HR corresponding to 70% $\dot{V}O_{2peak}$. The average actual exercise intensity that the participants exercised at was 68 ± 7% $\dot{V}O_{2peak}$ and the average EE during exercise was 815 ± 11 kcal per session. Participants were instructed not to perform any structured exercise during CON to ensure an ExEE of 0 kcal·day^{-1}.

Throughout each 7-day condition participants were asked to complete a combined written and photographic food diary for the assessment of daily EI, and wear an ActiHeart monitor (CamNtech, Cambridge, UK) for the assessment of daily free-living EE. The Actiheart device uses a system of branched modelling to combine both heart rate and activity to calculate AEE and TEE. From this point forward, AEE is the EE determined by the Actiheart device as activity. On the day prior to and 24 h after each intervention period (i.e. days 0 and 8), the appetite response to a fixed meal was assessed via a meal tolerance test. Participants replicated their food intake and refrained from exercising on the 24 h before the pre-intervention meal tolerance test, but it was not possible to control for dietary intakes for the post-assessment, as this was the final day of the 7-day intervention period.

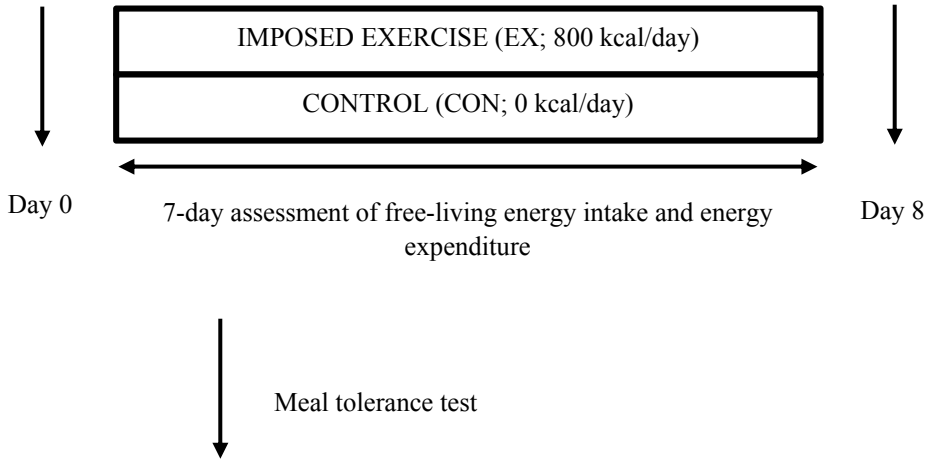

Figure 7. Study design with 2 x 7-day conditions.

5.2.3 Meal Tolerance Test

On day 0 and day 8 of each condition, participants arrived at the laboratory in the fasted state (at least a 12 h fast) for the assessment of appetite in relation to a test meal (see Figure 8). A Bodpod (Cosmed, Rome, Italy) measurement was taken to measure body fat percentage. After fasted measures, participants consumed a standard breakfast equating to 8 kcal per kg/body mass (consisting of bread, orange juice, milk, cheese, and jam: 17% protein, 35% fat, 48% carbohydrate, adapted from Martins et al. 2010) within 15 min. Following the three hour postprandial period, participants were given an *ad libitum* pasta meal consisting of 500g uncooked pasta cooked for 12 minutes and 500g chunky vegetable pasta sauce added to the pasta and cooked for a further four minutes. The pasta meal was presented to the participants in a standardised bowl and then self-served by the participants onto a standardised plate in an isolated food consumption area to avoid any social influences on food consumption. All participants were given the following instructions prior to consumption of the *ad libitum* pasta meal: 'We ask that you continue eating until you have satisfied your hunger' (Betts et al., 2011). The pasta was weighed before and after consumption to calculate the amount (g) that had been consumed. Blood samples were taken in the fasting state and every 30 minutes during the three hour postprandial period. Perceptions of hunger, satisfaction, fullness, prospective food consumption and were assessed using 100-mm visual analogue scales (VAS) in the fasting state, every 30 minutes postprandially and immediately after the *ad libitum* pasta meal (see Figure 8).

5.2.4 Blood sampling

A cannula (Vasofix, B. Braun, Sheffield, UK) was inserted into the antecubital vein. Blood samples were then drawn at fasting and every 30 minutes after the last mouthful of the standard breakfast (see Figure 8). Blood samples were collected into precooled 5ml EDTA vacuettes (Greiner Bio-One, Stonehouse, UK). Following collection, a 50µl combined solution of potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH) was added to the acylated ghrelin sample. Both vacuettes were then spun at 1500 x *g* for 10 minutes in a refrigerated centrifuge (Heraeus Labofuge 400R, Thermo-Fisher Scientific, Waltham, USA) at 4°C. The plasma supernatant from the acylated ghrelin sample was separated and dispensed into a 50 ml universal tube where 200 µl of 1 molar hydrochloric acid (HCL) was then added before the sample was spun for a further five minutes; after this 1 ml of the sample was stored in two 2 ml cryovials (Nunc, Thermo-Fisher Scientific, Waltham, USA). The plasma supernatant was also separated from the PYY sample and 1ml of plasma was placed into two separate 2 ml cryovials (Nunc, Thermo-Fisher Scientific, Waltham, USA).

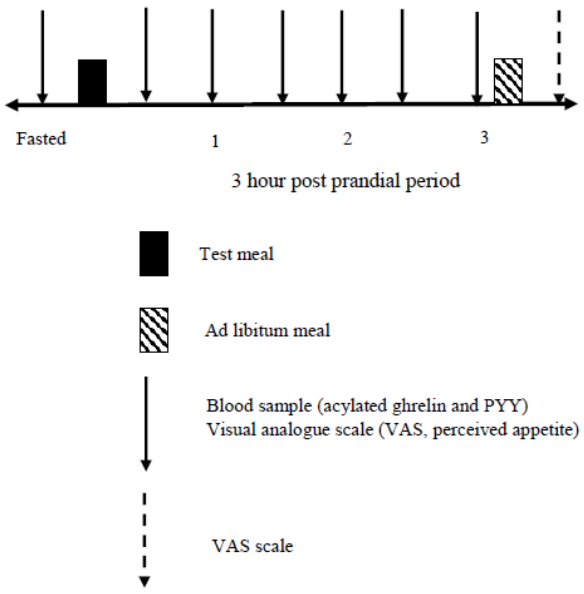

Figure 8. Meal tolerance test

5.2.5 Biochemical Analysis

Plasma acylated ghrelin concentrations were measured via commercially available enzyme linked immunosorbent assays (ELISA) (Bertin Pharma, France). PYY concentrations were also analysed via a commercially available ELISA (EMD Millipore, Missouri, USA). To eliminate inter-assay variation, samples from each participant were analysed on the same ELISA plate. Intra-assay coefficient of variation (CV) was calculated for one acylated ghrelin plate (sample for only one plate were completed in duplicate due to funding restrictions); the CV for this plate was 12 %. The inter-assay CV for acylated ghrelin was 5 % and for PYY was 7 %.

5.2.6 Statistical Analysis

Statistical analyses were completed using Statistical Package Social Sciences software version 22 (SPSS Inc., Chicago, IL, USA). Area under the curve (AUC) was calculated for all blood metabolite and subjective appetite rating variables using the trapezoidal method. Quantile-quantile (Q-Q) plots were used to test whether the data was normally distributed for all results. All data was analysed using linear mixed models; this method was chosen because of its ability to account for missing data points. Also, for repeated measures data, different covariate structures and between subject variability can be modelled accurately. For EI and EE analysis, the within-subject factors inserted were condition x day (2 x 7). For subjective appetite, *ad libitum* pasta meal intake, and appetite-regulating hormone AUC analysis, the within-subject factors inserted were condition x pre- to post- intervention test day (2 x 2). For the subjective appetite and appetite-regulating hormone raw data, the within-subject factors

inserted were condition x pre- to post- intervention test day x time (2 x 2 x 7). Statistical significance was accepted at P≤0.05.

The smallest Hurvich and Tsai's criterion was used to select the most suitable model. Normality and homogeneity of variance were measured on all residuals data for all variables using Q-Q plots. Only body mass was non-normally distributed following linear mixed model analysis and normality checks on residual data therefore, a Friedman's analysis was carried out on this variable.

5.3 Results

5.3.1 Energy Expenditure and Energy Intake

There was a main effect of condition for daily EI (F=8.549, P=0.005), but no main effect of day (F=0.281, P=0.943) and no condition by day interaction (F=0.271, P=0.948) (see Figure 9).

For daily TEE, there was a main effect of condition (F=280.472, P<0.0005), day (F=2.611, P=0.033) and an interaction between condition and day (F=30.22, P=0.017). Figure 10 indicates that the higher total EE in EX was less marked on day 4 of the intervention and that EE tended to fall over the course of EX, but not for CON.

AEE produced a main effect of condition (F=23.239, P=0.0002), but no main effect of day (F=2.133, P=0.076), and there was a condition by day interaction (F=2.512, P=0.037) (see Figure 11). Similar to TEE, the difference between conditions for AEE was less marked on day 4 (Figure 11).

NexEE produced a main effect of condition (F=23.967, P<0.0005), but no main effect of day (F=1.974, P=0.091), and there was no condition by day interaction (F=1.121, P=0.367) (see Table 5).

Table 4. Mean values for Physical activity level (PAL) and Energy intake:Resting metabolic rate ratio.

	CON	**EX**
Physical activity level	1.64 ± 0.2	2.55 ± 0.35
Energy intake:Resting metabolic rate ratio	1.10 ± 0.35	1.47 ± 0.47

Values are expressed as means ± SD.

Table 5. Mean values for total EE (TEE), Activity EE (AEE), energy intake (EI), non-exercise energy expenditure (NexEE) and the difference between conditions.

	CON	EX	Difference
Total EE (kcal)	2982 ± 388	5250 ± 349	*2268 ± 515
Activity EE (kcal)	947 ± 309	2681 ± 314	*1734 ± 454
EI (kcal)	1959 ± 155	2471 ± 156	*511 ± 240
NexEE (kcal)	2982 ± 388	4586 ± 172	*1485 ± 518

Values are expressed as means ± SD. *Significant difference between conditions (P≤0.05).

Table 6. Descriptions of imposed exercise bout

	Imposed exercise bout
Treadmill Speed (km/h)	7.6 – 8.5
Heart rate (bpm)	155 - 168

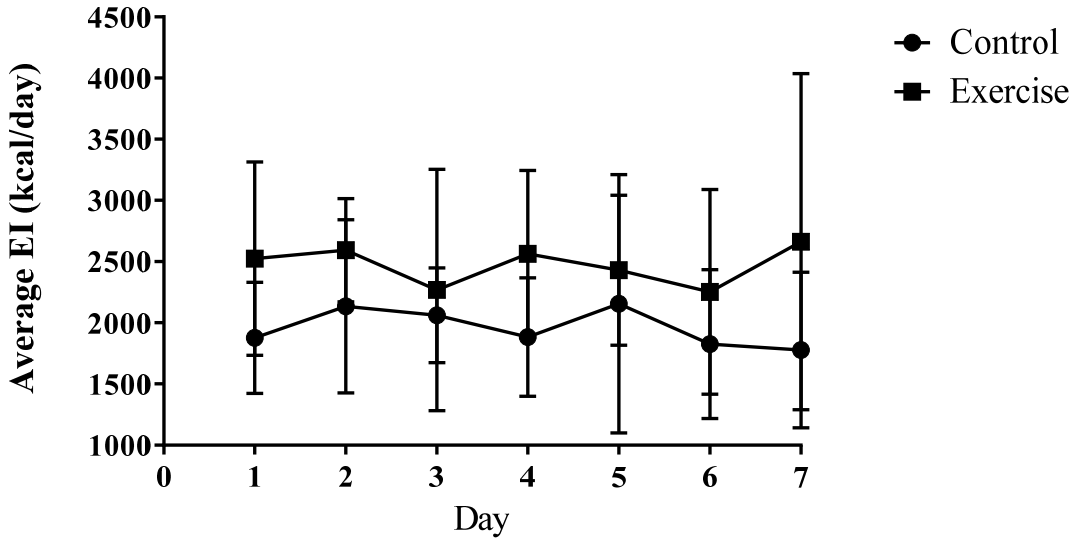

Figure 9. Energy intake (EI) in the exercise (EX) and control (CON) conditions. Significant difference between conditions (P=0.005)

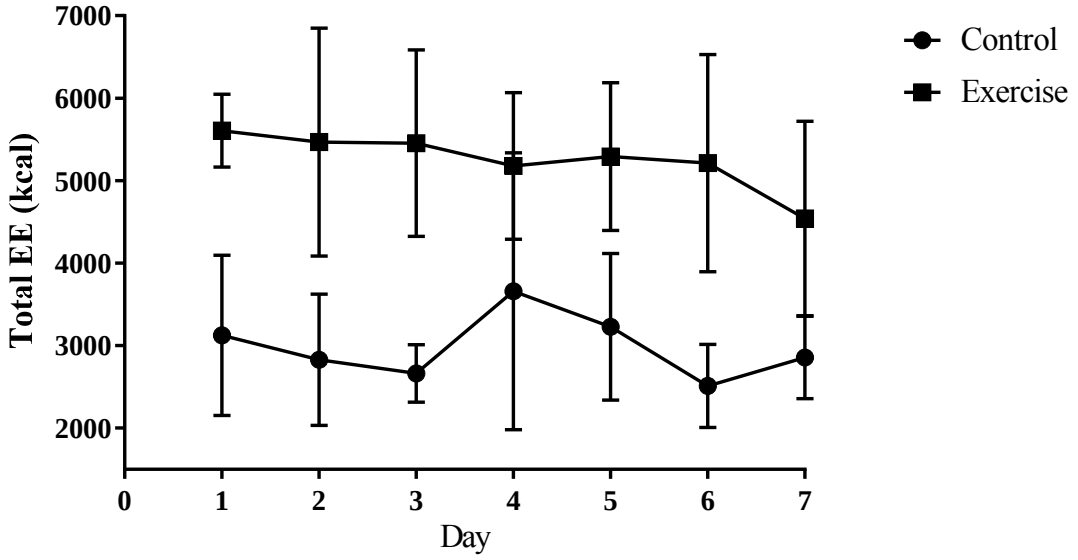

Figure 10. Total EE (TEE) in the exercise (EX) and control (CON) conditions. Significant differences between conditions, between days and an interaction between condition and day. (P>0.0005; P=0.033; P=0.017)

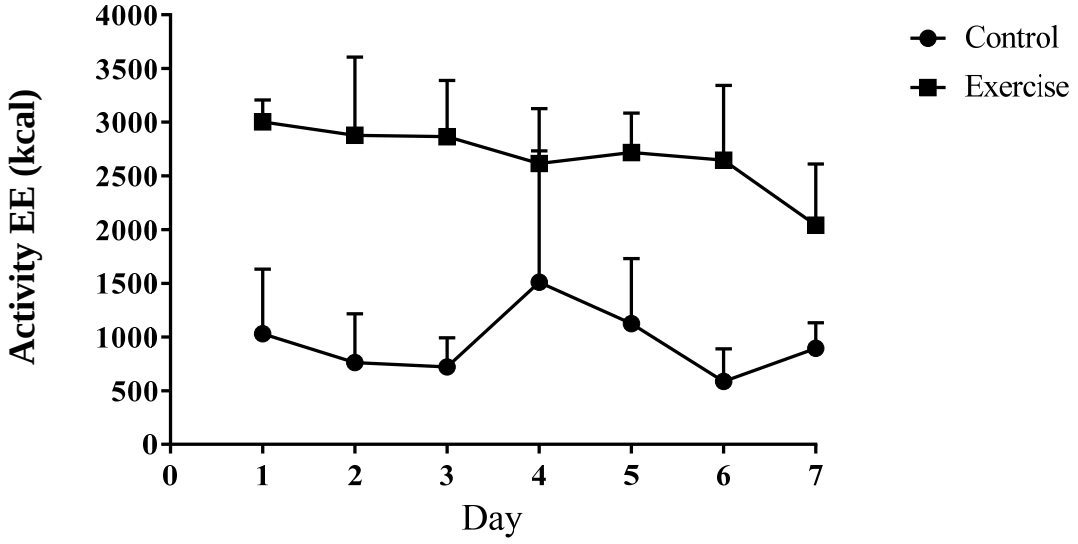

Figure 11. Activity EE between conditions. Significant differences between conditions (P=0.0002) and an interaction between condition and day (P=0.041)

5.3.2 Body Composition

Body mass residual data after linear mixed modelling was not normally distributed. Therefore, these results were analysed using a Friedman's analysis of variance (ANOVA), which showed body mass did not differ significantly between conditions (P=0.487) or between days (pre- to post- intervention) (P≥0.317). Similar findings were observed for the measurement of body fat percentage; there was no main effect of condition (F=2.57, P=0.134) or pre- to post- intervention test day (F=0.091, P=0.768).

5.3.3 Ad Libitum Meal

For total amount (g) consumed at the *ad libitum* pasta meal, there was no main effect of condition (F=1.254, P=0.285) or day (F=2.072, P=0.176) and no condition by day interaction (F=0.123, P=0.732) (see Figure 11). However, Figure 11 shows that the pre- to post- intervention increase in the amount consumed was more marked in the exercise condition. A more detailed examination of individual showed that 4 of the 5 participants increased their consumption when comparing pre- to post- values for EX, whereas the changes in the CON condition were variable (Figure 12). There was no main effect of condition (F=0.819, P=0.383) or day (F≤0.0005, P=0.993) for time to consumption of the *ad libitum* pasta meal.

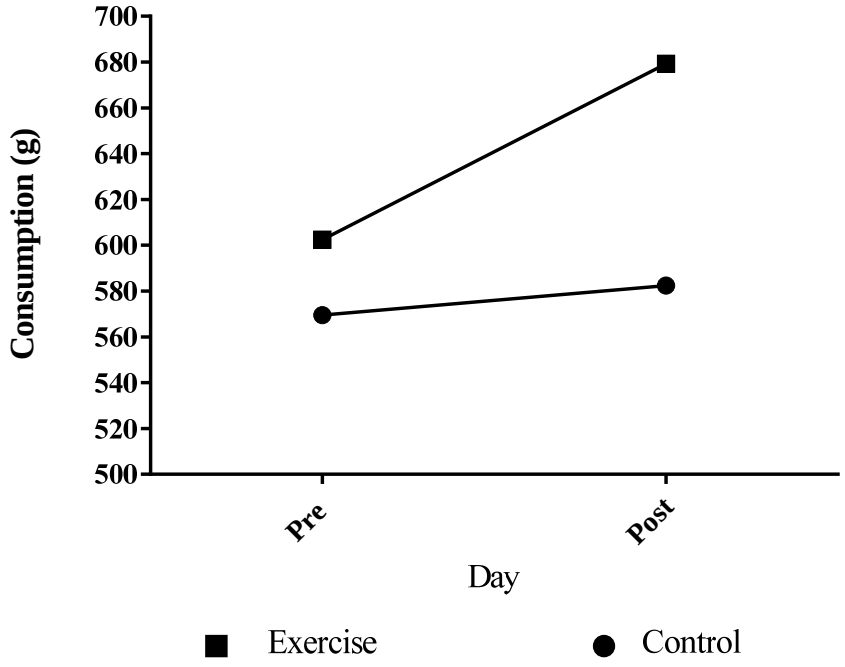

Figure 12. Total consumption of *ad libitum* meal.

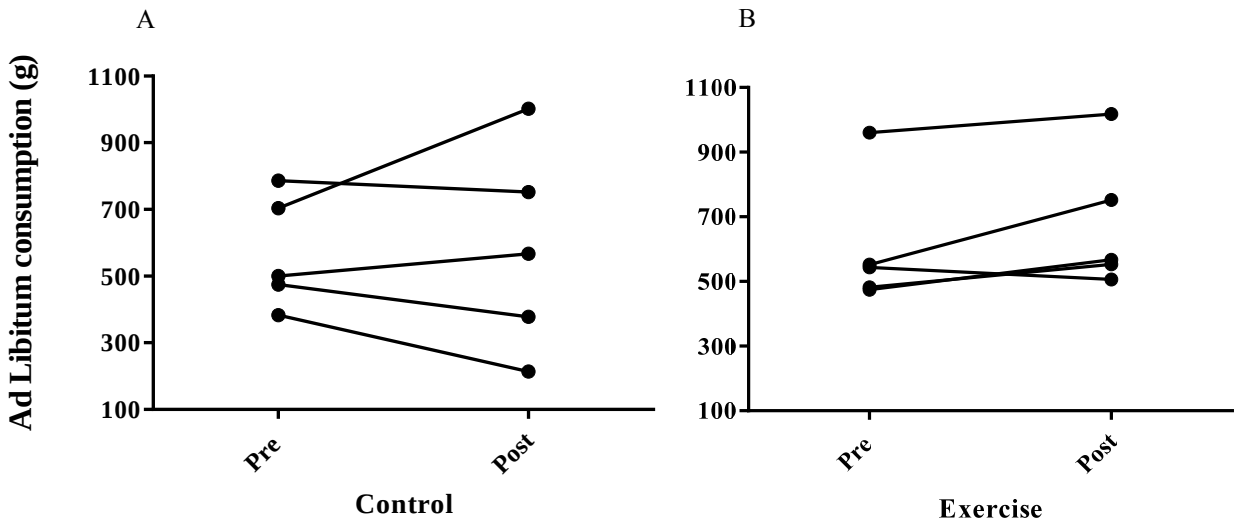

Figure 13. Individual ad libitum meal consumption.

5.3.4 Plasma Acylated Ghrelin and Total PYY

There was a main effect of condition (F=14.930, P<0.0005) and time (F=9.274, P<0.0005) on acylated ghrelin concentrations, but no main effect of test day was observed (F=0.133, P=0.716). No significant interactions were seen (P>0.072) (see Figure 14). The condition by time interaction approached significance (P=0.072); a closer examination of the results revealed that the time points 150 and 180 minutes for acylated ghrelin were significantly higher in EX compared with CON (P=0.005, P=0.001). There was a main effect of condition for fasting acylated ghrelin (F=6.144, P=0.031); concentrations were found to be significantly higher in the exercise condition. However, no main effects for test day (F=2.640, P=0.132) and no interaction between condition and test day (F=0.058, P=0.814) was found (see Figure 15). AUC analysis showed no main effect of condition (F=3.542, P=0.840) or test day (F=0.023, P=0.883) (see Figure 15).

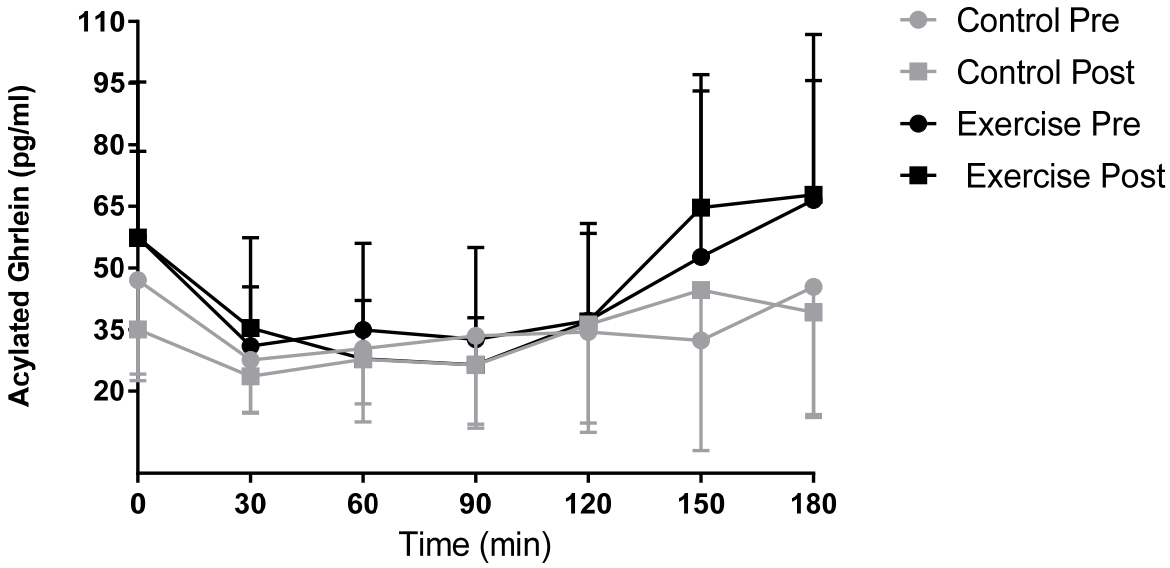

Figure 14. Acylated ghrelin hormone concentrations.

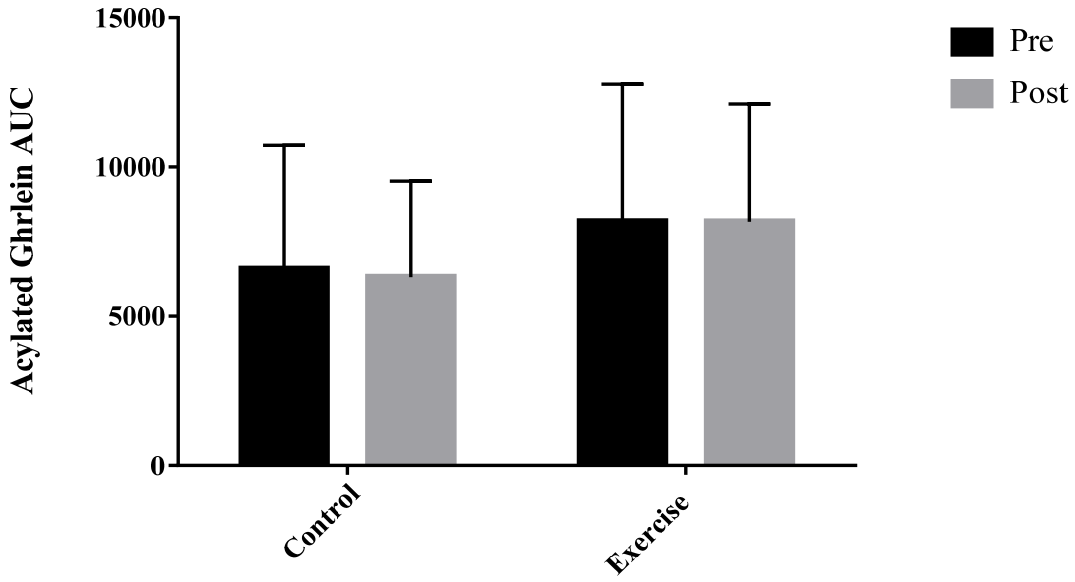

Figure 15. Acylated ghrelin AUC values.

For total PYY, there was no main effect of condition (F=2.110, P=0.150), test day (F=0.656, P=0.420) or time (F=1.301, P=0.264). No interactions were seen (P>0.549) for PYY concentrations. There was no main effect of condition for fasting PYY concentrations (F=1.749, P=0.218) or test day (F=1.000, P=0.348) and no interactions were seen between condition and test day (F=0.177, P=0.685) (see Figure 16). AUC analysis showed no main effect of condition (F=0.442, P=0.519) or test day (F=0.694, P=0.421) (see Figure 17).

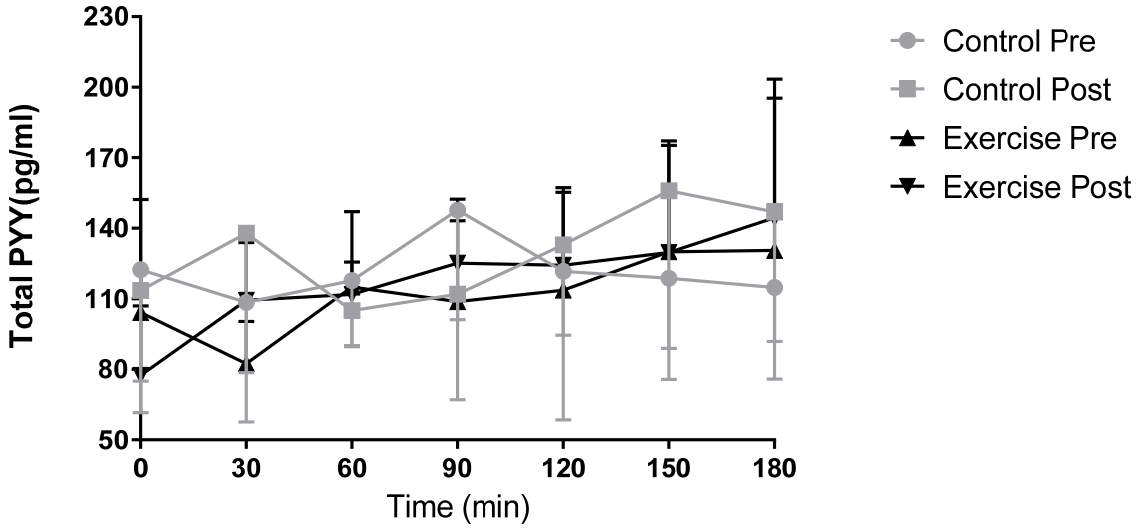

Figure 16. Total PYY hormone concentrations.

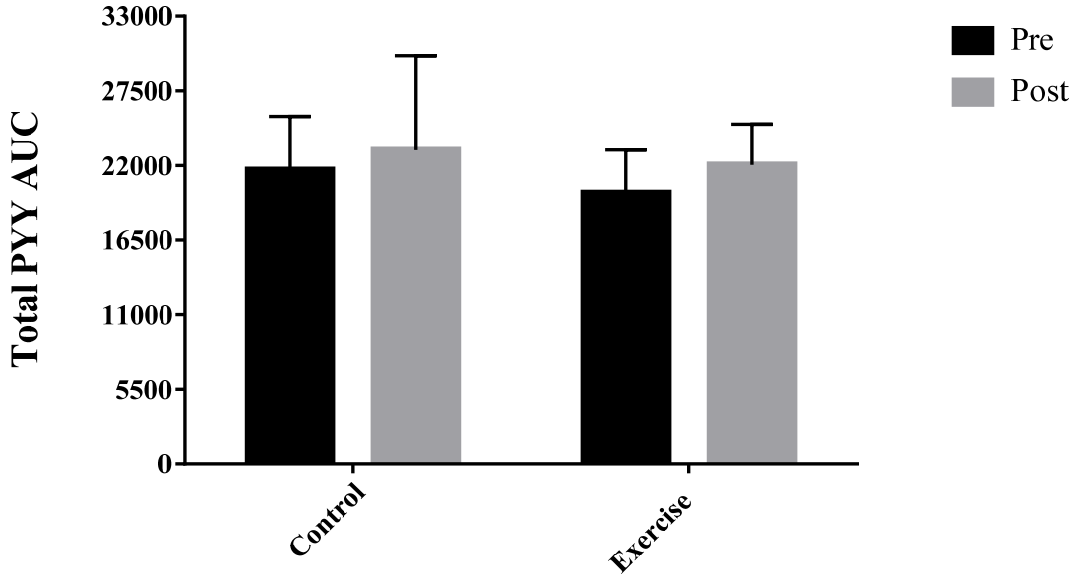

Figure 17. Total PYY AUC values.

5.3.5 Appetite Ratings

AUC linear mixed model analysis showed that there were no main effects of condition, test day and no condition by test day interaction for hunger, satisfaction, fullness and prospective food consumption ($P \geq 0.209$). Linear mixed model analysis showed there were no main effects of condition, test day and no condition by test day interaction for fasting hunger, satisfaction, fullness and prospective food consumption (≥ 0.092).

5.4 Discussion

The aim of the current study was to determine the effect of seven days of imposed exercise compared with a control no imposed exercise condition on energy balance, appetite and appetite-regulating hormones. The main findings were that 1) daily EI was higher in the exercise compared to control condition, as was EE, 2) no changes in the perceived appetite, acylated ghrelin or PYY response to a meal were seen as a result of the exercise intervention.

It is apparent from the current study that seven days of exercise eliciting an EE of ~815 kcal/day induces a state of negative EB, with participants not fully compensating either through an increase in EI, a reduction in physical activity EE or a combination of both. Nevertheless, there were indications of partial compensation, with EI being 511 kcal/day higher in the exercise condition compared with the control, which equated to 29% of TEE. This conclusion however, has to be met with caution as the method that was used to measure EI has a wide range of variability, as shown in the first study of this thesis (Chapter 4). Indeed, the LoA for EI estimated using this method were -1258 to 1545 kcal/day. Thus, the possibility that the variability within the method of measurement could partly explain the higher EI in the exercise condition compared with the control condition cannot be discounted from the current study. Nevertheless, using a more sensitive measure of EI, the *ad libitum* meal provided an indication of increased consumption following the exercise condition, adding support to the finding of higher free-living EI during the 7-day exercise intervention.

As expected, total EE was higher during the exercise condition compared with the control. It has been suggested that a reduction in activity outside of imposed exercise sessions would be a mechanism by which people compensate for increased EE (Church et al., 2009, Schneider et al., 2009). However, results from this current study showed that AEE was 1734 kcal/day higher in the exercise condition than the control condition. Since the imposed exercise only accounted for ~815 kcal/day of AEE, this finding this suggests that the participants did not compensate by becoming more sedentary in the exercise condition. Indeed, physical activity EE remained around 919 kcal/day higher in the exercise condition even when the EE from the imposed exercise was excluded. In contrast, a previous study that has measured EB over a 7-day exercise intervention in men suggested that, rather than increased EI, lower activity outside of imposed exercise is a potential mechanism to compensate for increased

exercise-induced EE (Stubbs et al., 2002b). However, this study and similar studies have only measured TEE (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b, Whybrow et al., 2008). Not only was AEE significantly higher, NexEE was 1485 kcal higher within the exercise condition, this may be due to the language used within the description of the control condition, in describing the instructions for the control week, the use of 'no exercise week' was used consistently, participants were asked to do no structured exercise and may have taken this along with the use of 'no exercise week' to mean no physical activity at all during the control condition and further reduced their activity than would be in their normal routine. Furthermore, in the exercise condition when they were given permission to do structured exercise outside of the imposed exercise participants may have also gone to the gym to do their resistance training as well as doing any extra physical activity they would normally participate in on a daily basis that they may have restricted during the control condition further amplifying the difference in NexEE within the exercise condition.

Stubbs et al. (2002a), Stubbs et al. (2004), Stubbs et al. (2002b), Whybrow et al. (2008) have observed compensation on average of 30% of the ExEE through EI, although they have seen increases of up to 50%. This current study observed an average increase of 64% (519 kcal/day), through EI, of the ExEE (~815kcal/day), suggesting that increased EI, rather than reduced activity outside of imposed exercise may be more important in explaining why longer term exercise interventions do not produce the weight loss that is theoretically expected, at least in our sample of five men (Thomas et al., 2012). Once again, this conclusion is made with caution due to the variability of the measurement of EI method. Despite this apparent partial EI compensation, the 1734 kcal/day higher AEE during the exercise condition compared with the CON condition would mean that participants compensated by 29%, through EI, of their activity EE, a result more in line with previous research investigating EB over this period of time (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b, Whybrow et al., 2008). Early studies investigating EB saw that EI matched EE in more active individuals (Mayer et al., 1956); this was also reflected in the present study, where EI began to rise and total EE began to fall as the exercise accumulated over seven days. These results may suggest that, if this intervention was to continue, total EE and EI may eventually become equal, inducing state of EB in our sample of habitually active men. In addition to the intervention being relatively short term (i.e., seven days) and thus not necessarily expected to induce large changes in body composition, the increase in EI during the exercise condition may also partly explain why body mass and body fat were unaffected by the exercise. Nevertheless, it must be emphasised that the increased in EI was not sufficient to fully compensate for the large energy deficit induced by the imposed exercise. With only partial compensation occurring, it can be suggested that this type of intervention would be a useful tool in the prevention of weight gain.

Despite the large differences between EE and EI during the exercise condition no significant weight loss occurred within this current study, even taking the imposed exercise bout out of the equation there is still over a 1000 kcal/day energy deficit that would be expected to induce significant weight loss

even over a 7-day period. Possible reasons for this lack of weight loss could be the overestimation of EE by the actiheart and underreporting of EI through the photographic food diary method. During the exercise condition the PAL of participants was 2.55 even with the imposed exercise this is considered to be too high for these participants (Goldberg et al., 1991). The EI:RMR ratio during the exercise week was 1.47 which again is low for these participants showing that there is likely some underreporting of EI within this condition and could account for the large differences in EI and EE and also why no weight loss occurred despite the apparent energy deficit. Calibration of the actiheart device could also suggest why there is such a high level of EE in the exercise week. The treadmill speeds during the imposed exercise bout all related to within 0.5km/h however, the heart rates during the imposed exercise bouts were considerably higher despite being at similar running speeds. The actiheart is calibrated to the energy expended at selected heart rates, an issue with this method is that it the actiheart software only allows energy expended to be at every 10bpm, this means that with the submaximal test used for calibration a participant may expend 14kcal/min running at 8km/h that relates to a heart rate of 144bpm therefore, this would be rounded down to 140bpm and could lead to an overestimation of EE. Having entered energy expended at heart rates relating to the four stages of the submaximal test the actiheart then predicts EE for all other heart rates. The process described could therefore, mean that predicted energy expended at all heart rates within the software is higher and could account for such a high EE obtained in the exercise condition. Not only this but with the imposed exercise bouts heart rates being considerably higher compared to the submaximal tests despite running at similar speeds potentially increasing the expenditure during the exercise condition.

A major criticism of past work assessing changes in EI and EE in response to seven days of exercise is that measures of appetite-regulating hormones have not been assessed to provide a mechanistic understanding of possible changes in EI (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b, Whybrow et al., 2008). The finding that both acylated ghrelin and PYY did not change in response to the exercise (or control) condition indicates that the partial compensation in EI observed during the exercise condition may not be due to changes in appetite-regulating hormones, at least in response to a test meal. The lack of change in acylated ghrelin and PYY is consistent with the findings of King et al. (2015), where no compensatory changes in these hormones was shown the day after a single bout of exercise. Measuring acylated ghrelin and PYY 48 or 72 hours post the final bout of exercise would allow for the control of diet before the test day and may provide a further way in which to assess the whether the exercise intervention affected appetite regulation. Despite no significant differences in hormone concentrations due to the exercise intervention, there was a condition by time interaction that approached significance and a closer examination showed that there was an increase in acylated ghrelin concentrations at the 150 and 180 minute postprandial time point in the exercise condition compared with the control. However, since the present study was well-controlled and included an assessment of pre- intervention appetite responses, it was apparent that the higher acylated ghrelin concentrations were not a response to the exercise intervention. Consumption at the *ad libitum* meal showed a larger increase in consumption from pre- to post- the exercise condition (12.9 g in the control, 76.8 g in the exercise condition); although significance was not reached, it is likely that this is due to an inadequate sample size in this study. Thus, the inclusion of more participants in the current study may result in

statistically significant increase in EI at the *ad libitum* meal following seven days of exercise, but not the control. It is also interesting that the postprandial rise in acylated ghrelin (150, 180 minutes time points) in the exercise condition may have acted as a hunger signal that could partly explain the higher *ad libitum* meal consumption when compared to the control condition at the post-intervention meal tolerance test. However, a larger sample size would be required to confirm these observations. In line with the lack of difference the acylated ghrelin and PYY response to the test meal from pre- to post-intervention, perceived appetite to the test meal was also unaffected. Therefore, seven days of exercise did not appear to result in a compensatory increase in acylated ghrelin or reduction in PYY to promote hunger in an attempt to restore energy balance.

Unfortunately, it is not possible to compare our appetite-regulating hormone findings with similar studies, as research using 7-day interventions and a within-subjects design is unavailable. In acute studies, appetite rating and acylated ghrelin concentrations have been consistently suppressed and inducing an energy deficit produced by the exercise (Broom et al., 2009, Deighton et al., 2013a, King et al., 2010a). Chronically, Martins et al. (2010) found an increase in fasting and postprandial acylated ghrelin concentrations in response to a 12 week intervention. This study, however, could not fully attribute the change in acylated ghrelin to the exercise intervention independently of possible changes in habitual diet, as the participants exerted some dietary restraint, being asked not to alter their food intake. It has been shown that an acute energy deficit induced by dietary restriction increases acylated ghrelin concentrations compared to and exercise induced energy deficit (King et al., 2011). Further to this, Martins et al. (2010) used a 12 week exercise intervention; it is possible to suggest that this is a long enough intervention to induce a change in acylated ghrelin, as it seems over a longer period of time EI begins to track EE more accurately (Mayer et al., 1956). Thus, the 7-day intervention used in the present study may not have been sufficient to elicit changes in appetite-regulatory hormones. Along with this, in this study hormones were assessed at least 48 hours after the final exercise bout to negate any acute effects of exercise (Martins et al., 2010). However, it has been shown that these hormones do not change in the day after exercise (King et al., 2015), a finding that could explain the lack of significant changes in hormone concentration within this current study and explain the differences between these findings and those of Martins et al. (2010). A major strength of the present study was that it employed a within-subjects design and was tightly controlled with a control condition as well as an exercise condition, where the exercise was supervised. As this was not the case within Martins et al. (2010) study, no comparison can be made between a control and exercise condition over this period of time to ensure the changes that were seen could be directly attributed to the exercise intervention. These differences in study design may have also contributed to discrepancies between our findings and those of Martins et al. (2010). Nevertheless, the lack of change in total PYY concentrations during the current study was is in line with Martins et al. (2010), despite the increase in acylated ghrelin that these authors observed.

Exercise at 70% $\dot{V}O_{2max}$ has been shown to acutely supress acylated ghrelin and increase PYY, inducing an energy deficit that lasts for 1-2 days (Broom et al., 2009, King et al., 2015, King et al., 2010a). Acylated ghrelin and PYY concentrations were not measured in response to the exercise bout at any stage within this current study, so it cannot be determined how the exercise bout affected acylated ghrelin or PYY on a daily basis. However, the higher EI in the exercise condition may suggest decreased or no suppression in acylated ghrelin and a decrease or no change in PYY during the intervention, although we did not measure this. Studies using acute exercise at 70%$\dot{V}O_{2max}$ have observed an inverse relationship between acylated ghrelin and PYY (Broom et al., 2009, Deighton et al., 2013a, King et al., 2010a); thus, it may not be surprising that both of these hormones showed a similar response to the exercise intervention in the present study (i.e., no effect).

Limitations of this current study include the method used to measure free-living EI, which has been shown to produce wide intra-individual variability in results (see Chapter 4). For example, using a photographic food diary within a free living environment may lead to inaccurate estimations of portion size and, especially when participants eat takeaway food for example, from less recognised establishments and there is no way to access the exact macronutrient content of the food. Another significant limitation of this current study is the low participant number. This means conclusions made from the results gained are to be taken with caution. Despite statistical significance being attained for some results, trends were observed for others and it is difficult to generalise these results to the population. Although Stubbs et al. (2002a) made strong conclusions with a similar sample size (i.e. 6) and study design, it should be acknowledged that the current study would benefit from a larger sample size to further examine the differences between conditions.

In conclusion, no study to the author's knowledge has investigated EB and appetite-regulating hormones over a seven day exercise intervention and directly compared this with a control condition. Conclusions from the present study are that seven days of imposed exercise at 70% $\dot{V}O_{2max}$ that elicited an EE of ~815 kcal resulted in an increase in EI of 511 kcal/day compared with an equivalent control period and, as expected, resulted in higher total daily energy expenditures. However, the exercise intervention did not appear to affect appetite regulation, with no change in the acylated ghrelin or PYY response to a meal.

5.4.1 Recommendations for Future Research

Further research would be valuable in extending the findings of the present study. Firstly, similar studies with higher sample sizes are required to attempt to further understand the mechanisms behind the increased EI that were observed herein. Further to this, assessing appetite and appetite hormone response 48 or 72 hour after the intervention would allow for the independent effect of the 7-day

intervention to be examined. Indeed, it is possible that 24 hours post-intervention measurements in the present study could have been affected by the final bout of exercise. Further to this, different populations and intensities of exercise corresponding to those populations are required to be investigated (i.e. females, obese/overweight, sedentary individuals). However, it may not be possible for some of these populations to run at as higher intensity as this current study over seven days, meaning that a more manageable intensity would be required to be used that would still create a large energy deficit. Also, there is a need for research to determine the differences between sexes, as this current study focused on males only and previous research has shown that men and women compensate for increased exercise-induced EE differently (Stubbs et al., 2002a, Stubbs et al., 2004, Stubbs et al., 2002b).

Research determining the effects of a longer intervention would be an addition to the current literature. Indeed, within this current study EI and EE showed signs that EB could be restored if the exercise intervention continued, but further research would be required to confirm this suggestion. This current study used treadmill running to create an energy deficit during the exercise intervention, determining whether other modes of exercise (cycling, resistance, rowing, swimming) have similar or differing effects compared to the current study would add significant knowledge to the current literature. Lastly, this current study used daily exercise bouts which, although not measured, may have caused significant fatigue within subjects. Therefore, using a different dose of exercise would be an interesting investigation (i.e. every other day or weekday exercise with weekend rest from imposed exercise); in particular, exercising at the intensity used within this current study across 'normal' 7-day period may be unrealistic for many individuals. Therefore, examining the effects of a more realistic intervention that individuals may carry out in their everyday life on energy balance and appetite regulation would be an interesting research area to inform public health guidelines.

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Appendices

Appendix A

Study 1

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INFORMATION SHEET

Title: The reliability of a 7 day photographic food diary to measure free-living energy intake

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The purpose of the study is to examine the reliability of a 7 day photographic food diary. This study is being undertaken as part of the requirements of MSc by Research (MRes) degree at the University of Bedfordshire.

What type of participant is needed?

The study requires 19-30 year old males who are physically active. It is possible that individuals with certain medical conditions may be excluded from the study but this will be decided at the first meeting.

What will participants be asked to do?

As a participant, you will be required to participate in two 7-day trials. During each 7-day trial, free-living energy intake (EI) will be measured through a food diary (with photographic evidence) and energy expenditure (EE) through wearing an Actiheart. You will be required to attend the University of Bedfordshire laboratories on 4 separate occasions.

Visit 1:	Resting Metabolic Rate (RMR), a submaximal exercise treadmill test (6 x 4 minute stages of increasing speed) and a maximal exercise treadmill test (Increasing gradient until volitional exhaustion) will all be completed for Actiheart calibration. You will be familiarised with the food diary, as well as the Actiheart equipment and fitted with the Actiheart.
0-6 d:	A food diary with digital photographs will be completed every day during trial 1. An Actiheart will also need to be worn for EE measurements.
Visit 2:	Actiheart will be removed and all data will be collected
7- 13 d:	No measurements will need to be taken (7 day washout period)
Visit 3:	Actiheart will be fitted
14 – 20 d:	Identical to trial 1: A food diary with digital photographs will be completed every day during trial 2. An Actiheart will also need to be worn for EE measurements.
Visit 4:	Actiheart will be removed and all data will be collected

What are the possible risks of taking part in the study?

Due to the nature of the study, participants will not be place under any unnecessary physical or mental stress throughout the duration of the reliability study.

- ***Participants*** – Participants will be informed of the study aims of methods; a consent form will be completed before test measurements commence. Data collected will be either locked

in a filing cabinet by a University member of staff or either in a password protected folder on a computer.

- ***Anonymity*** – The data collected would not in any way be linked to specific participants.

- ***Discomfort*** - The Actiheart may cause some discomfort from the strap positioned around the chest needed to hold the Actiheart in place. To minimise discomfort, participants will undergo a familiarisation session to get used to the initial discomfort caused by the Actiheart.

- ***Electrodes*** - Participants will be asked for any known allergies before skin preparation or wearing of the Actiheart. If any soreness or skin irritation develops then participants will stop using the Actiheart. Electrodes will be disposed of in accordance to the 'Collection and Disposal of Clinical Waste' guidelines and will not be re-used.

- ***Physical stress during exercise*** - Participants will be informed of the exercise protocol and all safety procedures will be explained before testing commences. A safety mat will always be present behind the treadmill and clear of any equipment, in order to minimise the risk of injury. A first aider will be present at all times within the laboratories so that if an incident occurs, a first aid will be immediately provided. A researcher will be present at all times during exercise to insure participants are not in any discomfort. Exercise will be stopped if participants feel ill or are in discomfort or pain and will be monitored.

What if you decide you want to withdraw from the project?

If, at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

What will happen to the data and information collected?

Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?

Questions are always welcome and you should feel free to ask myself Paul Mackie, my colleague, Chris Esh or supervisor John Hough any questions at any time. See details below for specific contact details.

Should you want to participate in this study then please complete the attached assent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

Paul Mackie (email: paul.mackie@study.beds.ac.uk)

Chris Esh (email: Christopher.esh@study.beds.ac.uk)

John Hough (email: John.hough@beds.ac.uk)

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Study 2

DEPARTMENT OF S
EXERCISE SCIENCE

Bedford Campus

Polhill Avenue

Bedford

MK41 9EA

INFORMATION SHEET

Title: The effect of a 7 day imposed exercise on energy balance, appetite and appetite-regulating hormones.

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The purpose of the study is to examine the effect of a 7 day imposed exercise training on energy balance, appetite and appetite regulating hormone responses to a meal. This study is being completed for an MSc by Research degree.

What type of participant is needed?

The study requires 19-30 year old males who are physically active. It is possible that individuals with certain medical conditions may be excluded from the study but this will be decided at the first meeting.

What will participants be asked to do?

As a participant, you will be required to participate in two 7-day trials. During one trial, you will be asked to complete 7 days of exercise, and for the other you will be asked not to complete any exercise. During each 7-day trial, free-living energy intake (EI) will be measured through a food diary (with photographic evidence) and energy expenditure (EE) through wearing an Actiheart. The day before, the day after and two days after each trial, you will be asked to come to the University to complete a meal tolerance test, which will involve a series of blood samples.

In total you will be required to attend the University of Bedfordshire laboratories on 13 separate occasions:

Visit 1:

Resting Metabolic Rate (RMR), a submaximal exercise treadmill test (6 x 4 minute stages of increasing speed) and a maximal exercise treadmill test (Increasing gradient until volitional exhaustion) will all be completed for Actiheart calibration. You will be familiarised with the food and exercise diary.

Visits 2, 10 11, 12 & 13:

You will be asked to fast for 12 h (i.e. overnight) before arriving at the lab. You will arrive at the lab for a 09:00 start. During this session we collect 7 blood samples via cannulation during each of these visits. You will be provided with a meal to consume during this visit and we will ask you about your appetite during this each trial. Each visit will last ~3 h (i.e. leaving at 12 noon).

Visits 3-9 (inclusive):

You will complete 7 days of imposed exercise during these visits. During each visit you will run on a treadmill at 70% $\dot{V}O_{2peak}$ for a duration to utilise 1000 kcal (~1 h). A food diary with digital photographs and exercise diary will be completed every day during trial this period. An Actiheart will also need to be worn for EE measurements.

What are the possible risks of taking part in the study?

Due to the nature of the study, participants will not be place under any unnecessary physical or mental stress throughout the duration of the reliability study.

- ***Participants*** – Participants will be informed of the study and what they can do. A consent form will be completed before test measurements commence. Data collected will be either locked in a filing cabinet by a University member of staff or either in a password protected folder on a computer.

- ***Anonymity*** – The data collected would not in any way be linked to specific participants.

- ***Discomfort*** - The Actiheart may cause some discomfort from the strap positioned around the chest needed to hold the Actiheart in place. To minimise discomfort, participants will undergo a familiarisation session to get used to the initial discomfort caused by the Actiheart.

- ***Electrodes*** - Participants will be asked for any known allergies before skin preparation or wearing of the Actiheart. If any soreness or skin irritation develops then participants will stop using the Actiheart. Electrodes will be disposed of in accordance to the 'Collection and Disposal of Clinical Waste' guidelines and will not be re-used.

- ***Physical stress during exercise*** - Participants will be informed of the exercise protocol and all safety procedures will be explained before testing commences. A safety mat will always be present behind the treadmill and clear of any equipment, in order to minimise the risk of injury. A first aider will be present at all times within the laboratories so that if an incident occurs, a first aid will be immediately provided. A researcher will be present at all times during exercise to insure participants are not in any discomfort. Exercise will be stopped if participants feel ill or are in discomfort or pain and will be monitored.

- ***Blood sampling*** - A certified first aider will be on-site whilst blood sampling occurs and all procedures will be given special care. Samples will be collected in a clean and sterile environment to avoid the chance of infection and all wounds will be treated until bleeding has stopped and then covered to reduce the risk of infection.

What if you decide you want to withdraw from the project?

If, at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

What will happen to the data and information collected?

Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?

Questions are always welcome and you should feel free to ask Paul Mackie, Chris Esh (both experimenters), Dr.John Hough (supervisor) or Dr. Julia Zakrzewski (supervisor) any questions at any time (contact details below). See details below for specific contact details.

Should you want to participate in this study then please complete the attached assent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

Paul Mackie (email: paul.mackie@study.beds.ac.uk)

Chris Esh (email: Christopher.esh@study.beds.ac.uk)

John Hough (email: John.hough@beds.ac.uk)

Julia Zakrzewski (email: Julia.Zakrzewski@beds.ac.uk)

Department of Sport and Exercise Sciences,

University of Bedfordshire

Bedford Campus,

Polhill Avenue,

Bedford

Appendix B

Study 1

SUBJECT CONSENT FORM

UNIVERSITY of BEDFORDSHIRE

The reliability of a 7 day photographic food diary to measure free-living energy intake

Date and approximate time: _____

I confirm that I understand the nature of the study above and what is involved in the protocol outlined. I further confirm that my health is normal and the information given on the health/medical questionnaire is accurate and complete.

My agreement to participate in the experiment is made of my own free will, and not in response to financial or other inducements (e.g. peer pressure). I confirm that I am not currently participating in another experimental trial. I confirm that I understand the risks involved in the protocol outlined and that all information and data collected will be held securely at the University of Bedfordshire.

The attention of volunteers is drawn to the fact that in the case of injury to persons or damage to property no claim for damages can succeed against University of Bedfordshire or against its employees unless legal liability resulting from negligence can be proved.

Name: _____

Signed:_____

Witness: _____

Signed:_____

Date: _____

Study 2

SUBJECT CONSENT FORM

UNIVERSITY of BEDFORDSHIRE

The effect of a 7 day imposed exercise on energy balance, appetite and appetite-regulating hormones

Date and approximate time: _____

I confirm that I understand the nature of the study above and what is involved in the protocol outlined. I further confirm that my health is normal and the information given on the health/medical questionnaire is accurate and complete.

My agreement to participate in the experiment is made of my own free will, and not in response to financial or other inducements (e.g. peer pressure). I confirm that I am not currently participating in another experimental trial. I confirm that I understand the risks involved in the protocol outlined and that all information and data collected will be held securely at the University of Bedfordshire.

The attention of volunteers is drawn to the fact that in the case of injury to persons or damage to property no claim for damages can succeed against University of Bedfordshire or against its employees unless legal liability resulting from negligence can be proved.

Name: _____

Signed:_____

Witness: _____

Signed:_____

Date: _____

Appendix C

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

→ **DELAY BECOMING MUCH MORE ACTIVE:**

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada / Santé Canada

continued on other side...

University of Bedfordshire

Sport & Exercise Science Laboratories
Polhill Avenue
Bedford MK41 9EA

PRE-TEST MEDICAL QUESTIONNAIRE

To be completed by all subjects before participating in practical sessions.

Name: .....
Age:..... Gender: M / F

1 Are you in good health? Yes / No
If no, please explain:

2 Are you pregnant or have you given birth in the last 6 months? Yes / No

3 How would you describe your present level of moderate activity?
< once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

4 Have you suffered from a serious illness or accident? Yes / No
If yes, please give particulars:

5 Are you recovering from an illness or operation? Yes / No
If yes, please give particulars:

6 Do you suffer, or have you ever suffered from:
Respiratory conditions (asthma, bronchitis, tuberculosis, other)? Yes / No
Diabetes? Yes / No
Epilepsy? Yes / No
High blood pressure? Yes / No
Heart conditions or circulation problems:
(angina, high blood pressure, varicose vein, aneurysm, embolism, heart attack, other)?
Do you have chest pains at any time? Yes / No
Do you suffer from fainting/blackouts/dizziness? Yes / No
Is there any history of heart disease in your family? Yes / No

7 Are you currently taking medication ? Yes / No
If yes, please give particulars:

8 Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? If yes, please give particulars: Yes / No

9 Have you had to consult your doctor, or had hospital treatment within the last six months? Yes / No

10 Have you, or are you presently taking part in any other laboratory experiment? Yes / No

11. Are you currently fitted with a pacemaker? Yes / No

12. Do you have any food allergies or intolerances? Yes / No

If yes, please state what this allergy or intolerance is.....

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment.
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella)
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and, if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum;

DECLARATION

I hereby volunteer to be a subject in experiments/investigations during the period of 20___.

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Name of subject (please print) _____

Signature of Subject _____ Date: _____

Name of Experimenter (please print) _____

Signature of Experimenter _____ Date: _____

BLOOD ANALYSIS – Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?

b. Have you had jaundice within the previous year?

c. Have you ever had any form of hepatitis?

d. Have you any reason to think you are HIV positive?

e. Have you ever been involved in intravenous drug use?

f. Are you a haemophiliac?

g. Is there any other reason you are aware of why taking blood might be hazardous to your health?

h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer **Yes** to any of questions a-g? Please tick your response.

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the tester guidance as to my suitability for the test.

Signed ……

Date ……

If there is any change in the circumstances outlined above, it is your responsibility to

tell the person administering the test immediately.

The completed Medical Questionnaire (Par Q) and this Blood Sampling Form will be held in a locked filing cabinet in the School of PE and Sport Sciences laboratories at the University for a period of one-three years. After that time all documentation will be destroyed by shredding.

If you wish to have a photocopy of any of the completed documents, please ask for one.

Appendix D

Food diary

Participant ID: _____

Start date of food diary: _____

Finish date of food diary: _____

Contacts:

Paul Mackie (Paul.Mackie@study.beds.ac.uk – Main researcher)

Chris Esh (Christopher.Esh@study.beds.ac.uk – Main researcher)

John Hough (John.Hough@beds.ac.uk – 1st supervisor)

Julia Zakrzewski (Julia.Zakrzewski@beds.ac.uk – 2nd supervisor)

FOOD DIARY INSTRUCTIONS

- **Everything** that you eat and drink over the course of the testing should be **recorded** in this **diary** and **photographed**.

- In the evening, you can **look through the photos** you took that day and use them to help you complete your food diary.

- If you **forgot to take a photo** of something you ate or drank in the day, you should still **add this** to the diary.

- Please make sure you fill in all the columns for each food/drink item:

    1. **Date and time of day** – the date and time you had the food/drink (you only need to write the date at the beginning of each day).
    2. **Description – as much detail as possible. Please tell us the manufacturer's name (e.g. Kelloggs, Heniz) and cooking method (e.g. grilled, roast, boiled).**
    3. **Amount** – approximate portion or weight, most snack foods will have the weight of the food on the packet so you can write this in your diary (e.g. full packet of crisps).
    4. **Leftovers** – the amount that you did not eat or drink (e.g. apple cores, crusts of bread). **Make sure that all left over food is also photographed.**

- This information is important for understanding our results from the study, so it is very important that you **avoid missing things out or making it up!** Thank you!

Day 1

What time did you wake up this morning? _____

What time did you turn off the light and go to bed this evening? _____

Date and time of day	Brand name (e.g. Heinz, Tesco, Kellogs)	Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)	Amount served (grams/ approx. portion)	Did you leave any? How much did you leave? (Photographed)

Day 2				
Date and time of day	**Brand name (e.g. Heinz, Tesco, Kellogs)**	**Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)**	**Amount served (grams/ approx. portion)**	**Did you leave any? How much did you leave? (Photographed)**

Day 3				
Date and time of day	**Brand name (e.g. Heinz, Tesco, Kellogs)**	**Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)**	**Amount served (grams/ approx. portion)**	**Did you leave any? How much did you leave? (Photographed)**

Day 4				
Date and time of day	**Brand name (e.g. Heinz, Tesco, Kellogs)**	**Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)**	**Amount served (grams/ approx. portion)**	**Did you leave any? How much did you leave? (Photographed)**

Day 5				
Date and time of day	**Brand name (e.g. Heinz, Tesco, Kellogs)**	**Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)**	**Amount served (grams/ approx. portion)**	**Did you leave any? How much did you leave? (Photographed)**

Day 6				
Date and time of day	**Brand name (e.g. Heinz, Tesco, Kellogs)**	**Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)**	**Amount served (grams/ approx. portion)**	**Did you leave any? How much did you leave? (Photographed)**

Day 7				
Date and time of day	**Brand name (e.g. Heinz, Tesco, Kellogs)**	**Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)**	**Amount served (grams/ approx. portion)**	**Did you leave any? How much did you leave? (Photographed)**

Day				
Date and time of day	**Brand name (e.g. Heinz, Tesco, Kel-logs)**	**Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)**	**Amount served (grams/ approx. portion)**	**Did you leave any? How much did you leave? (Photographed)**

Appendix E

Instructions for taking photographs

What do you need to photograph?

- Everything you eat and drink from when you wake up until to when you go to bed – **WE ONLY WANT PHOTOS OF FOOD AND DRINK!**
- Remember to take the photos when **eating out**, **eating snacks** and for **all drinks**.
- Angle the camera **diagonally down (65-75º)** at the food (see below for 'good examples').

How will we know how much you actually ate?

- Take the photographs **before** and **after** meals, so we can see your leftovers.
    - Include a **knife/fork/spoon** in the photos on the side of the plate/bowl, so we can work out the size of the plate or bowl.
    - Take a photo of the **whole plate** with some space around it (no close-ups!).
    - If you have a drink with your meal or snack, you can **include the drink in the same photo as the food**.

Good examples:

Before

After

Before

After

Bad examples

- ✘ **Photo not taken at correct angle.**
- ✘ **Knife and fork not by the side of the plate.**
- ✘ **Some of the meal has been eaten already.**
- ✘ **No 'after' picture.**

- ✘ **No knife or fork.**
- ✘ **No 'after' picture.**

What is this meal?
It is not possible to know! This is why we need you to complete your food diary…