INDEPENDENT EFFECTS OF 7-DAYS IMPOSED EXERCISE ON FREE-LIVING ENERGY BALANCE AND APPETITE-REGULATING HORMONES IN MALES

Paul Ian Mackie

INDEPENDENT EFFECTS OF 7-DAYS IMPOSED EXERCISE ON FREE-LIVING ENERGY BALANCE AND APPETITE-REGULATING HORMONES IN MALES

By

Paul Ian Mackie BSc (Hons)

A thesis submitted to the University of Bedfordshire, in fulfilment of the requirements for the degree of Masters of Science by Research at the Institute for Sport and Physical Activity Research (ISPAR).

April 2016

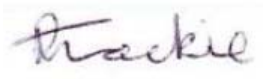
Abstract

Study 1 examined the reliability of a photographic food diary (with additional written details) on free-living energy intake (EI) in 13 healthy males. Daily average EI did not differ significantly between two, 7-day periods (p = 0.116) but a large systematic bias (143 ± 715 kcal.day^{-1}) and wide limits of agreement (LoA) (-1258 to 1545 kcal.day^{-1}) were found. Study 2 examined the influence of imposed exercise (7 days) on energy balance and the acylated ghrelin and total PYY response to a meal. Five healthy males completed two, 7-day trials in a crossover randomised design: no exercise (N-EX) and exercise (EX; ~69% $\dot{V}O_{2peak}$ expending an average 815 kcal.day^{-1}). EI and EE were assessed throughout each trial. Blood and appetite ratings (visual analogue scales; VAS) were collected the day prior to and 70 hours post each trial (fasting and for 3 hours postprandial; a final VAS after an *ad libitum* meal). Exercise significantly increased EI by 27% (p = 0.005), although participants remained in an energy deficit. Appetite regulating hormones and appetite ratings did not alter from pre- to 70 hours post-intervention. Thus, 7-days of imposed exercise induced a partial compensation through EI, without changes in appetite hormones or appetite ratings.

Author's declaration

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

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

Name of candidate: Paul Mackie Signature: 

Date: 06.04.2016

Table of contents

List of tables and figures

Acknowledgements

I would like to thank my supervisors Dr John Hough and Dr Julia Fruer for their support and advice throughout my MSc by Research. Thank you for your time and guidance. I would further like to thank Dr David Stensel for his contribution towards the study.

To all the postgraduates, I thank you for your advice, support and encouragement throughout my MSc. Chris, it was a pleasure working with you and thank you for all your help.

To the BSc students who helped with data collection and analysis, thank you for you for support.

To all the participants who volunteered and participated in the studies, thank you. Without your time and effort, the studies who not have been possible.

Finally, I would like to thank my parents and friends for their constant support and encouragement throughout my MSc.

List of abbreviations

%	Percentage
$\dot{V}CO_2$	Carbon dioxide produced
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximum oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
µL	Microlitres
^{18}O	Oxygen stable isotope
24HR	24 hour recall
^{2}H	Deuterium stable isotope
AEE	Activity energy expenditure
AgRP	Agouti related peptide
AICC	Hurvich and Tsai's criterion
ARC	Arcuate nucleus
AUC	Area under the curve
bpm	Beat per minute
CCK	Cholecystokinin
cm	centimetre
CNS	Central nervous system
CV	Coefficient of variation
DLW	Doubly labelled water
EDTA	Ethylenediaminetetraacetic acid

EE	Energy expenditure
EI	Energy intake
ELISA	Enzyme linked immunosorbant assays
EX	Imposed exercise
ExEE	Exercise energy expenditure
FFQ	Food frequency questionnaire
GHS-R	Growth hormone secretagogue receptor
GLP-1	Glucagon-like peptide 1
GOAT	Ghrelin O-acyltransferase
HCL	Hydrochloric acid
Hex	High exercise level
HR	Heart rate
HSE	Health survey for England
iAUC	Incremental area under the curve
ICC	Intraclass correlation coefficient
Kcal	Kilocalorie
kg	Kilograms
KJ	Kilojoule
Km/h	Kilometres per hour
$L.min^{-1}$	litres per minute
LoA	Limits of agreement
M	Mole

Mex	Moderate exercise level
Min	Minute
mM	Millimole
mm	millimetre
NaOH	Sodium hydroxide
N-EX	No exercise
NexEE	Non-exercise energy expenditure
NPY	Neuropeptide tyrosine
^{o}C	Degrees Celsius
PAEE	Physical activity energy expenditure
PAR-Q	Physical activity readiness questionnaire
PBS	Potassium phosphate buffer solution
PFC	Prospective food consumption
$pg.ml^{-1}$	Picogram per millilitre
PHMB	P-hydroxymercribenzoic acid
PYY	Peptide tyrosine-tyrosine
REI	Relative energy intake
RER	Respiratory exchange ratio
RFPM	Remote food photography method
RMR	Resting metabolic rate
RPE	Rating of perceived exertion
s	Seconds

SD	Standard deviation
SE	Standard error
tAUC	Total area under the curve
TEE	Total energy expenditure
VAS	Visual analogue scale
W	Watts
WHO	World health organisation

1.0 Introduction

The obesity epidemic represents a global health issue with worldwide obesity having doubled between 1980 and 2014 (World Health Organisation, 2015). In 2014 alone, 1.9 billion adults (18 years or older) were classified as overweight, with 600 million deemed obese. In the UK, prevalence of obesity in adults (16 years or older) has continued to rise from 13% to 24% in males and 16% to 24% in females between 1993 and 2006 as identified by the Health Survey for England (HSE) (Scantlebury and Moody, 2015). The HSE further classified 27% of women and 24% of men as obese in 2014 with an additional 31% of women and 41% of men being classed as overweight. Being obese and overweight is a consequence of sustained periods of positive energy balance, where energy intake (EI) exceeds energy expenditure (EE) (Guan et al., 1997) and is associated with diseases such as diabetes, cardiovascular disease and certain cancers (World Health Organisation, 2015). Strategies aimed at increasing EE or reducing EI to attenuate obesity are therefore required.

A promising strategy for the prevention and management of obesity is increasing EE through exercise in order to induce an energy deficit. Nevertheless, prescribed exercise programmes often produce weight loss that is less than expected theoretically (Thomas et al., 2012). This indicates resistance over the long term between the prescribed 'exercise energy expenditure' (ExEE; the energy expended during prescribed exercise only) and compensatory mechanisms, comprising of increased EI and/or decreased 'non-exercise energy expenditure' (NexEE; the energy expended outside of prescribed exercise which includes rest and all other physical activities) (Melanson et al., 2013). Thus, prescribing exercise in the management of weight could be more difficult than anticipated.

Evidence has largely shown that a single bout of prescribed aerobic exercise does not lead to an increase in EI or hunger in the short-term, for energy balance to be restored (Donnelly et al., 2014, Schubert et al., 2013). Furthermore, acute bouts of high-intensity exercise (~70% maximal oxygen uptake ($\dot{V}O_{2max}$)) often produce transient (during and up to 60 min post-exercise) declines in hunger (Broom et al.,

2009). The potential mechanisms regulating the transient declines in hunger are suggested to be through a suppression and elevation of the gut hormones acylated ghrelin and peptide tyrosine-tyrosine (PYY), respectively (Broom et al., 2009, King et al., 2010). In contrast, a single bout of prolonged (90 min) treadmill running (70% $\dot{V}O_{2max}$) does not lead to alterations in gut hormones acylated ghrelin and PYY, when measured 20-24 hours succeeding the exercise bout (King et al., 2010, King et al., 2015). An energy deficit, however, was still apparent 24 hours post the acute exercise bout (King et al., 2010, King et al., 2015). This is somewhat paradoxical since prescribed exercise interventions tend to produce less than expected weight loss. This might suggest that compensation, through increased EI and decreased NexEE, of the energy deficit induced by the prescribed ExEE may be observed after 24 hours following the commencement of an exercise regimen. However, few acute or long-term studies have examined the role of NexEE as well as EI, in opposing the negative energy balance induced by imposed exercise.

To date, studies examining the chronic (greater than one day) effect of prescribed exercise on energy balance are limited. It is documented that 9-14 days of chronic exercise produces modest compensation in free-living EI and NexEE, revealing the first stages of compensation to restore energy balance (Stubbs et al., 2002b, Whybrow et al., 2008). Unfortunately, these 9-14 day studies have typically not assessed appetite-regulating hormones to provide an objective indication of the mechanisms responsible for potential compensatory changes in EI.

The assessment of free-living EI endures many complexities which can lead to misleading conclusions in studies measuring the compensatory responses to chronic exercise. Self-reporting EI relies on the ability of an individual to accurately recall and record all foods consumed (Williamson et al., 2003) which can lead to underestimating and/or overestimating food intake. Photographing food consumed provides a valid estimate of EI when compared with weighed food and double labelled water (DLW) assessments (Martin et al., 2012, Williamson et al., 2003). Martin et al. (2012) reported that a photographic method using camera smartphones to collect EI data (referred to as Remote Food Photography Method (RFPM)) is a valid and reliable method for the assessment of free-living EI over a 6 day period.

The photographic food diary has been documented to underestimate free-living EI over a 3 day (by 6.6%) (Martin et al., 2009a) and a 6 day (3.7%) period (Martin et al., 2012) when compared to weighed foods and DLW measurement, respectively. However, the photographic method has been documented to overestimate meal portion size, although of small magnitude (~6g), over a single day (Williamson et al., 2003). The assessment of free-living EI also places a burden on individuals to record all food and beverages consumed and could therefore result in alterations in EI. Wang et al. (2006) reported that weighed food records were considered most burdensome, by 86% of the 28 female participants, and on average took the longest to record (37 min) when compared with a digital photographic diary (16 min) and 24 hour recall (24HR) (22 min), over 1 day. Therefore, the use of digital photographs of food to estimate EI allows for quick data acquisition and is a convenient method for both participants and researchers. However, the reliability of a photographic food diary to assess free-living EI across seven days has not been examined to date.

Despite the significance of regular exercise in maintaining a healthy body weight, few well-controlled studies have investigated the chronic (more than a single session) effects of imposed exercise on free-living EI and NexEE. Furthermore, the reliability of the methods employed to assess energy intake has not always been clear. In addition, alterations in fasting and postprandial appetite-regulating hormones have not been assessed within these studies (Stubbs et al., 2002b, Whybrow et al., 2008), which may aid in the explanation of any compensatory changes in habitual EI.

2.0 Literature review

This chapter will review and describe the current literature on methods used for measuring EI, as well as the effects of acute and chronic exercise doses on energy balance and gut hormone responses. The first section will describe the methods used for measuring EI and will summarise which is the most appropriate for measuring free-living EI. Energy balance and the influence of acute and chronic exercise on energy balance will then be examined. The importance of gut hormones in energy balance and the signalling pathways will be discussed and will lead onto the final section, which will describe the effects of acute and chronic exercise on appetite and gut hormone responses.

2.1 Methods of measuring EI and EE

The majority of research investigating free-living dietary intake often relies on the use of self-report methods. Self-report methods assessing EI are renowned for producing measurement error of two types; systematic and random error. Systematic error occurs through inaccurate reporting of food intake, which could lead to a bias in intake estimates (Rutishauser, 2005). Random error could arise from mistakes in writing or data processing and consequently lead to false-negatives being concluded (Trabulsi and Schoeller, 2001).

The most commonly used methods of free-living EI measurement are weighed food records, 24HR and food frequency questionnaires (FFQ) (Williamson et al., 2003). Weighed food records are regarded as a prospective recording method, where all foods and fluids must be recorded before consumption, whereas retrospective methods, such as the 24HR and FFQ, require individuals to recall foods previously consumed (Trabulsi and Schoeller, 2001). Accurately collecting and measuring free-living EI through self-report measures, however, is abundant with problems. Prospective methods, like the weighed food records, are burdensome to participants (Wang et al., 2006) and have been shown to influence an individual's food selections, subsequently altering EI (Trabulsi and Schoeller, 2001). Retrospective

methods could lead to problems of random and systematic error due to poor recall of foods previously consumed and underreporting EI (Trabulsi and Schoeller, 2001). Evidence suggests that an underestimation in EI can occur when weight reduction through calorie count is being assessed or an overestimation in EI when exercise is assessed (Purnell, 2006). Since accurately estimating free-living macronutrient and EI is essential to researchers investigating the role of interventions on body weight and EI, there is a need for an accurate method to assess free-living EI that does not place a large burden on the participant.

2.1.1 Doubly labelled water (DLW)

DLW is considered the gold standard measure of EI (Martin et al., 2009a, Martin et al., 2009b) and EE (Livingstone and Black, 2003) under free-living conditions. EE is calculated by giving individuals a quantity of water which is enriched with oxygen (^{18}O) and deuterium (^{2}H), stable isotopes (Livingstone and Black, 2003). Urine is collected prior to and following the assessment period (typically between 7 and 21 days) to determine both isotopes disappearance rates (Livingstone and Black, 2003). Production of carbon dioxide is then calculated for the equations of indirect calorimetric for estimation of EE (Livingstone and Black, 2003). The precision and accuracy of DLW is reported to be of 2-8% and 1-3%, respectively, in well controlled conditions (Livingstone and Black, 2003). During a period of energy balance, where EI equals EE and where body weight remains stable, total EE (TEE) is reflective of actual EI (Livingstone and Black, 2003). Therefore, it is possible for the DLW method to act as the criterion measure for EI during periods of energy balance. Nevertheless, short-term EI cannot accurately be assessed by DLW during periods of negative energy balance, even when changes in energy stores are considered (de Jonge et al., 2007). DLW, therefore, cannot accurately assess EI during periods of dieting. Furthermore, the DLW method is seldom used due to its high costs, inability to provide information on the compositions of foods ingested, and the need for highly skilled individuals and facilities for data analysis.

More cost effective and appropriate methods are required for the assessment of free-living EI.

2.1.2 Weighed food records

Weighed food records are another accurate method of measuring EI (Small et al., 2009) and have been used as a criterion measure to assess the validity of other dietary intake methods (Trabulsi and Schoeller, 2001). All beverages and foods consumed are weighed prior to and after consumption and are recorded daily, commonly over a 3-14 day timescale.

Several studies have examined the relationship between EI, as measured by weighed food records, against DLW. It is generally seen that weighed food records underreport EI when validated against DLW (Goran and Poehlman, 1992, Seale and Rumpler, 1997). For example, Seale and Rumpler (1997) showed average EI to be underreported by 23% in 24 healthy males and females aged 40-65 years who completed a 7 day weighed food record, when compared against DLW. Consistent with these results, Goran and Poehlman (1992) found an underestimation of 21% in older adults aged 56-78 years, when measured against DLW. These results were not explained by an energy imbalance since body weight did not significantly alter throughout the study period (Goran and Poehlman, 1992) which indicates an underestimation of EI when assessed using the weighed food diary.

One of the main limitations of weighed food records is that they are burdensome and time consuming for participants to complete accurately (Wang et al., 2006). Wang et al. (2006) found that weighed food records, on average, took the longest to record (37 minutes) when compared to the 24HR method (22 minutes) and digital photographic diary (16 minutes) during 1 day of EI assessment. In further agreement, the weighed food record was regarded most burdensome by 86% of participants. The 24HR was deemed somewhat burdensome by 50% of participants and 57% of participants considered the digital photographic diary as least burdensome, of the 3 methods. Therefore, new methods of assessing EI, such as the

digital photographic diary, are needed in order to reduce participant burden and associated changes in dietary intakes, for free-living EI to be measured.

2.1.3 Digital photography food diary

As the literature currently shows, there is a need for more appropriate measures of free-living EI. The digital photography method is regarded as a valid and reliable method for measuring free-living EI (Lassen et al., 2010, Martin et al., 2012, Williamson et al., 2003). This method allows photographs of participant's food selections and plate waste to be captured in real-time. The photographs can then be analysed by researchers to estimate portions sizes and further estimate EI.

Williamson et al. (2003) tested the validity of direct visual estimations and digital photography in measuring portion sizes against weighed foods. The digital photograph method yielded small overestimations (~6 g) across all meals in comparison to weighed foods, except for plate waste (Williamson et al., 2003). Portion sizes (g) of food intake, plate waste and food selections estimated through digital photography correlated highly with weighed food diaries. Overall grams revealed a correlation of 0.89 for digital photography, although visual estimation had a higher correlation of 0.97, when compared against weighed foods. These findings support the validity of the photographic food diary for measuring EI. However, this study was confined to a university cafeteria and therefore additional research is required on the use of the photographic food diary in free-living conditions.

In a paediatric population, 36% of participants reported EI accurately (within ± 5% of actual EI) following a 3-day photographic food diary, when validated against a weighed food diary (Higgins et al., 2009). However, 29% of participants underreported EI (by 16% (449 ± 22 kcal.day^{-1})) with 35% over-reporting EI (by 31% (663 ± 103 kcal.day^{-1})). Higgins et al. (2009) further reported that fat intake was overestimated by 72% of participants in comparison to 18% who underreported. Carbohydrate intake was underestimated by 57% of participants and

overestimated by 32% of participants. As fat contains more calories per gram (9 kcal.gram^{-1}) than carbohydrate (4 kcal.gram^{-1}) (Smith et al., 2000) it has a greater influence on the agreement with EI if estimates of fat intake are inaccurate. Therefore, the greater magnitude of over-reporting in comparison to underreporting by Higgins et al. (2009) could be explained by an inaccurate reporting of fat and carbohydrate intake. These results however cannot be generalised to other populations; therefore, future research is required in an adult population.

A similar method to that of the photographic food diary is the remote food photography method (RFPM) in which photographs of foods are sent via a wireless network to researchers. Martin et al. (2009a) examined the validity and reliability of the RFPM in 50 adults over a 3 day period, under free-living and laboratory conditions. Within free-living conditions, a cooler containing pre-weighed foods was provided to participants for their evening meals. The cooler was returned the next morning for post-weight to be obtained, and EI to be calculated. All lunch meals were consumed under laboratory conditions. Under free-living conditions, the RFPM underestimated EI significantly by 6.6% (97 kcal) whereas laboratory conditions showed no significant underestimation of EI (5.5%; 36 kcal). Estimated EI through the RFPM produced a high correlation with weighed EI under free-living conditions ($r = 0.95$) and laboratory conditions ($r = 0.93$) implying the RFPM is a valid method. In a follow-up study, Martin et al. (2012) conducted two studies investigating the validity and reliability of 6 days free-living EI assessment using the RFPM. Study 1 employed two different types of prompts to remind participants to capture images of foods before and after consumption. One group received standard prompts 2-3 times each day around normal meal times whereas the other group received customised prompts 3 to 4 times each day near individual's meal times. Additionally, the customised group received feedback quicker (within 24 hours *vs.* 1-2 days) if any data issues occurred. Customised prompts underestimated mean EI by 8.8% (270 ± 748 kcal.day^{-1}) compared to standard prompts which underreported mean EI by 34.3% (895 ± 770 kcal.day^{-1}), when measured against DLW. The findings by Martin et al. (2012) reveal that the more prompts received the greater the accuracy of the RFPM in measuring free-living EI. Study 2 investigated the validity and reliability of the RFPM, using customised prompts,

against DLW in 42 participants (Martin et al., 2012). Measurement of EI by the RFPM showed no significant difference when compared against DLW, although did reveal an underestimation of 3.7% (152 ± 694 kcal.day^{-1}). Additionally, the RFPM produced an intraclass correlation coefficient (ICC) of 0.74, which showed good reliability. These findings indicate that, the RFPM, with the use of customised prompts, could provide a valid and reliable method for measuring EI under free-living conditions. However, this finding is based on limited data and further research needs to assess the reliability and validity of the digital photographic method (with and without prompts) over 7 days or more under free-living conditions.

2.2 Energy balance

Fluctuations in body weight are associated with energy imbalances where EI does not equal EE. Weight loss, for example, results from a negative energy balance where EE exceeds EI, and vice versa for weight gain. The most commonly prescribed exercise intervention for inducing weight loss and attenuating weight gain is aerobic exercise. However, most exercise programmes produce less than expected weight loss (Melanson et al., 2013). The attenuated magnitude of weight loss in response to increased ExEE, may be due to the stimulation of physiological and behavioural compensatory responses of energy balance that oppose the exercise-induced energy deficit (Figure 2.1).

Energy imbalances however, are further affected through social and psychological influences on EI. Within a social context, individuals tend to consume foods in the presence of others, which may impact an individual's decision on food consumption. A mechanism for social contexts effects on eating behaviour may be through social norms which provide an appropriate guide of action (Higgs, 2015) to inform individuals of their food preferences (Higgs and Thomas, 2016). Further, research suggests that individuals tend to eat similarly to others as the emotional experience created is positive (Higgs and Thomas, 2016). Psychological factors, such as stress or depression, further influences EI and subsequently energy balance.

Literature tends to suggest an association between elevated levels of stress and an increased desire for more energy dense foods (Torres and Nowson, 2007). This could then lead to an overconsumption of EI and may consequently result in weight gain through a positive energy balance.

Compensatory responses

Physiological	Behavioural
Resting metabolic rate ↓	Non-exercise energy expenditure ↓
Non-exercise energy expenditure ↓	Hunger ↑
Exercise energy expenditure ↓	Fullness ↓
Fat mass ↓	Energy intake ↑
Fat free mass ↑	Alterations in macronutrient content
Alterations in appetite-related hormones	

Figure 2.1 Compensatory responses to increased exercise energy expenditure (ExEE). Adapted from Hopkins et al. (2014).

2.2.1 Acute effects of exercise on energy balance

The majority of literature examining the acute effects of a single exercise bout on energy balance has established that individuals do not compensate through an increase in EI; although due to differing protocols, results are inconsistent. In a meta-analysis, Schubert et al. (2013) concluded that absolute EI is unaffected by an acute bout of exercise, resulting in a subsequent energy deficit. A recent review by Donnelly et al. (2014) is in agreement with Schubert et al. (2013) and additionally states that any post-exercise increase in EI is a partial compensation.

The intensity of an exercise bout shows differing effects on energy balance and ratings of appetite. King et al. (1994) investigated the effects of low intensity (30% $\dot{V}O_{2max}$ ~60 mins; EE 359 ± 42 kcal) cycling compared with high intensity (70% $\dot{V}O_{2max}$ ~30 mins; EE 340 ± 28 kcal) cycling (performed 120 min postprandial) in 12 healthy males aged 21-27 years. Macronutrient content and absolute EI were not significantly influenced by either intensity following an *ad libitum* test meal, 15 min post exercise. Hunger however, was significantly suppressed during and immediately following the high intensity bout of exercise, returning to levels comparable to that of the control group immediately before the meal (15 min post-exercise). EI was further assessed, via a weighed food record, and did not differ significantly 48 hours after the intervention. Similarly, Imbeault et al. (1997) found no significant difference in absolute *ad libitum* EI (15 min post-exercise) when comparing a low intensity treadmill walk at 35% $\dot{V}O_{2max}$ (72 ± 14 mins; ExEE 491 ± 11 kcal) and a high intensity treadmill run at 75% $\dot{V}O_{2max}$ (34 ± 6 mins; ExEE 483 ± 9 kcal) in healthy males. Furthermore, no significant differences were found between conditions for fullness or hunger. High intensity treadmill running, however, significantly reduced relative EI (REI; EI minus the ExEE) in comparison to the control and low intensity groups. EE was matched for during the high and low intensity exercise bouts, suggesting that the results on REI were an independent effect of exercise intensity. Thus, REI is lower following high intensity exercise (75% $\dot{V}O_{2max}$) compared with lower intensity exercise (35% $\dot{V}O_{2max}$), although without any suppression of hunger. Accordingly, high intensity exercise bouts (≥ 70% $\dot{V}O_{2max}$) appear to reduce REI, without any influence on absolute EI on the day and 48 hours post the exercise bout. Moreover, hunger reveals contrasting results following either low or high intensity exercise bouts.

Exercise duration is another variable which can impact energy balance. King et al. (1994) investigated two high intensity trials (75% $\dot{V}O_{2max}$), one of long duration (average 52 min; EE 541 ± 52.2 kcal) and the other of short duration (average 26 min; EE 296 ± 38.4 kcal) in 12 male participants (22 – 31 years). Absolute EI in an *ad libitum* test meal (15 min post-exercise) was not significantly different following the exercise interventions. However, due to the larger ExEE, the longer duration

trial revealed a significantly lower REI during the *ad libitum* test meal and the remainder of the day when compared to the shorter duration and resting control trials. A more recent study found that high intensity (70% $\dot{V}O_{2peak}$; ExEE men, 975 ± 195 kcal; women, 713 ± 86 kcal), long duration (82 min men; 84 min women) cycling does not significantly influence EI compared to rest, in males and females (Hagobian et al., 2012). REI produced significantly lower results in the *ad libitum* meal 40 min post-exercise in both the males and females, in comparison to the control trial. Vatansever-Ozen et al. (2011) observed similar findings on REI and absolute EI following 120 min treadmill running (105 min at 50% $\dot{V}O_{2max}$ and 15 min at 70% $\dot{V}O_{2max}$) on *ad libitum* EI, 60 min post-exercise intervention. Absolute EI did not significantly differ between conditions in healthy elite males (n = 10). REI, as seen in other studies, was significantly reduced following exercise than rest showing that participants were in an acute state of energy deficit. A significantly reduced hunger rating immediately and 60 min post-exercise was also revealed. In another study examining the effects of exercise duration on EI, Erdmann et al. (2007) found that 120 min of cycling at 50 W (EE 342 kcal) significantly increased EI (15 min post-exercise) in healthy males (n = 4) and females (n = 3), compared to 30 min (EE 86 kcal) and 60 min (EE 171 kcal) cycling at 50 W. Satiety and hunger ratings however did not change over time following each exercise duration. The significant increase in absolute EI is in contrast to previous studies (Hagobian et al., 2012, King et al., 1994), which are of shorter duration (52-82 min). Nevertheless, Vatansever-Ozen et al. (2011) reported no change in absolute EI following the same exercise duration of 120 min. The type and intensity of exercise however was different between studies, thus potentially leading to the contradicting results. Regardless of this, most studies are in agreement that REI is significantly reduced following an exercise bout of duration ≥ 52 min.

Participant activity levels play a further role on the effects of an acute exercise bout on EI. For example, active male participants (n = 10) showed a compensatory effect (22.7%) on EI 60 min post-exercise (45 min cycle at 65-75% age-predicted maximum heart rate to expend 450 kcal) in comparison to a negative compensation (-35.4%) in inactive participants (n = 10) (Jokisch et al., 2012). Overall, active males compensated by 36% when including self-report intake throughout the day

and at the *ad libitum* lunch meal, whereas the inactive group demonstrated a 3% overall compensation. Interestingly, perceived hunger was significantly greater in the active males control trial, compared to the exercise trial in the inactive males at 80, 90, 100 and 110 min (after baseline measures), although no differences were noted within active and inactive conditions. Rocha et al. (2013) found similar results of EI compensation on the day of an acute bout of exercise. Fifteen active males, following an acute bout of exercise (60 min cycling at 50% $\dot{V}O_{2max}$), significantly increased their EI on the day of the exercise trial compared to a resting control trial. This compensatory increase, however, was seen during the remainder of the exercise day, and not at the 60 min post-exercise *ad libitum* meal. The inactive group (n = 15) had a delayed response, which only saw a significant increase in EI 3 days following the acute exercise bout compared to control. Consequently, active individuals may be more sensitive to changes in energy balance following an acute exercise bout, and, in response, compensate to a larger degree than inactive individuals.

An acute bout of moderate to high intensity (50% - 70% $\dot{V}O_{2max}$) exercise of long duration (>60 min) results in an unaltered absolute EI, reduced REI, and, therefore, an acute negative energy balance. Compensatory increases in EI are more often seen in active compared with inactive populations on the day of exercise; however additional research is required on the chronic (more than a single bout of exercise) effects of exercise on free-living EI, and in free-living conditions.

2.2.2 Chronic effects of exercise on energy balance

Chronic exercise training (5-14 days) generally produces modest increases in EI in an attempt to restore energy balance. The majority of studies, however, show that these increases in EI are the first stages of compensation and do not compensate enough to restore energy balance from the energy deficit induced during the study (Staten, 1991, Stubbs et al., 2002b, Whybrow et al., 2008). Staten (1991) reported that 5 days of 60 min treadmill running (~70% $\dot{V}O_{2max}$) per day significantly

increased EI in males (n = 10) (208 ± 64 kcal.day^{-1}) compared to a 5 day non-exercise control period, whereas the female group (n = 10) did not significantly alter EI between conditions. Irrespective of this, both males and females remained in a state of energy deficit due to the exercise induced increases in EE (ExEE 596 kcal.day^{-1} and 382 kcal.day^{-1} in the males and females, respectively). Results of a greater magnitude were found by Stubbs et al. (2002b) in which an energy deficit, on average, of 1194 kcal.day^{-1} was recorded in males, following high exercise (Hex) (ExEE 764 kcal.day^{-1}; 120 min cycling) and 262 kcal.day^{-1} following moderate exercise (Mex) (ExEE 382 kcal.day^{-1}; 80 min cycling) over 9 days. EI did not significantly differ between conditions, although EI in the Mex declined during the later stages of the intervention, from day 3. Interestingly, TEE declined throughout the duration of the study in all groups with a further decline in NexEE following Hex and Mex throughout the study. This could be regarded as a compensatory mechanism to limit TEE and restore energy balance, although the authors attributed the decline of TEE to fatigue. Nevertheless, a measure of fatigue was not collected in this study and therefore this possible explanation is only speculative.

A study of longer duration (14 days) found that both Mex (2 x 40 min bouts.day^{-1}) and Hex (3 x 40 min bouts.day^{-1}) exercise (on a cycle ergometer or treadmill) significantly increases daily EI, however only in males and not females (Whybrow et al., 2008). Additionally, a 30% partial compensation of the exercise induced EE was reported in participants (6 males, 6 females). Nevertheless, the Hex group (ExEE males 1170 kcal; females 907 kcal) and the Mex group (ExEE males 668 kcal; females 477 kcal) remained in an energy deficit. Thus, a partial compensation in EI (~30%) of the exercise induced EE is observed over a longer duration (> 7 days), although without energy balance being restored. However, further research would be required to confirm this finding as other studies have not drawn the same conclusions in males and females, respectively (Staten, 1991, Stubbs et al., 2002b). Intensity of exercise was not stated by Stubbs et al. (2002b) or Whybrow et al. (2008) and could explain the unaltered EI and lack of compensation observed in these studies, respectively. As a result, future research using well-controlled study designs is required to investigate the effects of high-intensity exercise over ≥ 7 days on energy balance.

2.3 The role of gut hormones on appetite regulation

The hypothalamus is a key component in the regulation of appetite (Suzuki et al., 2010), which communicates with adipose tissue and gastrointestinal organs (King et al., 2010). Anticipation of or in response to a meal stimulates the release of short term signalling gut hormones, which are known as episodic hormones (Howe et al., 2014). These hormones include PYY, glucagon-like peptide 1 (GLP-1) and cholecystokinin (CCK) which all suppress appetite (King et al., 2010). These are satiation (anorexigenic) hormones. Ghrelin is the only known episodic hormone to stimulate appetite (orexigenic hormone) (Suzuki et al., 2010). Conversely, leptin and insulin are tonic hormones that indicate energy status over the long term and further supress appetite (Howe et al., 2014). Table 2.1 summarises the key adiposity signal and gut hormones that are associated with EI.

Table 2.1 Summary of predominate adiposity signals and gut hormones in appetite control (Adapted from Suzuki et al. (2010))

	Feeding	Receptor	Major secretion site	Other actions
Gut hormones				
PYY_{3-36}	↓	Y2	L cells in gut	Delays gastric emptying
GLP-1	↓	GLP-1	L cells in gut	Incretin, decreases blood glucose, delays gastric emptying, neurotrophic effect
CCK	↓	CCK 1, 2	I cell of small intestine	Gall bladder contraction, relaxation of sphincter of Oddi, pancreatic enzyme secretion
Ghrelin	↑	GHS	Stomach	Growth hormone secretion
Adiposity signals				
Insulin	↓	Insulin	Pancreatic β cell	Decreases blood glucose levels, stimulates glycogen synthesis
Leptin	↓	Leptin (Ob-R)	Adipocyte	Regulation of energy metabolism

PYY = peptide YY; GLP-1 = glucagon-like peptide-1; CCK = cholecystokinin

2.3.1 Peptide tyrosine-tyrosine (PYY)

PYY is from the neuropeptide Y (NPY) family and released by endocrine L cells in the large and small bowel, following food intake (Neary et al., 2004). The mechanisms linking the increased levels of PYY, following food ingestion, are thought to be either hormonal or neural (Karra and Batterham, 2010). PYY_{1-36} and PYY_{3-36} are the 2 forms of PYY with the latter being the more active form (Zwirska-Korczala et al., 2007). Postprandially, circulating PYY_{3-36} is the predominant form (Zwirska-Korczala et al., 2007) and supresses food intake (Neary et al., 2004). These effects are mediated via the Y_2 receptors, located in the hypothalamus to which PYY binds (Neary et al., 2004) and exhibits gastrointestinal motility suppression and inhibition of pancreatic enzyme and gastric acid secretion (Zwirska-Korczala et al., 2007). Although the active form of PYY, PYY_{3-36}, is seen to be of greater concentration in the circulation (Zwirska-Korczala et al., 2007), total PYY produces similar plasma patterns following a meal (Batterham et al., 2006).

2.3.2 Ghrelin

Ghrelin is a 28 amino acid peptide (Andrews, 2011) which is a result of the proteolytic process of preproghrelin (precursor peptide) (Gahete et al., 2014). Ghrelin's physiological functions include appetite stimulation, modulation of gastric functions (motility and acid secretion), exocrine and endocrine pancreatic secretions and growth hormone secretion (Delporte, 2013). A decrease in circulating ghrelin levels following chronic overfeeding are seen, with increases in circulating ghrelin levels following a chronic energy deficit (Neary et al., 2004).

Ghrelin is predominately synthesised by endocrine X/A-like cells within the oxyntic glands of the stomach (Kishimoto et al., 2012) and in plasma exists in two forms; des-acylated ghrelin and acylated ghrelin (Andrews, 2011). Acylation of ghrelin occurs post-translationally by the ghrelin O-acyltransferase (GOAT) enzyme (Sato et al., 2012), in which an octonoic acid (eight chain carbon-fatty acid)

attaches at the serine 3 residue of ghrelin (Lim et al., 2011). Acylated ghrelin, which is ~10% of the total ghrelin concentration (Patterson et al., 2005), causes it's action by activating growth hormone secretagogue receptor 1a (GHSR1a) (Andrews, 2011). The orexigenic effects of acylated ghrelin are exerted in the hypothalamus (Gahete et al., 2014), which is crucial in the regulation of appetite (Suzuki et al., 2010). Growth hormone secretagogue receptor (GHS-R) mRNA is expressed highly within the arcuate nucleus (ARC) of the hypothalamus (Guan et al., 1997) in which food stimulating neurons (Suzuki et al., 2010), are activated (Nakazato et al., 2001). Additionally, circulating ghrelin levels within the blood stimulates food intake via the vagus nerve of the central nervous system (CNS) (Sato et al., 2012). Figure 2.2 shows the pathways of ghrelin along the gut-brain axis.

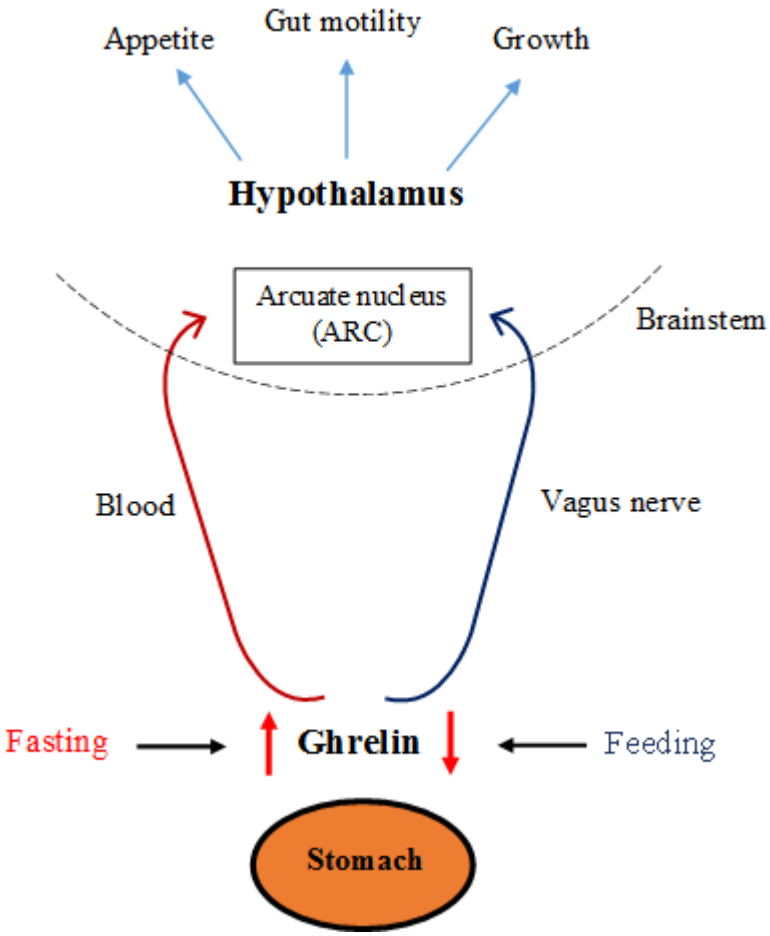


Figure 2.2 The gut-brain axis showing the pathways of ghrelin. Adapted from Inui et al. (2004).

2.3.3 The acute effects of exercise on total PYY and acylated ghrelin

It is predominately seen that PYY increases during and for up to 60 minutes after an acute bout of aerobic exercise (~ 60 min) (Martins et al., 2007, Broom et al., 2009), whereas acylated ghrelin is suppressed following acute exercise (Broom et al., 2007, Broom et al., 2009, Deighton et al., 2013, King et al., 2010).

Various studies have investigated the effects of moderate and high intensity (> 65% $\dot{V}O_{2max}$), long duration (60 min) exercise on both total PYY and acylated ghrelin concentrations (Broom et al., 2009, Deighton et al., 2013, King et al., 2015). Deighton et al. (2013) examined the responses of PYY and acylated ghrelin following a 60 min cycling bout (65% $\dot{V}O_{2max}$) compared to a 30 min interval sprint training (6 x 30s sprints against 7.5% body mass) session in healthy males (n = 12), following a standardised meal. Acylated ghrelin was significantly suppressed following both exercise sessions compared with a resting control trial. PYY was only elevated on completion of the 60 min cycle bout relative to the control. Similar results were found by Broom et al. (2009) in healthy fasted males (n = 11) in which 60 min treadmill running (70% $\dot{V}O_{2max}$) elevated PYY concentrations in comparison to rest and a resistance exercise session. This elevation in PYY concentrations was confirmed by a PYY AUC that was higher in the pre-prandial (0 – 2 h), postprandial (2-5 h) and the full 8 hour trial periods compared to control and the resistance session. A suppression of acylated ghrelin was observed at 0.75 hours in the 60 min exercise bout and 90 min resistance training session, and further at the end of the resistance training bout. The difference in the PYY response during the aerobic condition could be due to the greater ExEE (3,832 ± 97 kJ *vs.* 1,473 ± 114 kJ) or disturbance in the gut compared to that in the resistance training condition. Thus, aerobic exercise of ~ 60 min in duration, at an intensity of at least 65% $\dot{V}O_{2max}$ significantly reduces acylated ghrelin and further results in an elevated PYY concentration.

Individual assessment of total PYY and acylated ghrelin have provided further evidence of increased and reduced concentrations, respectively, following high intensity exercise bouts (Broom et al., 2007, Martins et al., 2007). Intermittent

cycling (65% max heart rate) of 60 min duration significantly increases PYY concentration, during exercise, in healthy males (n = 6) and females (n = 6), 60 min after a standardised meal (Martins et al., 2007). Decreases in hunger ratings were also noted; however, both increases in PYY concentrations and decreases in hunger ratings were diminished on completion of the exercise bout. In contrast to the majority of the literature (Schubert et al., 2013), *ad libitum* EI 2 hours following the exercise bout significantly increased in comparison to the control condition, without subsequent changes in hunger or PYY concentration. This implies that PYY may not always provide a direct indication of food intake, although other factors (physiological, social and psychological factors) can further affect food intake (Hall et al., 2012). Unlike PYY, an acute bout of exercise suppresses acylated ghrelin. High intensity (75% $\dot{V}O_{2max}$) treadmill running shows a significant decrease in acylated ghrelin during a 60 min exercise bout compared to rest in 9 fasted healthy males (Broom et al., 2007). Specifically, acylated ghrelin was reduced during the first 30 min of exercise and AUC additionally showed that acylated ghrelin was lower during the first 3 hours of the 9 hour trial (38% lower) and during the entire trial (0 - 9 hours) (35% lower) compared to a resting control trial. Ratings of hunger decreased during the exercise bout whereas during the control trial, hunger increased. Furthermore, AUC for hunger was significantly lower during the first 3 hours of the exercise trial (24 *vs.* 32 mm) although was significantly greater (50 *vs.* 44 mm) for the 6 hours postprandial against the rest condition. The increase in hunger 6 hours postprandial during the exercise condition could be explained by a possible delayed effect of the exercise induced energy deficit, despite the low concentrations of acylated ghrelin during the exercise trial, which would suggest that hunger would remained suppressed in comparison with the control condition. King et al. (2010), however, reported concentrations of acylated ghrelin to be significantly suppressed during and immediately following a 90 min treadmill run (70% $\dot{V}O_{2max}$) with a non-significant suppression of hunger during the exercise compared to control condition, in healthy males. A significantly lower AUC for acylated ghrelin was reported during the exercise condition, for the initial 2.5 hours (40%) and the full 10 hour condition (25%), although did not significantly differ between conditions following the meal after 2.5 hours. EI did not differ between

trials for the three *ad libitum* buffet meals (at 2.5, 5.5 and 9 hours) or 22.5 hours post the exercise bout, providing evidence that the potential compensatory effects of exercise on EI are still not seen the day after exercise. King et al. (2010) further reported a non-significant suppression of hunger only during the exercise compared to control condition. Accordingly, acylated ghrelin and total PYY are significantly altered following a high intensity (> 65% $\dot{V}O_{2max}$) exercise bout, however the results imply that these alterations in gut hormone concentrations may not always provide a direct indication on hunger and EI responses.

Implementation of a prolonged exercise bout (90 min at 70% $\dot{V}O_{2max}$) the day before appetite and gut hormone assessment shows no significant differences in appetite ratings (satisfaction, prospective food consumption (PFC), hunger and fullness), fasting acylated ghrelin or fasting total PYY concentrations (King et al., 2015). Acylated ghrelin and total PYY concentration did not differ throughout the 7 hour testing day between conditions. However, a significantly lower AUC for acylated ghrelin (14%) was noted in the exercise condition after the meal provided at the 4 hour time point. Contrasting results were reported by Heden et al. (2013) who reported lower (18%) fasting concentrations of acylated ghrelin in normal weight males and females (n = 8; n = 6, respectively) on the day after a 60 min moderate intensity bout of exercise (55-60% $\dot{V}O_{2peak}$). Incremental AUC during the 4 hours postprandial was lower (39%) in the rest condition in comparison to the exercise condition, which is in contrast to King et al. (2015) and King et al. (2010). Although the mechanisms behind the contradicting results are unknown, it could be attributed to the differences in studies; intensity and duration and subsequently ExEE, gender differences (males and females in Heden et al. (2013) *vs.* only males in King et al. (2015)) and sampling time after exercise/rest condition (12 hours in Heden et al. (2013) compared to 20 hours post exercise in King et al. (2015)).

The literature on total PYY and acylated ghrelin tends to suggest that continuous exercise at ≥ 65% $\dot{V}O_{2max}$ of long duration (≥ 60 min) significantly increases PYY and decreases acylated ghrelin concentrations during and immediately after the exercise bout. However, these alterations are removed 20 hours post-exercise. Thus, additional research is required on the days succeeding an exercise bout as a potential

delayed response could be seen. Further research is also warranted on the effects of more than a single bout of exercise on total PYY and acylated ghrelin concentration, appetite ratings and additionally *ad libitum* EI for objective measures of appetite. Indeed, the potential compensatory effects of exercise on EI do not appear to be shown following just a single session.

2.3.4 The chronic effects of exercise on total PYY, acylated ghrelin concentration

The available literature on the chronic (greater than one day) effects of exercise on acylated ghrelin and total PYY are limited. To the current knowledge of the author, only a few studies have investigated the chronic effects of exercise on acylated ghrelin and total PYY in response to a meal (> 24 hours post the final exercise bout) (Martins et al., 2010, Kanaley et al., 2014).

Martins et al. (2010) conducted a 12-week exercise intervention, consisting of running or walking (75% of maximal heart rate) 5 days a week (expending 500 kcal per session) in 15 obese and overweight individuals. Body fat and body mass significantly reduced on completion of the exercise intervention, without any alterations on habitual EI, as measured by food diaries over 3 days. Fasting acylated ghrelin increased significantly from pre-intervention to 48 hours post the final exercise bout (post-intervention), with unaltered fasting PYY concentrations. The increase in fasting acylated ghrelin may be viewed as a compensatory response to promote EI following the exercise intervention. In response to a standardised meal, however, both acylated ghrelin and total PYY concentrations did not significantly differ from pre-intervention to post-intervention. Fasting and postprandial ratings of hunger significantly increased post-intervention in comparison to pre-intervention ratings, implying participants were perceived as more hungry post-intervention. A more recent study of shorter duration found similar findings for fasting total PYY concentrations in obese males and females, with no significant changes in fasting PYY concentrations post a 15 day exercise intervention (60 min walking at 70% $\dot{V}O_{2peak}$ per day) from baseline (Kanaley et al. (2014). Total PYY

concentrations and total area under the curve (tAUC) from pre- to post-intervention did not differ significantly. Across all meals (6 meals each separated by 2 hours), PYY concentration was significantly higher, 40 min post meals 2 and 3 (160 and 280 min, respectively) in comparison to the first and final meal (0 and 600 min, respectively). Reported fullness and hunger were not significantly altered from pre- to post- the exercise intervention. Post-exercise examination of PYY concentrations were completed 24-36 hours post the final bout in order to reduce the acute effects of exercise on the PYY response to a meal, similar to that by Martins et al. (2010). These studies, however, did not examine the results against a control condition and therefore only a within condition effect can be noted and it is not possible to directly attribute the reported effects to the exercise intervention.

Exercise interventions of shorter duration (4 days) report differing results on fasting and postprandial acylated ghrelin (Hagobian et al., 2009). Four days of moderate intensity (50 – 65% $\dot{V}O_{2peak}$) exercise (expending 30% of daily EE), in an energy deficit or with a restored energy balance, did not significantly change fasting acylated ghrelin concentration in previously obese or overweight men and women, compared to a control condition. Appetite ratings in response to the standardised meal, however, were significantly reduced when energy balance was restored, compared to the energy deficit condition in men. Conversely, females displayed a significantly greater acylated ghrelin concentration following the standardised meal, in comparison to the men, in the exercise conditions compared to control, without any change in appetite ratings between conditions. The increase in acylated ghrelin suggests a possible compensatory effect in females, but not in males; thus, it may be important to examine males and females separately in future research. AUC showed a 32% and 25% increase of acylated ghrelin in the energy deficit and energy balance condition, respectively, relative to control. These findings however are in contradiction to that of Martins et al. (2010). The increased fasting acylated ghrelin concentrations noted by Martins et al. (2010) in comparison to unaltered concentrations in Hagobian et al. (2009), could be explained by the study duration; 12 week *vs.* 4 days, respectively. Moreover, the greater acylated ghrelin concentration following a meal in females could be partly due to the acute effects of exercise (measured 24 hours post final exercise bout) whereas as Martins et al.

(2010) examined the chronic effects of exercise on the acylated ghrelin in response to a meal (48 hours post final exercise bout), and further did not examine sex differences.

Fasting total PYY concentrations produce contradicting results following longer term exercise interventions (> 12 weeks) (Jones et al., 2009, Gueugnon et al., 2012). Jones et al. (2009) examined the effects of an 8 month aerobic exercise programme containing 3 x 45 min exercise bouts per week (60-85% $\dot{V}O_{2peak}$) on fasting PYY concentrations in 12 overweight adolescents. A reduction in body fat percentage (2.2%) was noted with an increased fasting PYY concentration (23%) following the 8 month exercise programme. This suggests that fasting appetite is reduced following the 8 month training programme in overweight adolescents, which is in contrast to short-term interventions in adults (Martins et al., 2010), although free-living EI was not recorded throughout the duration of the study. Therefore, the reduced body fat percentage may not completely be attributed to the impact of raised fasting PYY concentrations on EI. In contrast, Gueugnon et al. (2012) found concentrations of PYY in a fasted state did not significantly alter, although tended to increase following 9 months of 45-60 min of exercise (5 times.week^{-1}) in obese adolescents than normal weight adolescents. Gueugnon et al. (2012) controlled total EI at around 2300 – 2500 kcal.day^{-1} during the 9 month training programme suggesting that the combined training and controlled EI programme tended towards an increase in fasting PYY concentration. Body fat percentage and body mass significantly reduced over the 9 month training period (31.4% and 10.9%, respectively) which is of a greater magnitude than that found by Jones et al. (2009). The difference noted in body mass and fat could be attributed to a greater exercise-induced energy deficit and restricted EI, created by predominately more exercise completed each week. These observations, however, were only examined in obese adolescent populations and may not relate to normal weight healthy adults where the prevention of weight gain is important. Additionally, neither Jones et al. (2009) nor Gueugnon et al. (2012) examined the effects of long term exercise training on total PYY concentrations in response to a meal.

Current literature, albeit confined to a small number of studies, has found that longer exercise interventions increase fasting acylated ghrelin in comparison to shorter interventions (12 weeks *vs.* 4 days, respectively). Fasting total PYY does not significantly alter following exercise interventions of ≤ 12 weeks. In response to a meal, total PYY and acylated ghrelin tended not to significantly increase from pre- to post-interventions. Hagobian et al. (2009) however was the only study to examine a between condition effect, which resulted in a significantly greater acylated ghrelin concentration in response to a meal, in females, although this effect may be confounded by the acute effects of exercise (24 hours post final exercise bout). Thus, additional research on fasting and postprandial acylated ghrelin and total PYY concentrations is warranted. Evidence tends to suggest that compensation is more sensitive in active individuals (Jokisch et al., 2012, Rocha et al., 2013), therefore research in normal weight individuals and investigating the chronic effects of exercise in response to a meal (>36 hours post final exercise bout) is required.

2.4 Summary

The methods used to assess free-living EI and the acute and chronic effects of exercise on energy balance and gut hormones response (acylated ghrelin and total PYY) were examined in this chapter. This review showed that commonly used methods to assess free-living EI, such as the weighed food diary, are burdensome and time consuming for participants. This suggests that participants may alter EI in response to the EI method, rather than through an intervention. Furthermore, this review tended to reveal that chronic exercise interventions (≥ 7 days) produced partial compensations in EI of the exercise induced energy deficit. Nevertheless, few studies have examined the chronic effects of exercise on free-living EI in conjunction with gut hormones and ratings of appetite in response to a meal (> 24 hours post final exercise bout). Therefore, the aims of the studies presented in this thesis study are:

- To assess the reliability of a 7-day photographic food diary (with additional written details) in free-living men.

- To examine the effect of 7-days of imposed exercise compared to a no exercise control on daily EI and EE in habitually active men.

- To examine the effect of 7-days of imposed exercise compared to a no exercise control on perceived appetite and appetite-regulating hormones (acylated ghrelin and PYY) in habitually active men.

3.0 General methods

This section details methods that were used in both studies 1 and 2. Methods specific to individual studies are detailed separately in studies 1 and 2 (Chapters 4 and 5, respectively).

3.1 Ethical approval

Both of the studies completed in this thesis gained ethical approval from the University of Bedfordshire's Institute of Sport and Physical Activity Research Ethics Committee before any testing commenced. All testing was completed in the Sport Science Laboratories and Aspire gym on the Polhill Campus, University of Bedfordshire.

In total, each participant completed five visits to the laboratory during study 1, which consisted of one preliminary visit, two visits for combined HR/accelerometer (Actiheart) set up and 2 for Actiheart, camera and food diary collection. In study 2, each participant completed 12 visits to the laboratory. Participants had one preliminary assessment visit, two visits for the non-exercise condition (testing day visits) and nine for the exercise condition (2 testing day visits and 7 visits to complete the exercise training bouts).

3.2 Preliminary assessment

On the first visit to the laboratory the participants completed preliminary assessments to provide data required for Actiheart calibration. These preliminary assessments consisted of a resting metabolic rate (RMR) measure and a submaximal and maximal oxygen uptake ($\dot{V}O_{2max}$) test. Participants arrived at the laboratories at 09:00 in a fasted state (following a 12 hour fast) having refrained from any alcohol, caffeine or strenuous physical activity for 24 hours pre-visit. Anthropometric measures (including stature using a stadiometer (Holtain Ltd,

Crymych, Dyfed, UK) (cm) and body mass using TANITA scales (Hoogoorddreef 56E, Amsterdam, Netherlands) (kg)) were collected on arrival followed immediately by a RMR measure.

3.2.1 Resting metabolic rate (RMR)

Following a 10 min supine rest period, participants lay for an additional 10 min period in which expired air was sampled using an online gas analyser (Metalyzer 3b, Leipzeg, Germany). The first 5 min of gas analysis was discarded to allow the participant to reach steady-state. The final 5 min was then used to average oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). RMR was then calculated using the Weir equation [(3.941)($\dot{V}O_2$) + (1.106)($\dot{V}CO_2$)] to calculate human energy requirements (kcal.min^{-1}) (Weir, 1949). Heart rate (HR) was recorded using short-range radio telemetry (Polar, FS1, Warwick, England) throughout the testing period to obtain a resting HR for Actiheart calibration.

3.2.2 Submaximal exercise test

In addition to providing individual data required for Actiheart calibration, the submaximal exercise test was conducted to choose the starting speed for the $\dot{V}O_{2max}$ test and also to predict the treadmill running speed corresponding to 70% $\dot{V}O_{2max}$ for the exercise intervention in study 2. The submaximal exercise test was completed on a motorised treadmill (Woodway, PPS55 Med-i, D-79576 Weil am Rhein, Germany) and consisted of 4 x 4 min stages. The stages were completed at speeds of 4.5 km.h^{-1} (stage 1), 5.8 km.h^{-1} (stage 2) and from 7 – 11 km.h^{-1} (stages 3 & 4) (similar to Brage et al. (2005)). The range of running speeds in the final 2 stages were to insure a HR of 150 beats.min^{-1} or rating of perceived exertion (RPE) of 12 (using the 6-20 Borg scale) and further to insure an RPE of 12 was not exceeded during the submaximal exercise test. Each test was completed at a 1% gradient to reflect the outdoor energy cost of running (Jones and Doust, 1996) and

expired gas was continuously sampled throughout the treadmill test by breath-by-breath cardiopulmonary exercise testing system (MetaLyzer 3B, Cortex, Leipzig, Germany). HR and RPE were monitored continuously throughout the exercise test. RPE was collected every min and HR every 10 s. A maximal exercise test was then completed after a 15 min rest period.

Maximal gradient exercise test ($\dot{V}O_{2max}$)

At the start of the $\dot{V}O_{2max}$ test the treadmill was set at a 1% incline and the gradient was increased by 1% every min until volitional exhaustion. The initial speed was set corresponding to a HR of approximately 150 beats.min^{-1} or a RPE of 12 on the submaximal exercise test and remained constant throughout. Expired gas was sampled during the entire test using a breath-by-breath cardiopulmonary exercise testing system (MetaLyzer 3B, Cortex, Leipzig, Germany) and $\dot{V}O_2$ and $\dot{V}CO_2$ values were recorded. HR and respiratory exchange ratio (RER) were collected every 10 s and RPE was collected 15 s before the end of each stage. When $\dot{V}O_{2max}$ was not reached as a plateau or levelling of $\dot{V}O_2$ (l.min^{-1}), two of the following three criteria were used to determine whether a true $\dot{V}O_{2peak}$ was achieved: **1.** RER $\geq$ 1.15, **2.** HR within ± 10 bpm of age-predicted maximum, **3.** RPE $\geq$ 18. $\dot{V}O_{2peak}$ was taken as the maximum 30s rolling average $\dot{V}O_2$ (L.min^{-1}) from the final 4 stages of the protocol.

3.3 Assessment of free-living energy expenditure

On completion of all preliminary measures, participants were fitted with a combined HR/accelerometer (Actiheart, CamNtech, Cambridge, UK) which was fitted on day 0 and removed on day 8 for both study 1 and study 2. The skin was prepared using a wet paper towel and alcohol wipe to remove the top layer of skin (stratum corneum) to reduce noise levels and allow for appropriate R wave signals. Two ECG electrode pads (Bio Protech ECG electrode E5 Tele815) were then placed

on the participant's chest with the medial electrode been attached on the skin at the base of the sternum (positioned below and between V1 and V2 (4^{th} intercostal space, either side of sternum)) and the lateral electrode horizontally to the left side (positioned at V4 (5^{th} intercostal space in line of mid-clavicular) or V5 (5^{th} intercostal space in line with anterior axillary line)). The Actiheart wire was straight but not taut. A signal test was performed to check for appropriate R wave signals before participants were setup for any long-term recordings.

At the point when the monitor was fitted, participants were provided with the following message verbally 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."

Participants in study 1 and 2 wore the Actihearts for 2 x 7-day periods separated by a 7 day washout period. Participants could remove the Actiheart for a maximum of 20 min each day during a shower period. All recordings began at midnight and finished at midnight of the final day. The Actiheart was set to record HR in the 'Advanced Energy Expenditure' recording mode continuously over 15 s epochs (recording resolution). The Actiheart measures activity energy expenditure (AEE) and TEE. Individual calibration was performed by inserting the following values from preliminary testing into the software: RMR, the energy expenditure values (calculated from $\dot{V}O_2$ and $\dot{V}CO_2$) corresponding to the four different exercise intensities (expressed as HR) performed during the submaximal treadmill test, and the $\dot{V}O_{2peak}$. Data was downloaded after each trial using a reader interface unit and analysed using Actiheart software (Version 2.132, Cambridge Neurotechnology Ltd, Cambridge, UK).

3.4 Photographic food diary

Participants completed 2 x 7-day periods with a 7-day washout between, for both studies 1 and 2. Throughout each 7-day condition, participants were provided with a digital camera (Vivitar, ViviCam 46, China) and instructed to photograph all foods and beverages before and after consumption. Briefly, each participant was instructed to take photographs of their food diagonally down (65-75 degrees) before and after their meals, and to include a knife/fork/spoon to the side of the plate/bowl to confirm the size of the plate or bowl. A written food diary was also completed in conjunction with the photographic diary where participants 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 (Appendix A). During the preliminary testing, participants received a tutorial on the written food diaries and digital cameras. Additionally, instructions were provided for participants to accompany the tutorial (Appendix B).

Amounts (g) of each food and beverage consumed were estimated by comparing the digital photographs taken by the participants' with the Young Person's Food Atlas (Foster et al., 2010) (Appendix C). The Young Person's Food Atlas allowed researchers to estimate the food portion size both before and after the participant has eaten. Subsequently, the 7-day food diaries were analysed using Dietplan 6.70 (Forestfield Software, Horsham, UK) to estimate EI ($kcal.day^{-1}$) during each condition (Appendix D).

4.0 Study 1

The reliability of a 7-day food diary combined with digital photography to assess free-living EI

4.1 Introduction

Accurately collecting and measuring free-living dietary intake is abundant with problems (Purnell, 2006). Self-reporting dietary intake relies on the ability of an individual to accurately recall and record all foods consumed (Williamson et al., 2003) whereas 'gold standard' measures through DLW or weighed food diaries require expert training for the experimenters. Furthermore 'gold standard' measures, such as weighed food diaries, are burdensome and time consuming for participants and may impact on free-living EI (Wang et al., 2006). New methods of measuring free-living EI are, therefore, required for the valid and reliable assessment of free-living EI.

A valid and reliable method of measuring free-living food intake, over 3 to 6 days, is through digital photography (Martin et al., 2009a, Martin et al., 2012). The advantages of using digital photography are that it reduces the disruption of participants eating patterns (Ngo et al., 2009) and is more convenient for participants due to rapid data collection (Williamson et al., 2003). However, the reliability of using digital photography combined with descriptive food diaries to assess free-living EI, over 7 days, has not been examined and requires confirmation.

The primary aim of this first study is:

- To assess the reliability of a 7-day photographic food diary (with additional written details) in free-living men

The findings obtained from this study will inform the second study within this thesis (Chapter 5), which will use digital photography combined with descriptive written food diaries to assess the effect of an exercise intervention on free-living EI. Specifically, it will be possible to determine whether any changes in free-living EI

are a meaningful effect of the intervention or could be due to natural variance in EI as assessed using this method.

4.2 Methods

4.2.1 Participants

Thirteen healthy males volunteered to take part in this study. Table 4.1 outlines the participant's characteristics. All participants were provided with information sheets (Appendix E) detailing the nature and purpose of the study. A consent form (Appendix F), Physical Activity Readiness Questionnaire (PAR-Q) (Appendix G) form and pre-test Medical Questionnaire (Appendix H) were read and signed by each participant before any testing commenced.

Table 4.1 Participant's anthropometric and physiological characteristics

Variables	*n* = 13
Age (years)	23 ± 1
Body mass (kg)	82.1 ± 15.1
Height (cm)	176.5 ± 5.9
RMR (joules/kg/min)	60.3 ± 8.1
$\dot{V}O_{2max}$ (ml/kg/min)	44.3 ± 6.9

Values are means ± standard deviation (SD); RMR = resting metabolic rate; $\dot{V}O_{2max}$ = maximal oxygen uptake

4.2.2 Preliminary measures

On arrival (09:00 in a 12 hour fasted state), anthropometric measures and a 10 min RMR measure was collected, as outlined in Chapter 3.2. Participants then consumed a snack, and a submaximal treadmill test and graded treadmill $\dot{V}O_{2max}$

test followed, as described in Chapter 3.2. The Actiheart was calibrated from the values collected and attached to participants for the subsequent main trials (as explained in Chapter 3.3). A tutorial, as detailed in Chapter 3.4, of the photographic and written food diary was provided to all participants before each 7-day period.

4.2.3 Main trial

Participants completed 2 x 7-day trials separated by a 7-day washout period (Figure 4.1). An Actiheart monitor was worn throughout (see Chapter 3.3) and a photographic and written food diary were completed (as detailed in Chapter 3.4). During each 7-day period, participants were under free-living conditions and could participate in any forms of exercise and could consume any foods or fluids.

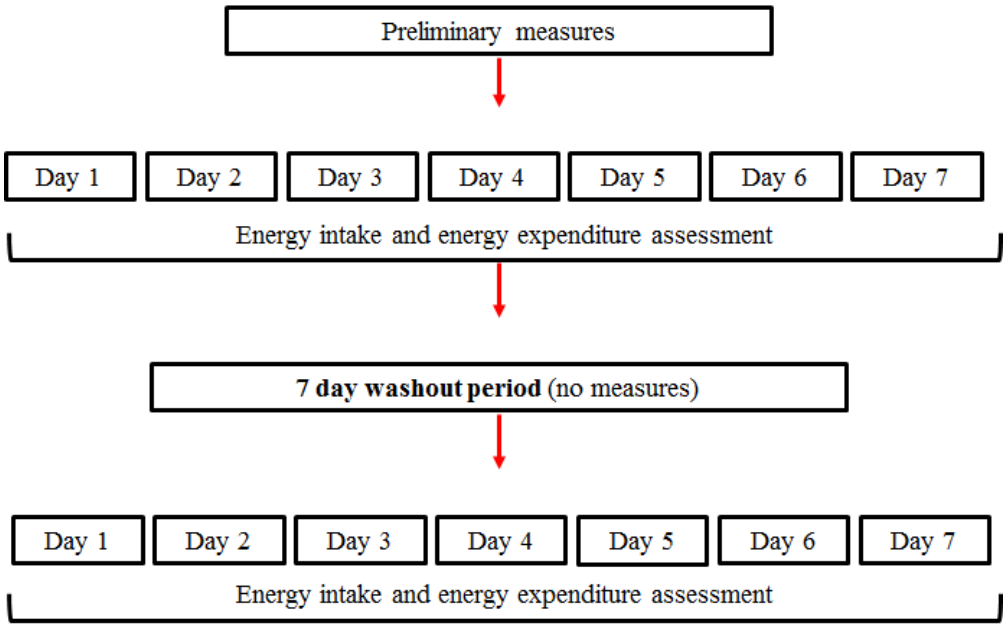


Figure 4.1 Schematic of study 1

Statistical analysis

Data was analysed using IBM SPSS statistics 22 (SPSS Inc., Chicago, USA) and Microsoft Excel (Microsoft, United Kingdom) (to calculate limits of agreement (LoA)). Normality of data for EI and EE were checked using Q-Q plots. EE and EI were deemed non-normally distributed and log transformed before rechecking for normality. Log transformed data was deemed normally distributed and was used for analysis. Raw data is reported in text and tables for a meaningful representation of data. All data is reported as mean ± standard deviation (SD).

Linear mixed models analysed for any differences in daily average (average over the 7-day period for each participant) EE, EI and energy balance between weeks. Day (each individual day of the 7-day periods) and week x day interactions were further assessed using linear mixed models. The model most suitable for the analysis was chosen by the smallest Hurvich and Tsai's criterion (AICC). Normality of residuals using Q-Q plots further confirmed the fit of the model.

For individual-level analyses, LoA were used to compare the daily average EI (individual average over the 7 days) values between week 1 and week 2 and the daily average EE values between week 1 and week 2 (Bland and Altman, 1986). Systematic error (bias) was calculated using the mean difference between weeks 1 and 2 and random error was determined by the standard deviation of the bias (bias ± (1.96 × RE)).

4.3 Results

4.3.1 EI

Daily EI did not differ between week 1 (2236 ± 462 kcal) and week 2 (2380 ± 383 kcal) ($F = 2.505$; $p = 0.116$) or between days ($F = 1.127$; $p = 0.349$) and no week x day interaction was found ($F = 1.763$; $p = 0.110$). A systematic bias ± random error of 143 ± 715 kcal.day^{-1} resulted in a 95% LoA of -1258 to 1545 kcal.day^{-1} between the daily average EI of weeks 1 and 2 (Figure 4.2).

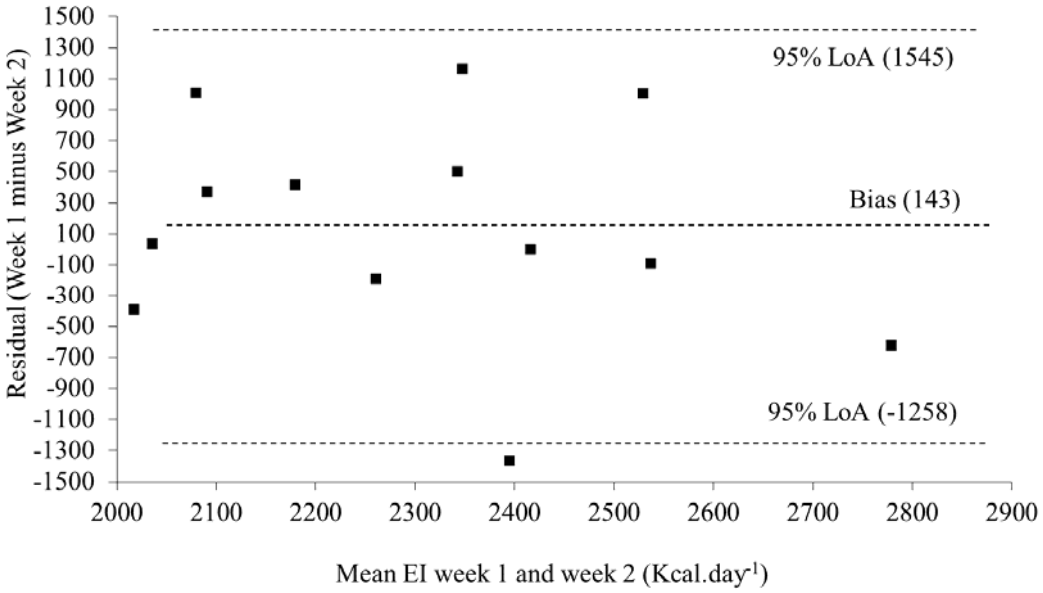


Figure 4.2 Bland-Altman plot of energy intake for week 1 and week 2

4.3.2 EE

Daily TEE revealed no significant effect of week ($F = 0.017$; $p = 0.897$; week 1, 4383 ± 1548 kcal; week 2, 4368 ± 1503 kcal) or day ($F = 0.341$; $p = 0.914$) and no week x day interaction ($F = 0.753$; $p = 0.608$). Systematic bias ± random error of -15 ± 455 kcal.day^{-1} resulted in a 95% LoA of -907 to 876 kcal.day^{-1} for average daily EE between the 2 weeks (Figure 4.3).

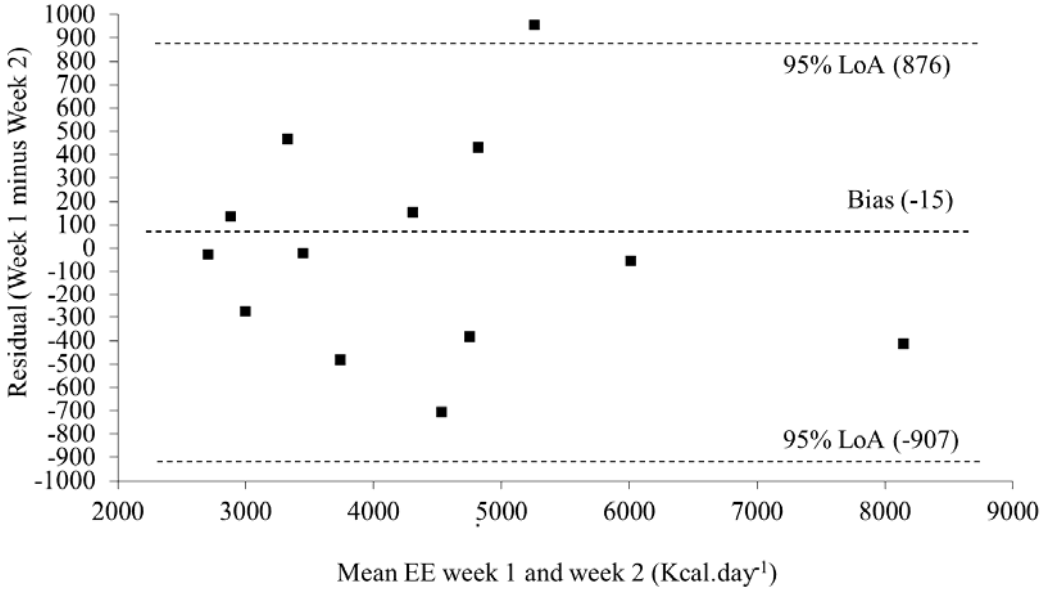


Figure 4.3 Bland-Altman plot of total energy expenditure for week 1 and week 2

4.3.3 *Energy balance*

Energy balance revealed no significant effect of week ($F = 0.174$; $p = 0.677$; week 1, -2148 ± 2051 kcal.day^{-1}; week 2, -2074 ± 1709 kcal.day^{-1}), day ($F = 1.000$; $p = 0.427$) or week x day interaction ($F = 1.670$; $p = 0.132$).

4.4 Discussion

This study investigated the reliability of a photographic food diary, in conjunction with a written diary, on free-living EI over a 7-day period. The primary finding of the present study was that estimated free-living EI produced a systematic bias of 143 kcal.day^{-1} and wide LoA over a 7-day period. Furthermore, EE produced a wide LoA over 7-days, although with a small systematic bias of -15 kcal.day^{-1}. Daily average EI, EE and energy balance, however, did not significantly differ between the 2 weeks when compared at the group level.

These findings are in contrast with previous research which found the photographic food diary a reliable method for measuring free-living EI over 3 (Martin et al., 2009a) and 6 days (Martin et al., 2012). In comparison to the present study, Martin

et al. (2009a) and Martin et al. (2012) evaluated the reliability of the photographic food diary using ICC, thus suggesting that the inconsistent results produced may be due to the types of analysis used. These studies further assessed the validity of the photographic food diary against weighed food records and DLW, respectively, and deemed the photographic food diary a valid method. Although the present study revealed that daily average EI did not significantly alter between the two, 7-day testing periods when compared at the group level, the Bland-Altman plot showed a bias of 143 kcal.day^{-1} and wide LoA of -1258 to 1545 kcal.day^{-1}. This indicates that a large intervention effect would be required to deem whether the impact of an exercise intervention on free-living EI was meaningful and not due normal short-term variation in EI when using this method. Similar results were found for EE, with a small bias of -15 ± 455 kcal.day^{-1}, but wide LoA -907 to 876 kcal.day^{-1}, as determined using combined HR-accelerometry. It may be recommended that a prescribed exercise intervention alters EI to a large magnitude to confirm that the intervention has produced a meaningful effect. Therefore, these results illustrate that caution should be used when using the photographic food diary for measuring changes in free-living EI within an individual.

Energy balance further confirms that at the group level, there were no significant differences noted between the 2 weeks. A negative energy balance however, was observed during both weeks (week 1, -2148 ± 2051 kcal.day^{-1}; week 2, -2074 ± 1709 kcal.day^{-1}), although cannot be confirmed through changes in body fat and body mass as measures were not completed pre to post-weeks. Therefore, it could be speculated that free-living EI may have been underestimated using the photographic diary and or that EE was overestimated by the Actiheart. The photographic food diary, as well as other free-living EI assessments (food diaries) have previously been shown to underestimate free-living EI (Martin et al., 2009a, Martin et al., 2012, Seale and Rumpler, 1997), thus speculating that EI was underestimated in the present study. EE however, as measured by the Actiheart, tends not to show any discrepancies in the assessment of EE (Brage et al., 2005, Villars et al., 2012). The present study further individually calibrated the Actiheart from preliminary data to improve validity of the measure and therefore, the potential overestimation observed in the present study is unclear.

In an attempt to explain the discrepancies in the findings of this current study in comparison to previous literature, the use of prompts needs to be highlighted. Martin et al. (2012) used frequent prompts to ensure participants remembered to photograph all foods, compared to the current study which used no prompts. More frequent prompts had already been observed to produce greater estimations of free-living EI than less frequent prompts with an error of 625 ± 762 $kcal.day^{-1}$ (Martin et al., 2012). Therefore, it may be speculated that more prompts could mean that participants are less likely to forget to photographs all foods and drinks consumed. Nevertheless, neither Martin et al. (2012) or the current study logged the amount of missing images, but used a written food diary in conjunction with the photographic diary in order to record food intake if any photos of foods consumed were missed. The current study, however, used no prompts in comparison to Martin et al. (2012), which could attribute to the wide LoA, although Martin et al. (2012) did not use LoA to determine intra-individual variability, making direct comparisons difficult.

The benefits of using the photographic food diary method is that participant's do not need to estimate portion sizes, which is associated with inaccurate estimations (Beasley et al., 2005). Furthermore, photographing foods consumed is less burdensome and time-consuming than a weighed food diary (Wang et al., 2006). This study, however, did present some limitations. Firstly, the quality of some pictures were poor, making it difficult to identify individual foods for analysis. Although the written food diary identified individual's food consumed, poor picture quality further made it difficult to estimate individual weights of food items. Secondly, prompts were not used within this study and therefore images of some foods were not captured by participants. This resulted in the inability to accurately assess weights of foods and fluids consumed.

In conclusion, the use of a photographic food diary, in conjunction with a written diary, appears to be a convenient tool for the assessment of free-living EI in healthy men. However, this method may not be sensitive enough to detect small intervention effects within an individual, although group level comparisons revealed no difference between two 7-day periods. Caution should, therefore, be

taken when using this method to determine differences in free-living EI between different conditions or examine changes over time, in healthy men.

Future research should further examine the validity of the photographic food diary against a ‘gold standard’, such as DLW. Additionally, the use of prompts should also be investigated on the reliability and validity of the photographic food diary on free-living EI.

5.0 Study 2

Independent effects of 7-days imposed exercise on free-living energy balance and appetite hormones in males

5.1 Introduction

Obesity is a global health issue, with the 2014 Health Survey for England (HSE) classifying 27% of women and 24% of men as obese (Scantlebury and Moody, 2015). Obesity is the result of sustained periods of positive energy balance in which EI exceeds EE. Aerobic exercise has become a common method prescribed to induce weight loss and attenuate weight gain. However, most exercise programmes produce less than expected weight loss (Melanson et al., 2013). Thus, weight management through prescribed exercise may be more complex than expected.

A bout of acute aerobic exercise has largely been shown to have no influence on EI, subsequently resulting in an acute energy deficit (Donnelly et al., 2014). Furthermore, an acute bout of high-intensity exercise (~70% $\dot{V}O_{2max}$) results in a brief decline in hunger, during and 60 min post-exercise (Broom et al., 2009). Appetite regulating hormones, PYY and acylated ghrelin have been identified to suppress and stimulate hunger, respectively (Schubert et al., 2013). Thus, possible mechanisms regulating the suppression of hunger are suggested to be through an elevation in PYY and suppression of acylated ghrelin (Broom et al., 2009). EI however, does not increase, eliciting an energy deficit which lasts up to 22.5 hours post an acute exercise bout (King et al., 2010). This lack of compensation in response to acute exercise is somewhat contradictory to chronic exercise interventions which produce weight loss that is less than expected. Thus, compensation of the exercise induced energy deficit may be observed through a combination of increased EI and decrease NexEE at some point 22.5 hours following the start of an exercise intervention and in response to more than just a single exercise session. Nevertheless, limited acute or long term studies have examined the role of NexEE, in conjunction with EI, in opposing the exercise induced energy deficit.

Literature on chronic (greater than one day) exercise interventions on energy balance is limited. Present research reveals that chronic exercise interventions of 9-14 days in duration result in partial compensate (~30%) for the exercise induced energy deficit through increasing EI and reducing NexEE (Stubbs et al., 2002a, Whybrow et al., 2008). Unfortunately, appetite regulating hormones have not been assessed within these studies, but could provide much-needed information on the possible mechanisms responsible compensatory responses.

Previous investigations have yet to ascertain the chronic effects of imposed exercise on free-living EI and NexEE, in combination with appetite regulating hormones. The assessment of fasting and postprandial appetite regulating hormones may provide a possible mechanism to any compensatory changes in free-living EI specifically. Therefore, the aims of the present study are:

- To examine the effect of 7-days of imposed exercise compared with a no exercise control on daily EI and EE in habitually active men
- To examine the effect of 7-days of imposed exercise compared to a control on perceived appetite and appetite-regulating hormones (acylated ghrelin and PYY) in response to a meal 70 hours post the final exercise bout in habitually active men

5.2 Methods

5.2.1 Participants

Seven healthy physically active males were recruited to complete the study. All participants confirmed verbally that they had sufficient running experience in order to participate within the study (running training of ≥ 3 h/week and could manage 60 min continuous running). Two participants completed one 7-day trial and were unable to complete the remainder of the study due to illness. Five healthy,

physically active males completed the study duration. Participant characteristics are shown in Table 5.1.

Information's sheets were given to participants (Appendix I) detailing the nature and purpose of the study. A consent form (Appendix J), PAR-Q form, Pre-test Medical Questionnaire and a blood screening form (Appendix K) were completed and signed by each participant before any testing commenced. This ensured that all participants had no underlying health concerns or issues that would put them or the experimenter at risk (e.g. blood borne diseases).

Table 5.1 Participant anthropometric and physiological characteristics

Variables	*n* = 5
Age (Years)	23 ± 1
Height (cm)	179 ± 7
RMR (Joules/kg/min)	69.9 ± 14.0
$\dot{V}O_{2max}$ (ml/kg/min)	48.9 ± 4.4

Values are means ± standard deviation (SD); RMR = resting metabolic rate; $\dot{V}O_{2max}$ = maximal oxygen uptake

5.2.2 Preliminary measurements

Participants arrived at the laboratories at 09:00 in a fasted state (no food or drink for at least 12 hours). Anthropometric measures were collected as described in Chapter 3.2. RMR was then measured as detailed in Chapter 3.2. Following a light snack, a submaximal exercise test and $\dot{V}O_{2max}$ test described in 3.2.2 and 3.2.3, respectively, were then completed to calibrate the Actiheart and to calculate the treadmill speed and duration for the participants running exercise intervention.

5.2.3 Study design

Using a randomised repeated measures design, participants completed 2 x 7 day trials (imposed exercise (EX) and no exercise control (N-EX)). The 7 day trials were separated by a 7-day washout period: (Figure 5.1). Every day in the EX condition participants completed a treadmill run at a speed eliciting 70% $\dot{V}O_{2peak}$ to expend 800 kcal, under supervision. The 800 kcal exercise bout was split into 2 x 400 kcal exercise sessions, to ensure participants would compete the full 800 kcal bout each day. Expired air was collected during the initial 10 min and final 5 min of each stage to ensure participants were running at the correct intensity. Analyses of expired air revealed that participants exercised at an intensity of 69% $\dot{V}O_{2peak}$ and ExEE was on average 815 kcal.day^{-1}. A 20 min resting recovery period separated each session. Body mass was collected before and after the completion of the 800 kcal exercise bout. Water was consumed *ad libitum* and was weighed before and after the completion of the 800 kcal bout. These measures were used to calculate fluid loss and participants were then instructed to consume 150% of sweat lost during the exercise bout to aid the participant's return to a euhydrated state for the next day's exercise session. During the N-EX 7 day period, participants were instructed not to complete any exercise session to ensure an exercise thermogenesis of 0 kcal·day^{-1}.

On the day prior to and 70 h after each 7 day trial (i.e. days 0 and 10), perceived appetite and gut hormone responses to a fixed meal were assessed, followed by an *ad libitum* test meal. A photographic food diary of all food and drinks consumed 24 hours preceding the initial testing day (i.e. day 0) was recorded. Participants were instructed to replicate their dietary intakes on the day prior to testing days 0 and 10 for both conditions.

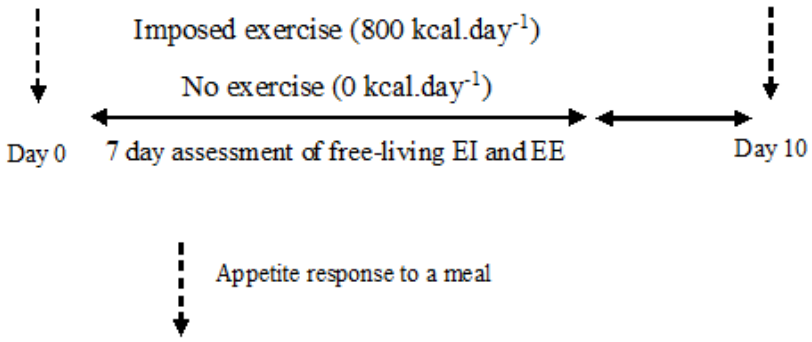


Figure 5.1 Study schematic of the 7-day trials and testing days. Dotted arrows represent the pre- and post- intervention testing days. EI = Energy intake; EE = energy expenditure.

5.2.4 Assessment of free-living energy intake

Throughout each 7-day condition, participants were provided with a digital camera (Vivitar, ViviCam 46, China) and instructed to photograph all foods and beverages consumed, in conjunction with a written food diary (see Chapter 3.4). Food diaries were dated and did not indicate which condition participants were in as to partially blind researchers to allow for a more subjective nature of food diary analysis.

5.2.5 Assessment of free-living energy expenditure

Participants were fitted with a combined heart-rate/accelerometer (Actiheart, CamNtech) in order to accurately record total and physical activity EE for the duration of each 7 day trial, as explained in Chapter 3.3. The Actiheart monitor was fitted on day 0 and removed on day 8 of each trial; further details are provided in Chapter 3.3.

5.2.6 Appetite response to a test meal

On day 0 and day 10 of each condition, participants arrived to the laboratory at 08:30 in the fasted state (at least a 12 hour fast) for the assessment of appetite in relation to a test meal (Figure 5.2). Participants were instructed to consume 500 ml of water at least 1 hour before arriving, to help to ensure participants were in a euhydrated state at the beginning of the trial. Hydration status was not measured via urine osmolality. Body fat percentage was measured immediately on arrival via the Bod pod (Bod pod, 2000A, Surrey, UK), and participants were asked to empty bladder prior to entering the Bod pod. Participants consumed a standard breakfast (consisting of bread, orange juice, milk, cheese, and jam: 8 $kcal.kg^{-1}$ of body mass, 17% protein, 35% fat, 48% carbohydrate) within 15 min. Participants consumed the same amount of food within the same time for all test days. After the 3 hour postprandial period an *ad libitum* buffet meal was consumed (consisting of 500g penne pasta (Tesco, everyday value, UK) with a 500g vegetable sauce (Tesco, everyday value, UK)) in isolation, as to reduce social influences. The pasta was boiled for approximately 12 min, then drained and weighed, and was further cooked with a vegetable pasta sauce for 4 minutes and again weighed. Participants were instructed to dish up their food into a separate bowl and then provided with this statement 'We ask that you continue eating until you have satisfied your hunger' (Betts et al., 2011). The *ad libitum* meal was weighed before and after to determine the quantity (grams) of the *ad libitum* meal consumed. Participants were also timed from their first mouthful to their final mouthful. Participants were not allowed to consume any water from the time of arrival (08:30 am) until completion of the *ad libitum* pasta meal.

Perceptions of hunger, satisfaction, fullness and PFC were assessed using the 100-mm visual analogue scales (VAS) at baseline (fasted) and every 30 min post-meal, for 3 hours. On completion of the *ad libitum* meal, participants completed a final VAS measure.

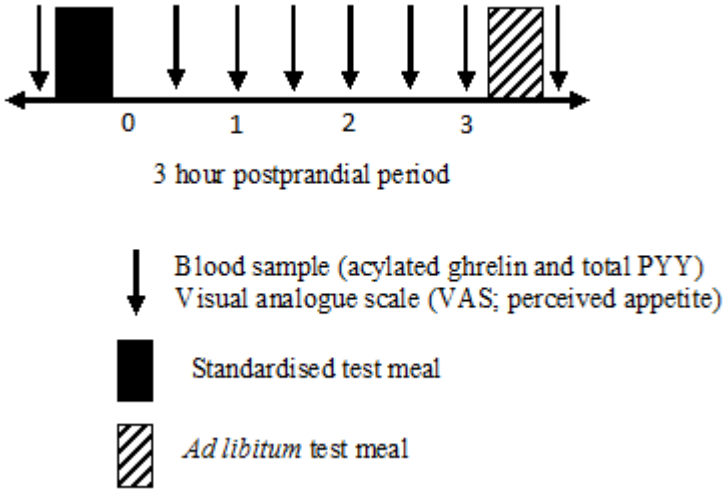


Figure 5.2 Schematic of testing day

5.2.7 Blood sampling

On each test day (days 0 and 10), an iv cannula (B. Braun, Sheffield, UK) was inserted into an antecubital vein after body fat measures were collected. Two fasting baseline blood samples were collected 5 min after the cannula was inserted and before the standardised meal was consumed. On completion of the standardised breakfast meal, 2 blood samples were collected every 30 min for a period of 3 hours.

Blood samples were collected into 2 x 5 ml pre-chilled EDTA tubes (Greiner Bio-One, Stonehouse, UK) for the assessment of acylated ghrelin and PYY. In order to prepare the samples for the assessment of acylated ghrelin, 50 µL of solution containing 10 M sodium hydroxide (NaOH), 0.1 M potassium phosphate buffer (PBS) and 100 mM P-hydroxymercribenzoic acid (PHMB) was added to 1 EDTA tube for the analysis of acylated ghrelin. The EDTA tube then spun for 10 minutes at 1500 *g* in a refrigerated centrifuge (4ºC) (Thermo-Fisher Scientific, Leicestershire, UK). Two ml of plasma supernatant from the acylated ghrelin tube was pipetted into a universal tube containing 200 µL of 1M hydrochloric acid (HCL). The sample then spun for a further 5 minutes at 1500 g at 4°C before storage. Plasma supernatant was aliquoted into 2 separate cryovials of ~1 ml. The

second EDTA tube for PYY analysis spun for 10 minutes at 1500 g in a refrigerated centrifuge (4°C). Two ml of the plasma supernatant was then aliquoted into separate 2 ml cryovials. All samples were stored at -80°C until later analysis. All samples were analysed within 4 months of collection.

5.2.8 Biochemical analysis

Commercially available, pre-standardised, enzyme linked immunosorbant assays (ELISA) were used to analyse acylated ghrelin (Bertin Pharma, France), and total PYY (Millipore, Germany, Darmstadt) concentrations. Good clinical practise was adhered to through following ELISA kit instructions and demonstrating reproducibility through pipette practice. Only one participant had samples measured in duplicate, due to funding constraints, revealing an intra-assay coefficient of variation (CV) of 12%. Completing acylated ghrelin and PYY analysis in singles is not unusual in this field. All standards were completed in duplicate. The within batch inter-assay CV for PYY was 7% and was 5% for acylated ghrelin.

5.2.9 Statistical analysis

IBM SPSS statistics 22 was used to analyse all data (SPSS Inc., Chicago, USA). Normality of data was checked using Q-Q plots.

Linear mixed models analysed any differences in the gut hormone responses between each condition (EX and N-EX), between test days (pre-intervention to post-intervention (day 10)), time (across all time points in each test day) and any interactions. Daily average EE, EI and energy balance was analysed between the two conditions and seven days (i.e., across the 7 day intervention). Quantity of food consumed during the *ad libitum* meal and time to consume this meal, body mass and body fat were analysed between the two conditions and two test days (i.e., pre-

intervention to post-intervention). Area under the curve (tAUC) was calculated using the trapezoidal method for all gut hormones analysed and VAS measures collected across each test day. Bonferroni post-hoc pairwise comparisons were calculated for significant interactions. The model most suitable for the analysis was chosen by the smallest Hurvich and Tsai's criterion (AICC). Normality of residuals using Q-Q plots further confirmed the fit of the model.

Body mass was deemed non-normally distributed and log transformation did not alter normality of data; therefore, Freidman's non-parametric test was used to analyse the raw data. Raw data is reported in text and tables for a meaningful representation of data. All data in text and tables is represented as mean ± SD and as mean ± standard error (SE) in figures. EE during 1 week was missing for 1 participant and therefore that participants EE data was discarded ($n = 4$).

5.3 Results

5.3.1 Body mass and body fat

No significant main effect across the study duration ($\chi^2(3) = 3.490$; $p = 0.322$) was noted for body mass in either condition (Table 5.2). Additionally, there was no significant main effect of condition ($F = 0.035$; $p = 0.856$) or pre- to post-intervention test day ($F = 0.022$; $p = 0.884$) for body fat (Table 5.2).

Table 5.2 Body mass and body fat

	N-EX		EX	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Body mass (kg)	79.28 ± 14.54	79.01 ± 14.51	80.39 ± 15.50	79.51 ± 15.80
Body fat (%)	15.16 ± 2.80	15.86 ± 3.64	16.04 ± 4.37	15.18 ± 4.57

Values are means ± standard deviation (SD)

5.3.2 Energy balance

A significant effect of condition ($F = 28.405$; $p < 0.0005$; EX -2828 ± 1236 kcal.day^{-1}; N-EX -993 ± 1420 kcal.day^{-1}) for energy balance was noted. No significant effect of day ($F = 0.965$; $p = 0.462$) or condition x day interaction ($F = 1.527$; $p = 0.195$) occurred.

5.3.3 Free-living EI

There was a significant effect of condition ($F = 8.595$; $p = 0.005$; EX, 2471 ± 346 kcal.day^{-1}; N-EX, 1959 ± 289 kcal.day^{-1}) for free-living EI. No significant effect of day ($F = 0.195$; $p = 0.977$) or interaction of condition x day ($F = 0.271$; $p = 0.948$) occurred. Figure 5.3 shows individual daily average EI between the N-EX condition and EX condition.

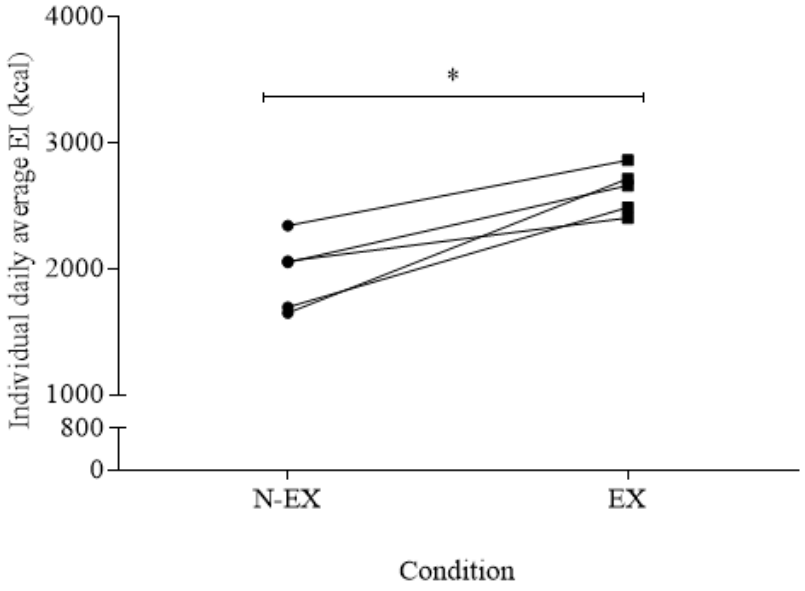


Figure 5.3 Individual daily average energy intake (EI) in the N-EX and EX conditions. A significantly higher EI was found in EX compared with N-EX. * = Significant difference between conditions.

5.3.4 EE

There was a significant main effect of condition on TEE ($F = 216.963$; $p < 0.0005$; EX, 5250 ± 994 kcal.day^{-1}; N-EX, 3450 ± 954 kcal.day^{-1}), with no main effect of day ($F = 1.575$; $p = 0.183$). There was a condition x day interaction found ($F = 2.748$; $p = 0.027$) with post-hoc comparisons revealing TEE, during the exercise condition, to be lower ($p = 0.034$) on day 7 (4540 ± 1180 kcal) than day 1 (5608 ± 444 kcal).

A significant main effect of condition ($F = 282.185$; $p < 0.0005$; EX, 2681 ± 392 kcal.day^{-1}; N-EX, 1103 ± 456 kcal.day^{-1}) was also observed for AEE, but no significant main effect of day ($F = 1.857$; $p = 0.113$). A significant condition x day interaction was also noted ($F = 2.923$; $p = 0.019$). Further inspection through post-hoc comparisons revealed a significantly greater AEE on day 1 (3002 ± 205 kcal), 2 (2878 ± 727 kcal) and 3 (2866 ± 522 kcal) compared to day 7 (2041 ± 570 kcal) in the exercise condition (all $p \leq 0.023$).

Furthermore, physical activity EE outside of prescribed exercise (PAEE; AEE minus imposed exercise) revealed a significant main effect of condition ($F = 38.574$; $p = 0.0005$) but no significant main effect of day ($F = 1.809$; $p = 0.125$). A significant condition x day interaction occurred ($F = 2.545$; $p = 0.037$) with post-hoc comparisons showing a significantly lower PAEE on day 7 (1226 ± 570 kcal) compared to day 1 (2187 ± 205 kcal) in the EX condition.

5.3.5 Ad libitum meal

No main effect of condition ($F = 0.090$; $p = 0.770$) or test day ($F = 3.485$; $p = 0.087$) was observed for the total quantity of food consumed during the *ad libitum* meal (Table 5.3). Time to consume the *ad libitum* meal showed no significant main effect of condition ($F = 0.112$; $p = 0.744$) or test day ($F = 0.522$; $p = 0.484$) (Table 5.3).

Table 5.3 Quantity consumed and time to consume *ad libitum* meal

	N-EX condition		EX condition	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Total quantity consumed (g)	569.50 ± 168.61	711.20 ± 211.36	602.50 ± 202.91	648.14 ± 219.12
Time to consumption (s)	499 ± 190	548 ± 175	490 ± 156	521 ± 232

Values are means ± standard deviation (SD); g = grams; s = seconds

5.3.6 Gut hormone concentrations

The resting concentrations and gut hormone responses in the test days pre and post the 7-day intervention period (N-EX and EX) are reported in Table 5.4. Fasting acylated ghrelin and total PYY concentrations did not significantly differ between conditions (all $p \geq 0.879$) or reveal a main effect of pre- to post- intervention test day (all $p \geq 0.164$).

Table 5.4 Pre and post-intervention gut hormone concentrations for fasting concentrations and tAUC

	N-EX condition		EX condition	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Fasting total PYY ($pg.ml^{-1}$)	122.3 ± 60.8	71.1 ± 45.8	104.2 ± 48.1	95.7 ± 53.3
Total PYY tAUC	23213 ± 5887	23574 ± 5488	21389 ± 4829	24737 ± 5228
Fasting acylated ghrelin ($pg.ml^{-1}$)	47.0 ± 24.5	58.2 ± 32.6	57.4 ± 21.0	50.6 ± 24.5
Acylated ghrelin tAUC	6786 ± 4007	9457 ± 5455	8230 ± 4514	6227 ± 3564

Values are means ± standard deviation (SD); tAUC, total area under the curve

No significant main effect of condition ($F = 2.103$; $p = 0.150$) or test day ($F = 0.297$; $p = 0.587$) was observed for acylated ghrelin concentrations (Figure 5.4). A significant main effect of time was observed ($F = 6.419$; $p = 0.0005$) between baseline (53.3 ± 24.3 pg.ml^{-1}) 30 min (31.0 ± 18.4 pg.ml^{-1}) and 60 min (31.9 ± 20.8 pg.ml^{-1}) postprandial ($p \leq 0.009$), and also between 180 min (58.4 ± 36.8 pg.ml^{-1}) and 30, 60, 90, 120 min (31.0 ± 18.4, 31.9 ± 20.8, 36.0 ± 20.9, 39.5 ± 27.8 pg.ml^{-1}, respectively) postprandial ($p \leq 0.028$) following post hoc analysis. There were no significant main effects of condition ($F = 0.551$; $p = 0.472$) or test day ($F = 0.077$; $p = 0.786$) for acylated ghrelin when analysed as tAUC (Table 5.4).

Total PYY concentrations did not show any significant main effects of condition ($F = 0.409$; $p = 0.524$), test day ($F = 1.217$; $p = 0.273$) or time ($F = 1.759$; $p = 0.115$) (Figure 5.4). In addition, when tAUC for PYY was analysed no significant condition ($F = 0.021$; $p = 0.888$) or test day ($F = 0.656$; $p = 0.434$) effect was observed (Table 5.4).

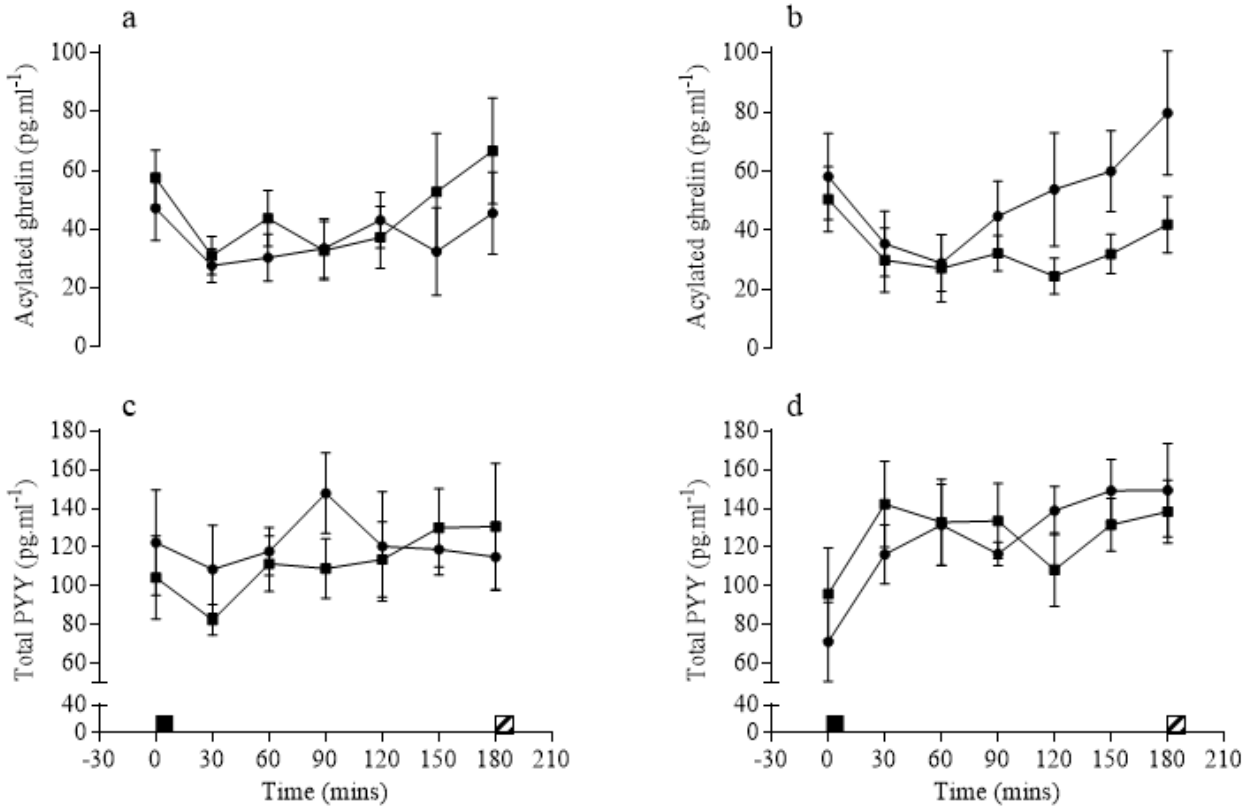


Figure 5.4 Pre-intervention acylated ghrelin (a), post-intervention acylated ghrelin (b), pre-intervention total PYY (c) and post-intervention total PYY (d) responses to N-EX (●) and EX (■) conditions. Black square indicates standardised breakfast, diagonally shaded rectangle indicates *ad libitum* pasta meal.

5.3.7 VAS response

A significant main effect of time (all $p \leq 0.0005$) was observed for each appetite perception (hunger, fullness, satisfaction, PFC), indicating a response to the test meals over time (see Figure 5.5). Hunger revealed a significant main effect of day ($F = 6.183$; $p = 0.014$; pre-intervention, 45 ± 13 mm; post-intervention, 51 ± 15 mm); nevertheless, no condition x day interaction occurred ($F = 1.180$; $p = 0.280$). There were no significant main effects of condition (all $p \geq 0.379$) or day (all $p \geq 0.212$) for hunger, fullness, satisfaction or PFC tAUC.

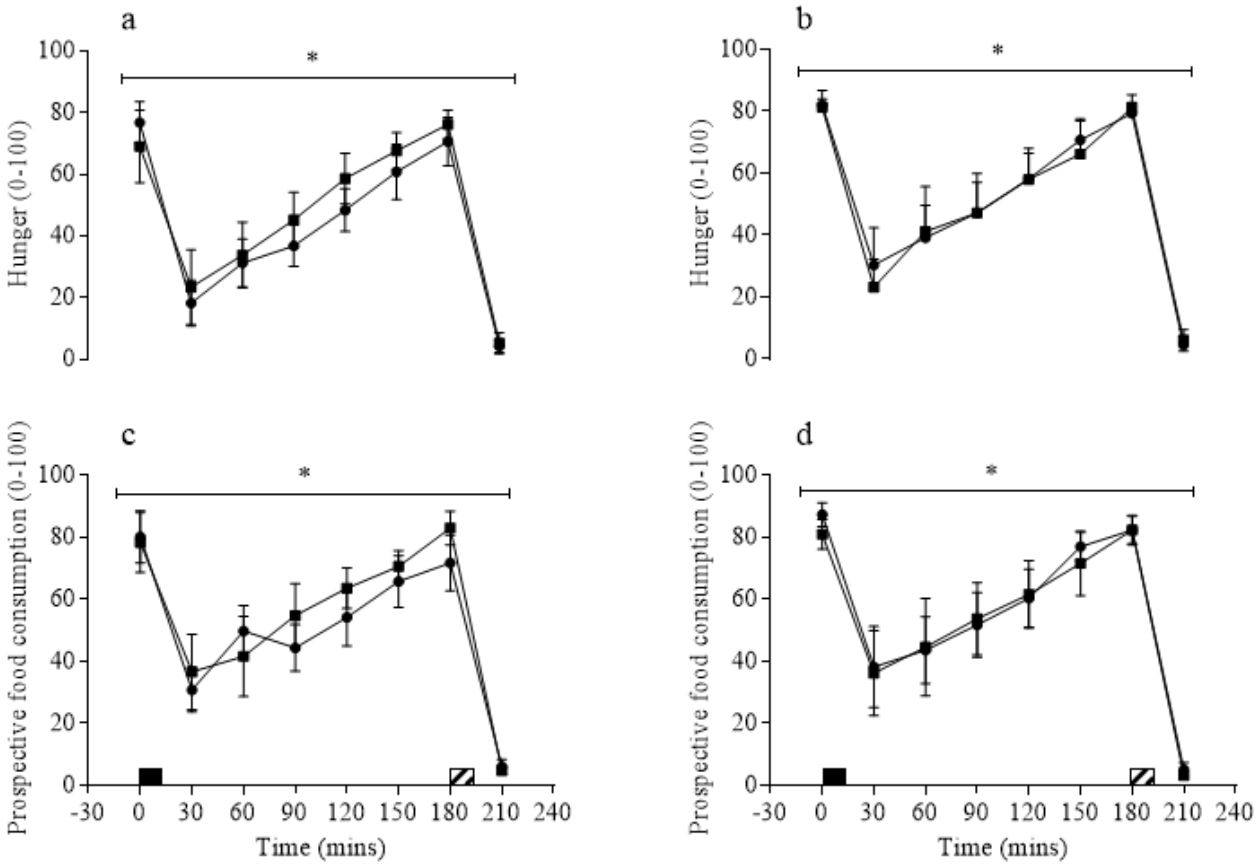


Figure 5.5 Appetite perceptions of pre-intervention hunger (a), post-intervention hunger (b), pre-intervention prospective food consumption (c), post-intervention prospective food consumption (d), pre-intervention fullness (e), post-intervention fullness (f), pre-intervention satisfaction (g) and post-intervention satisfaction (h) in N-EX condition (●) and EX condition (■). Black square indicates standardised breakfast, diagonally shaded rectangle indicates *ad libitum* pasta meal. * = significant effect of time.

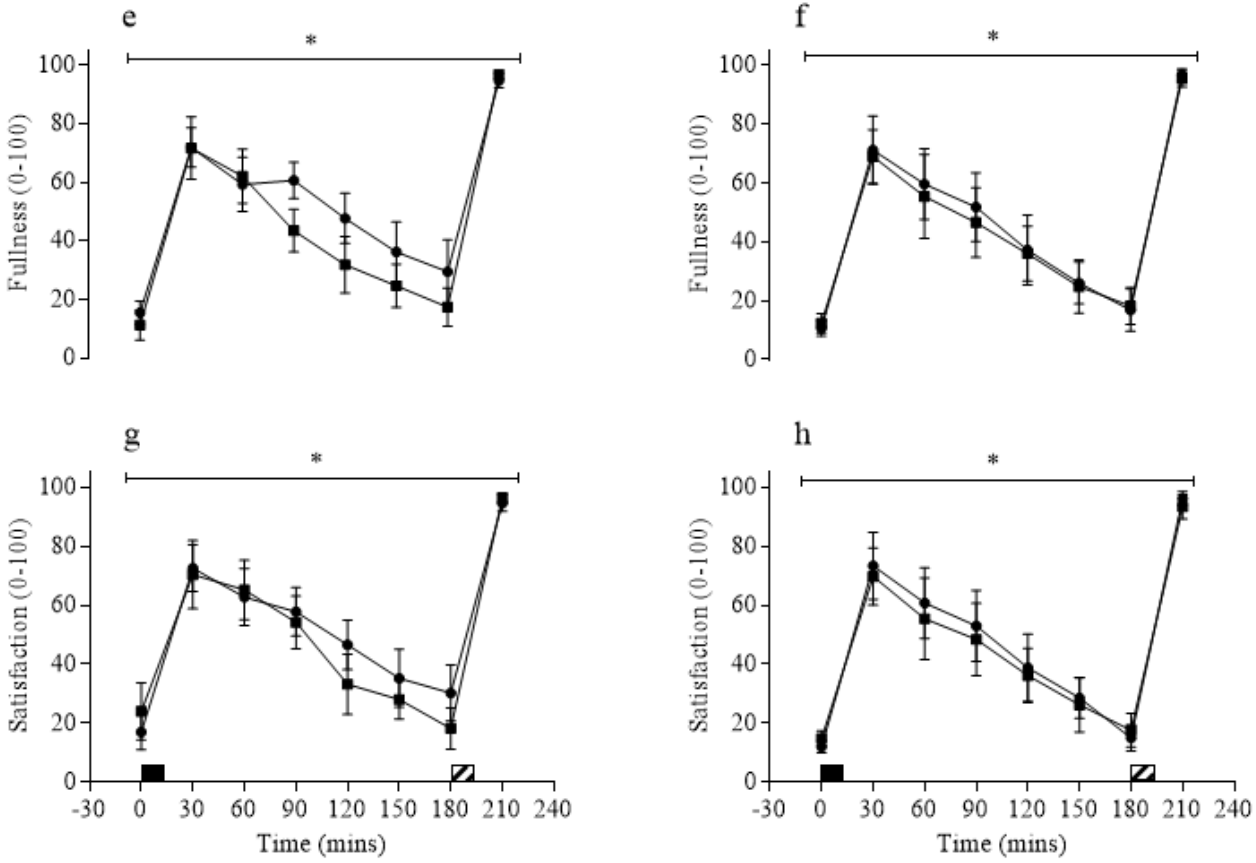


Figure 5.6 Continued.

5.4 Discussion

The primary purpose of this investigation was to examine the effect of 7 days of imposed aerobic exercise on energy balance. Importantly, this novel investigation is the first to examine the accumulative effects of 7 days of imposed exercise on acylated ghrelin, total PYY and appetite perceptions in response to a meal (70 hours post the final imposed aerobic exercise bout). The primary finding was that free-living EI was significantly greater in the EX condition when compared with the N-EX control condition, as was energy balance, TEE and PAEE. Secondly, the acylated ghrelin, total PYY and perceived appetite response to a meal measured 70 hours post the final exercise bout was unaffected by either intervention.

Free-living EI increased by 27% (~500 kcal) when comparing the N-EX condition to the EX condition, which is a partial compensation of ~65% of the prescribed exercise-induced EE (815 kcal.day^{-1}). This is in accordance with previous research, which on average saw a 30% compensation of the exercise induced EE over 7-14 days (Stubbs et al., 2002a, Whybrow et al., 2008). Furthermore, the ~500 kcal increase in EI in the present study is greater than that seen in 50% of short term studies (~200-335 kcal.day^{-1}) over 2-14 days, in response to prescribed exercise (Donnelly et al., 2014). The partial compensation observed in the current and previous studies could be attributed to the macronutrient content ingested, specifically, consumption of high fat, energy dense foods. Tremblay et al. (1994) reported that consumption of high fat foods following an acute bout of exercise (60 min) significantly increased EI to restore energy balance whereas low fat or mixed diets did not increase EI to restore energy balance. Moreover, chronic exercise interventions also reveal a significant increase in fat intake in combination with a partial increase in EI in women (Stubbs et al., 2002a) and men (Whybrow et al., 2008). Although an increase in carbohydrate and carbohydrate and protein (Stubbs et al., 2002a, Whybrow et al., 2008), respectively, was reported, fat has a higher density (fat, 9 kcal.g^{-1}) than carbohydrate and protein (carbohydrate and protein, 4 kcal.g^{-1}) (Smith et al., 2000), thus the influence of fat on EI is greater than carbohydrate and protein. Nevertheless, the current study did not examine macronutrient content and therefore the increase in EI can only be speculated to be

through a greater fat intake. Furthermore, caution should be taken when interpreting these findings on EI as the photographic food diary produced wide LoA (-1258 to 1545 kcal.day^{-1}) in study 1 (Chapter 4). These findings suggest that a large intra-individual variability exists in this measure of EI. The difference in EI reported in study two (518 kcal) across both conditions (N-EX and EX) lay inside of the LoA, but, more encouragingly, are greater than the systematic bias (143 ± 715 kcal.day^{-1}) reported in study 1. This suggests that it may not be possible to directly attribute the higher free-living EI to the exercise intervention and that the possibility of short-term variation in the assessment of EI contributing to the effect should not be discounted. However, more encouraging, examination of individual data showed that all of the participants demonstrated a higher EI during EX compared with N-EX (Figure 5.3).

Compensatory responses to restore energy balance have further been observed through a reduction in NexEE (Stubbs et al., 2002a, Stubbs et al., 2002b). Prescribed exercise interventions, however, tend not to influence NexEE, apart from elderly populations (Westerterp, 1998). In agreement, Whybrow et al. (2008) reported no significant effect of prescribed ExEE on NexEE. Thus, the reduction in NexEE reported in Stubbs et al. (2002a) and Stubbs et al. (2002b) may be due to fatigue, rather than a compensatory mechanism to restore energy balance. The present study however found contrasting results, as observed by a higher TEE and PAEE in the EX compared with N-EX condition; even when accounting for the ExEE, PAEE was still 763 kcal.day^{-1} higher in EX. The increase in PAEE may be due to an increase in motivation to engage in further activities, produced by the imposed exercise training (Thompson and Blanton, 1987). Thompson and Blanton (1987) proposed that this response could be attributed to an increase in sympathetic arousal. Nevertheless, further inspection of the EX condition revealed a tendency for PAEE to decrease throughout the 7-day period of the present study. On day 7, PAEE was significantly lower in comparison to day 1, suggesting that the imposed exercise intervention began to affect PAEE during the final days of the 7-day intervention. This result may further be attributed as compensatory mechanism to restore energy balance or through fatigue; although the present study did not measure fatigue. Extending the imposed exercise intervention over more than 7-

days may lead to further reductions in PAEE. Nevertheless, an increase in PAEE from the N-EX to EX condition is a novel finding as the majority of literature reports no significant alterations (Westerterp, 1998, Whybrow et al., 2008).

Investigation of energy balance is imperative in the assessment of weight management. The present study revealed a greater energy deficit (negative energy balance; EX -2828 ± 1236 kcal.day^{-1}; N-EX -993 ± 1420 kcal.day^{-1}) produced in the EX condition compared to the N-EX condition, further confirming the resulted partial compensation seen through EI. Nevertheless, in comparison to previous research (Stubbs et al., 2002b, Whybrow et al., 2008), body mass and body fat did not significantly alter following 7-days imposed exercise, which is less than expected. Thus, the discrepancies in results could be due to either an overestimation in EE through the combined HR-accelerometer (Actiheart) and or an underestimation of free-living EI through the photographic food diary. Although the validity of the combined photographic written food diary was not directly measured in the present study, a similar photographic food diary has previously been shown to underestimate free-living EI when measured against DLW (Martin et al., 2009a, Martin et al., 2012), suggesting the current study may have potentially underestimated free-living EI. The underestimation of free-living EI is also common in other measures of EI, such as weighed food diaries (Seale and Rumpler, 1997). Assessment of EE however, as measured by the Actiheart, has been shown not overestimate EE when assessed against indirect calorimetry and DLW (Brage et al., 2005, Villars et al., 2012). Furthermore, the present study individually calibrated Actihearts with preliminary measured data as to increase the validity of EE measurements. Therefore, it is unclear why EE appeared to be overestimated in the present study.

Acute exercise bouts (~70% $\dot{V}O_{2max}$) have been shown to cause a transient (during and up to 60 min post-exercise) decline in hunger ratings (Broom et al., 2009). The potential mechanisms for the hunger rating decline has been suggested to be through a suppression and elevation of the gut hormones acylated ghrelin and total PYY, respectively (Broom et al., 2009, King et al., 2010). Despite these reports, longer duration exercise interventions (of 4-15 days in duration) often report no

significant change in appetite perception and fasting or postprandial gut hormone (acylated ghrelin and PYY) responses when compared to resting control trials (Hagobian et al., 2009, Kanaley et al., 2014). Our findings are in similarity with studies examining chronic exercise interventions on ratings of appetite. The high intensity (~69% $\dot{V}O_{2peak}$) chronic exercise (7 days) intervention revealed no changes in ratings of appetite in response to the imposed exercise or control condition. Thus, the findings of the present study are consistent with previous chronic exercise interventions, as further confirmed by no change in acylated ghrelin and total PYY concentrations between conditions.

The novelty of the design of this current study was the examination of appetite perception and gut hormone (acylated ghrelin and PYY) responses following a period of imposed exercise while allowing for an appropriate recovery period (70 hours) between the final exercise session and the test day. Thus, the potential confounding effects of the final exercise session were negated and dietary intakes were controlled prior to all appetite assessments. Studies similar in duration to this study (i.e. exposing an exercise training period of ~7 days) have examined the acute effects of exercise on gut hormone concentrations and appetite perceptions in response to a meal ≤ 24 hours post the final exercise bout (Gueugnon et al., 2012, Hagobian et al., 2009, Jones et al., 2009). Moreover, studies investigating the chronic effects of exercise on gut hormones and appetite perceptions in response to a meal (24-48 hours post the final exercise bout) have been longer term interventions (12 weeks) (Martins et al., 2010) or only examined total PYY, and not in conjunction with acylated ghrelin (Kanaley et al., 2014). Thus, the present study is novel in that it examined total PYY and acylated ghrelin simultaneously, 70 hours post the final exercise bout, following a 7 day exercise intervention. Moreover, the present study included a control condition, unlike previous research (Kanaley et al., 2014, Martins et al., 2010). Martins et al. (2010) reported a significantly increased fasting acylated ghrelin, but not PYY, 48 hours post the final exercise bout, in addition to a significantly reduced body fat and body weight following the 12 week intervention. The duration of the intervention, however, was greater than the present study and further placed restraints on participant's food intake, potentially contributing to the observed differences in results. Nevertheless, as expected,

acylated ghrelin significantly increased before the *ad libitum* meal with a fasting acylated ghrelin significantly greater up to 60 min post the standardised breakfast meal, in the present study. A shorter exercise intervention (15 days) found similar results on fasting and postprandial total PYY (24-36 hours post the final exercise bout), although no change in body fat and body weight was noted (Kanaley et al., 2014). The unaltered total PYY concentrations found during fasting and postprandially in the present study and fasting in previous studies (Kanaley et al., 2014, Martins et al., 2010) could be due to the assessment of total PYY, instead of its more active form PYY_{3-36} (Zwirska-Korczala et al., 2007). Furthermore, Zwirska-Korczala et al. (2007) stated that PYY_{3-36} is the predominant form of total PYY in postprandial circulation, thus suggesting that PYY_{3-36} may be more sensitive to the effects of the imposed exercise intervention. The findings reported here suggest that extending the investigation duration of gut hormones response to a meal 70 hours after the final exercise bout has no added benefit on acylated ghrelin and total PYY concentrations, thus any effects of exercise on these gut hormones appear to be acute and there is no independent effect of exercise training performed over seven days on appetite control.

Several limitations were evident in the present study. Firstly, the power to find any significant relationships within the study was limited, due to a small sample size. Additionally, due to the small sample size, any significant relationships found may just be indicating a tendency of significance. An increase in sample size would allow for a more appropriate indication of any significant relationships. The limited number of participants recruited were primarily due to the duration of the study and further time constraints of the MSc by Research. Secondly, the photographic food diary used to assess free-living EI was previously shown (study 1) to produce wide LoA, concluding that any effect of EI should be interpreted with caution. The present study may have produced smaller LoA through greater participant commitment and thus better reliability however, due to the differing conditions, LoA could not be analysed. Further, incorporation of standardised cutlery and plates may allow for a more accurate analyses of plate weight and plate waste. Thirdly, gut hormones response to a meal was only assessed 70 hours post the final exercise bout. An assessment 24-48 hours post the final exercise bout, or during the

intervention, may reveal an effect of the exercise intervention. An extended intervention duration may have shown energy balance to be restored or a greater compensatory increase in EI. Conversely, reducing the intervention duration to < 7 days would allow for the inclusion of another condition (i.e. rest (no physical activity), control (habitual physical activity), exercise (habitual physical activity)). Finally, assessment of individual's normal physical activity levels during both the N-EX and EX condition would reveal whether the imposed exercise altered individual's normal activity levels. Additionally, inclusion of a 7 day control condition (normal physical activity levels), as well as the EX condition, would allow for an independent assessment of the imposed exercise.

In conclusion, high intensity exercise over 7 days produces a partial compensation of EI. However, this partial compensation should be interpreted with caution due to the individual variability in free-living EI measures. PAEE remained increased during the EX condition, suggesting no compensation through EE for the exercise induced energy deficit; although tended to decline throughout the 7-day period. Additionally, fasting and postprandial acylated ghrelin and total PYY concentrations, and the perceived appetite response to a meal, were not affected when assessed 70 hours post the final exercise bout.

6.0 Conclusions

The main conclusions from this thesis are:

- The photographic method for measuring free-living EI produces wide LoA, indicating that further research would be valuable in establishing and improving the reliability of free-living EI assessment using this method.
- Prescribing high intensity exercise bouts (~69% $\dot{V}O_{2peak}$; ExEE, 815 kcal.day^{-1}) for 7 days produces a partial compensation of the exercise induced EE through EI.

- Fasting and postprandial acylated ghrelin and total PYY concentration do not alter following 7 days imposed exercise, as assessed 70 hours following the final exercise bout.
- Furthermore, ratings of appetite in response to a meal do not alter significantly in response to 7 days imposed exercise.

7.0 Recommendations for future research

To extend the findings of the present study to further understand the effects of 7-days imposed exercise on energy balance and appetite-regulating hormones, future research is warranted. Firstly, further assessment of a more reliable and valid method for the assessment of free-living EI is required, to establish any significant exercise intervention effects.

Further research examining a larger sample size is also required to further understand the mechanisms involved in the partial compensation of EI found in the present study. This study investigated the chronic effects of exercise (70 hours post final exercise bout) revealing the independent effects of the exercise intervention. Further assessment of the acute effects (≤ 24 hours post the final exercise bout) of exercise on appetite regulating hormones further needs to be investigated in conjunction with the chronic effects. This would be valuable in establishing the acute and chronic effects of an imposed exercise intervention. Furthermore, investigating the more active form of PYY, PYY_{3-36}, may result in an alteration and would allow for further insight into the mechanisms of the exercise intervention. Additionally, only males were investigated in the current study; thus, further research is warranted on the differences between males and females, as females and males have both been shown to compensate for an exercise induced EE (Stubbs et al., 2002a, Whybrow et al., 2008).

Assessment of exercise interventions greater than 7-days on free-living EI and PAEE is also required. The findings of the present study displayed signs of restoring

energy balance through EI and EE. It is possible that continuation of the imposed exercise intervention (> 7-days) may have resulted in a restoration of energy balance, but additional research is required to investigate this speculation. The daily exercise bouts and mode of exercise (treadmill) used within the present study may be impractical for certain individuals (i.e. obese/overweight, elderly) when completing ≥ 7-days imposed exercise. Thus, investigating different modes of exercise (rowing, resistance, cycling) to create an exercise induced energy deficit on energy balance would allow a greater understanding on the role of different exercise modes and allow for investigation into other populations (i.e. obese/overweight, elderly). Furthermore, extending the daily exercise bouts to greater than 7-days may result in fatigue within participants. Therefore, research on the different doses of imposed exercise is further required for investigation (i.e. 5 days imposed exercise a week). This will allow for further investigation into other populations (obese/overweight) and further inform health care professionals.

References

Andrews, Z. B. (2011). Central Mechanisms Involved in the Orexigenic Actions of Ghrelin. *Peptides,* 32**,** 2248-2255.

Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., Le Roux, C. W., Thomas, E. L., Bell, J. D. & Withers, D. J. (2006). Critical Role for Peptide Yy in Protein-Mediated Satiation and Body-Weight Regulation. *Cell metabolism,* 4**,** 223-233.

Beasley, J., Riley, W. T. & Jean-Mary, J. (2005). Accuracy of a Pda-Based Dietary Assessment Program. *Nutrition,* 21**,** 672-677.

Betts, J. A., Thompson, D., Richardson, J. D., Chowdhury, E. A., Jeans, M., Holman, G. D. & Tsintzas, K. (2011). Bath Breakfast Project (Bbp)-Examining the Role of Extended Daily Fasting in Human Energy Balance and Associated Health Outcomes: Study Protocol for a Randomised Controlled Trial [Isrctn31521726]. *Trials,* 12**,** 172.

Bland, J. M. & Altman, D. (1986). Statistical Methods for Assessing Agreement between Two Methods of Clinical Measurement. *The lancet,* 327**,** 307-310.

Brage, S., Brage, N., Franks, P., Ekelund, U. & Wareham, N. (2005). Reliability and Validity of the Combined Heart Rate and Movement Sensor Actiheart. *European journal of clinical nutrition,* 59**,** 561-570.

Broom, D. R., Batterham, R. L., King, J. A. & Stensel, D. J. (2009). Influence of Resistance and Aerobic Exercise on Hunger, Circulating Levels of Acylated Ghrelin, and Peptide Yy in Healthy Males. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 296**,** R29-R35.

Broom, D. R., Stensel, D. J., Bishop, N. C., Burns, S. F. & Miyashita, M. (2007). Exercise-Induced Suppression of Acylated Ghrelin in Humans. *Journal of Applied Physiology,* 102**,** 2165-2171.

De Jonge, L., Delany, J. P., Nguyen, T., Howard, J., Hadley, E. C., Redman, L. M. & Ravussin, E. (2007). Validation Study of Energy Expenditure and Intake During Calorie Restriction Using Doubly Labeled Water and Changes in Body Composition. *The American journal of clinical nutrition,* 85**,** 73-79.

Deighton, K., Barry, R., Connon, C. E. & Stensel, D. J. (2013). Appetite, Gut Hormone and Energy Intake Responses to Low Volume Sprint Interval and Traditional Endurance Exercise. *European journal of applied physiology,* 113**,** 1147-1156.

Delporte, C. (2013). Structure and Physiological Actions of Ghrelin. *Scientifica,* 2013.

Donnelly, J. E., Herrmann, S. D., Lambourne, K., Szabo, A. N., Honas, J. J. & Washburn, R. A. (2014). Does Increased Exercise or Physical Activity Alter Ad-Libitum Daily Energy Intake or Macronutrient Composition in Healthy Adults? A Systematic Review. *PloS one,* 9**,** e83498.

Erdmann, J., Tahbaz, R., Lippl, F., Wagenpfeil, S. & Schusdziarra, V. (2007). Plasma Ghrelin Levels During Exercise—Effects of Intensity and Duration. *Regulatory peptides,* 143**,** 127-135.

Gahete, M. D., Rincón-Fernández, D., Villa-Osaba, A., Hormaechea-Agulla, D., Ibáñez-Costa, A., Martínez-Fuentes, A. J., Gracia-Navarro, F., Castaño, J. P. & Luque, R. M. (2014). Ghrelin Gene Products, Receptors, and Goat Enzyme: Biological and Pathophysiological Insight. *Journal of Endocrinology,* 220**,** R1-R24.

Goran, M. I. & Poehlman, E. T. (1992). Total Energy Expenditure and Energy Requirements in Healthy Elderly Persons. *Metabolism,* 41**,** 744-753.

Guan, X.-M., Yu, H., Palyha, O. C., Mckee, K. K., Feighner, S. D., Sirinathsinghji, D. J., Smith, R. G., Van Der Ploeg, L. H. & Howard, A. D. (1997). Distribution of Mrna Encoding the Growth Hormone Secretagogue Receptor in Brain and Peripheral Tissues. *Molecular Brain Research,* 48**,** 23-29.

Gueugnon, C., Mougin, F., Nguyen, N. U., Bouhaddi, M., Nicolet-Guénat, M. & Dumoulin, G. (2012). Ghrelin and Pyy Levels in Adolescents with Severe Obesity: Effects of Weight Loss Induced by Long-Term Exercise Training and Modified Food Habits. *European journal of applied physiology,* 112**,** 1797-1805.

Hagobian, T. A., Sharoff, C. G., Stephens, B. R., Wade, G. N., Silva, J. E., Chipkin, S. R. & Braun, B. (2009). Effects of Exercise on Energy-Regulating Hormones and Appetite in Men and Women. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 296**,** R233-R242.

Hagobian, T. A., Yamashiro, M., Hinkel-Lipsker, J., Streder, K., Evero, N. & Hackney, T. (2012). Effects of Acute Exercise on Appetite Hormones and Ad Libitum Energy Intake in Men and Women. *Applied Physiology, Nutrition, and Metabolism,* 38**,** 66-72.

Hall, K. D., Heymsfield, S. B., Kemnitz, J. W., Klein, S., Schoeller, D. A. & Speakman, J. R. (2012). Energy Balance and Its Components: Implications for Body Weight Regulation. *The American journal of clinical nutrition,* 95**,** 989-994.

Heden, T. D., Liu, Y., Park, Y., Dellsperger, K. C. & Kanaley, J. A. (2013). Acute Aerobic Exercise Differentially Alters Acylated Ghrelin and Perceived Fullness in Normal-Weight and Obese Individuals. *Journal of Applied Physiology,* 115**,** 680-687.

Higgins, J., Lasalle, A., Zhaoxing, P., Kasten, M., Bing, K., Ridzon, S. & Witten, T. (2009). Validation of Photographic Food Records in Children: Are Pictures Really Worth a Thousand Words&Quest. *European journal of clinical nutrition,* 63**,** 1025-1033.

Higgs, S. (2015). Social Norms and Their Influence on Eating Behaviours. *Appetite,* 86**,** 38-44.

Higgs, S. & Thomas, J. (2016). Social Influences on Eating. *Current Opinion in Behavioral Sciences,* 9**,** 1-6.

Hopkins, M., Stensel, D., King, N. & Blundell, J. (2014). The Bases Expert Statement on the Effects of Aerobic Exercise on Appetite Control and Energy Intake.

Howe, S. M., Hand, T. M. & Manore, M. M. (2014). Exercise-Trained Men and Women: Role of Exercise and Diet on Appetite and Energy Intake. *Nutrients,* 6**,** 4935-4960.

Imbeault, P., Saint-Pierre, S., Alméras, N. & Tremblay, A. (1997). Acute Effects of Exercise on Energy Intake and Feeding Behaviour. *British Journal of Nutrition,* 77**,** 511-521.

Inui, A., Asakawa, A., Bowers, C. Y., Mantovani, G., Laviano, A., Meguid, M. M. & Fujimiya, M. (2004). Ghrelin, Appetite, and Gastric Motility: The Emerging Role of the Stomach as an Endocrine Organ. *The FASEB Journal,* 18**,** 439-456.

Jokisch, E., Coletta, A. & Raynor, H. A. (2012). Acute Energy Compensation and Macronutrient Intake Following Exercise in Active and Inactive Males Who Are Normal Weight. *Appetite,* 58**,** 722-729.

Jones, A. M. & Doust, J. H. (1996). A 1% Treadmill Grade Most Accurately Reflects the Energetic Cost of Outdoor Running. *Journal of sports sciences,* 14**,** 321-327.

Jones, T. E., Basilio, J., Brophy, P., Mccammon, M. & Hickner, R. (2009). Long-Term Exercise Training in Overweight Adolescents Improves Plasma Peptide Yy and Resistin. *Obesity,* 17**,** 1189-1195.

Kanaley, J. A., Heden, T. D., Liu, Y., Whaley-Connell, A. T., Chockalingam, A., Dellsperger, K. C. & Fairchild, T. J. (2014). Short-Term Aerobic Exercise Training Increases Postprandial Pancreatic Polypeptide but Not Peptide Yy Concentrations in Obese Individuals. *International Journal of Obesity,* 38**,** 266-271.

Karra, E. & Batterham, R. L. (2010). The Role of Gut Hormones in the Regulation of Body Weight and Energy Homeostasis. *Molecular and cellular endocrinology,* 316**,** 120-128.

King, J. A., Garnham, J. O., Jackson, A. P., Kelly, B. M., Xenophontos, S. & Nimmo, M. A. (2015). Appetite-Regulatory Hormone Responses on the Day Following a Prolonged Bout of Moderate-Intensity Exercise. *Physiology & behavior,* 141**,** 23-31.

King, J. A., Miyashita, M., Wasse, L. K. & Stensel, D. J. (2010). Influence of Prolonged Treadmill Running on Appetite, Energy Intake and Circulating Concentrations of Acylated Ghrelin. *Appetite,* 54**,** 492-498.

King, N., Burley, V. & Blundell, J. (1994). Exercise-Induced Suppression of Appetite: Effects on Food Intake and Implications for Energy Balance. *European journal of clinical nutrition,* 48**,** 715-724.

Kishimoto, I., Tokudome, T., Hosoda, H., Miyazato, M. & Kangawa, K. (2012). Ghrelin and Cardiovascular Diseases. *Journal of cardiology,* 59**,** 8-13.

Lassen, A. D., Poulsen, S., Ernst, L., Andersen, K. K., Biltoft-Jensen, A. & Tetens, I. (2010). Evaluation of a Digital Method to Assess Evening Meal Intake in a Free-Living Adult Population. *Food & nutrition research,* 54.

Lim, C. T., Kola, B., Grossman, A. & Korbonits, M. (2011). The Expression of Ghrelin O-Acyltransferase (Goat) in Human Tissues. *Endocrine journal,* 58**,** 707-710.

Livingstone, M. B. E. & Black, A. E. (2003). Markers of the Validity of Reported Energy Intake. *The Journal of nutrition,* 133**,** 895S-920S.

Martin, C. K., Correa, J. B., Han, H., Allen, H. R., Rood, J. C., Champagne, C. M., Gunturk, B. K. & Bray, G. A. (2012). Validity of the Remote Food Photography Method (Rfpm) for Estimating Energy and Nutrient Intake in near Real-Time. *Obesity,* 20**,** 891-899.

Martin, C. K., Han, H., Coulon, S. M., Allen, H. R., Champagne, C. M. & Anton, S. D. (2009a). A Novel Method to Remotely Measure Food Intake of Free-Living Individuals in Real Time: The Remote Food Photography Method. *British Journal of Nutrition,* 101**,** 446-456.

Martin, C. K., Kaya, S. & Gunturk, B. K. Quantification of Food Intake Using Food Image Analysis. Engineering in Medicine and Biology Society, 2009. EMBC 2009. Annual International Conference of the IEEE, 2009b. IEEE, 6869-6872.

Martins, C., Kulseng, B., King, N., Holst, J. J. & Blundell, J. (2010). The Effects of Exercise-Induced Weight Loss on Appetite-Related Peptides and Motivation to Eat. *The Journal of Clinical Endocrinology & Metabolism,* 95**,** 1609-1616.

Martins, C., Morgan, L. M., Bloom, S. R. & Robertson, M. D. (2007). Effects of Exercise on Gut Peptides, Energy Intake and Appetite. *Journal of Endocrinology,* 193**,** 251-258.

Melanson, E. L., Keadle, S. K., Donnelly, J. E., Braun, B. & King, N. A. (2013). Resistance to Exercise-Induced Weight Loss: Compensatory Behavioral Adaptations. *Medicine and science in sports and exercise,* 45**,** 1600.

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K. & Matsukura, S. (2001). A Role for Ghrelin in the Central Regulation of Feeding. *Nature,* 409**,** 194-198.

Neary, N. M., Goldstone, A. P. & Bloom, S. R. (2004). Appetite Regulation: From the Gut to the Hypothalamus. *Clinical endocrinology,* 60**,** 153-160.

Ngo, J., Engelen, A., Molag, M., Roesle, J., García-Segovia, P. & Serra-Majem, L. (2009). A Review of the Use of Information and Communication Technologies for Dietary Assessment. *Br J Nutr,* 101**,** S102-S112.

Patterson, M., Murphy, K. G., Le Roux, C. W., Ghatei, M. A. & Bloom, S. R. (2005). Characterization of Ghrelin-Like Immunoreactivity in Human Plasma. *The Journal of Clinical Endocrinology & Metabolism,* 90**,** 2205-2211.

Purnell, B. (2006). Photographic Food Diaries for Nutritional Analysis: A Multi-Dimensional Picture. *Practical Diabetes International,* 23**,** 51-51.

Rocha, J., Paxman, J., Dalton, C., Winter, E. & Broom, D. (2013). Effects of an Acute Bout of Aerobic Exercise on Immediate and Subsequent Three-Day Food Intake and Energy Expenditure in Active and Inactive Men. *Appetite,* 71**,** 369-378.

Rutishauser, I. H. (2005). Dietary Intake Measurements. *Public health nutrition,* 8**,** 1100-1107.

Sato, T., Nakamura, Y., Shiimura, Y., Ohgusu, H., Kangawa, K. & Kojima, M. (2012). Structure, Regulation and Function of Ghrelin. *Journal of biochemistry,* 151**,** 119-128.

Scantlebury, R. & Moody, A. (2015). Adult Obesity and Overweight.

Schubert, M. M., Desbrow, B., Sabapathy, S. & Leveritt, M. (2013). Acute Exercise and Subsequent Energy Intake. A Meta-Analysis. *Appetite,* 63**,** 92-104.

Seale, J. & Rumpler, W. (1997). Comparison of Energy Expenditure Measurements by Diet Records, Energy Intake Balance, Doubly Labeled

Water and Room Calorimetry. *European journal of clinical nutrition,* 51**,** 856-863.

Small, L., Sidora-Arcoleo, K., Vaughan, L., Creed-Capsel, J., Chung, K.-Y. & Stevens, C. (2009). Validity and Reliability of Photographic Diet Diaries for Assessing Dietary Intake among Young Children. *ICAN: Infant, Child, & Adolescent Nutrition,* 1**,** 27-36.

Smith, S. R., De Jonge, L., Zachwieja, J. J., Roy, H., Nguyen, T., Rood, J., Windhauser, M., Volaufova, J. & Bray, G. A. (2000). Concurrent Physical Activity Increases Fat Oxidation During the Shift to a High-Fat Diet. *The American journal of clinical nutrition,* 72**,** 131-138.

Staten, M. A. (1991). The Effect of Exercise on Food Intake in Men and Women. *The American journal of clinical nutrition,* 53**,** 27-31.

Stubbs, R., Sepp, A., Hughes, D., Johnstone, A., King, N., Horgan, G. & Blundell, J. (2002a). Short Communication: The Effect of Graded Levels of Exercise on Energy Intake and Balance in Free-Living Women. *International journal of obesity,* 26**,** 866-869.

Stubbs, R. J., Sepp, A., Hughes, D. A., Johnstone, A. M., Horgan, G. W., King, N. A. & Blundell, J. E. (2002b). The Effect of Graded Levels of Exercise on Energy Intake and Balance in Free-Living Men, Consuming Their Normal Diet. *European journal of clinical nutrition,* 56**,** 129-140.

Suzuki, K., Simpson, K. A., Minnion, J. S., Shillito, J. C. & Bloom, S. R. (2010). The Role of Gut Hormones and the Hypothalamus in Appetite Regulation. *Endocrine journal,* 57**,** 359-372.

Thomas, D., Bouchard, C., Church, T., Slentz, C., Kraus, W., Redman, L., Martin, C., Silva, A., Vossen, M. & Westerterp, K. (2012). Why Do Individuals Not Lose More Weight from an Exercise Intervention at a Defined Dose? An Energy Balance Analysis. *Obesity Reviews,* 13**,** 835-847.

Thompson, J. & Blanton, P. (1987). Energy Conservation and Exercise Dependence: A Sympathetic Arousal Hypothesis. *Medicine and science in sports and exercise,* 19**,** 91-99.

Torres, S. J. & Nowson, C. A. (2007). Relationship between Stress, Eating Behavior, and Obesity. *Nutrition,* 23**,** 887-894.

Trabulsi, J. & Schoeller, D. A. (2001). Evaluation of Dietary Assessment Instruments against Doubly Labeled Water, a Biomarker of Habitual Energy Intake. *American Journal of Physiology-Endocrinology And Metabolism,* 281**,** E891-E899.

Tremblay, A., Almeras, N., Boer, J., Kranenbarg, E. K. & Despres, J. (1994). Diet Composition and Postexercise Energy Balance. *The American journal of clinical nutrition,* 59**,** 975-979.

Vatansever-Ozen, S., Tiryaki-Sonmez, G., Bugdayci, G. & Ozen, G. (2011). The Effects of Exercise on Food Intake and Hunger: Relationship with Acylated Ghrelin and Leptin. *Journal of sports science & medicine,* 10**,** 283.

Villars, C., Bergouignan, A., Dugas, J., Antoun, E., Schoeller, D. A., Roth, H., Maingon, A.-C., Lefai, E., Blanc, S. & Simon, C. (2012). Validity of Combining Heart Rate and Uniaxial Acceleration to Measure Free-Living Physical Activity Energy Expenditure in Young Men. *Journal of applied physiology,* 113**,** 1763-1771.

Wang, D.-H., Kogashiwa, M. & Kira, S. (2006). Development of a New Instrument for Evaluating Individuals' Dietary Intakes. *Journal of the American Dietetic Association,* 106**,** 1588-1593.

Weir, J. D. V. (1949). New Methods for Calculating Metabolic Rate with Special Reference to Protein Metabolism. *The Journal of physiology,* 109**,** 1.

Westerterp, K. R. (1998). Alterations in Energy Balance with Exercise. *The American journal of clinical nutrition,* 68**,** 970S-974S.

Whybrow, S., Hughes, D. A., Ritz, P., Johnstone, A. M., Horgan, G. W., King, N., Blundell, J. E. & Stubbs, R. J. (2008). The Effect of an Incremental Increase in Exercise on Appetite, Eating Behaviour and Energy Balance in Lean Men and Women Feeding Ad Libitum. *British Journal of Nutrition,* 100**,** 1109-1115.

Williamson, D. A., Allen, H. R., Martin, P. D., Alfonso, A. J., Gerald, B. & Hunt, A. (2003). Comparison of Digital Photography to Weighed and Visual Estimation of Portion Sizes. *Journal of the American Dietetic Association,* 103**,** 1139-1145.

World Health Organisation. 2015. *Obesity and Overweight* [Online]. Available: http://www.who.int/mediacentre/factsheets/fs311/en/.

Zwirska-Korczala, K., Konturek, S., Sodowski, M., Wylezol, M., Kuka, D., Sowa, P., Adamczyk-Sowa, M., Kukla, M., Berdowska, A. & Rehfeld, J. (2007). Basal and Postprandial Plasma Levels of Pyy, Ghrelin. *Journal of physiology and pharmacology,* 58**,** 13-35.

8.0 Appendices

8.1 Appendix A

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				
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)**

Day 2				
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)**

Day 3				
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)**

Day 4				
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)**

Day 5				
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)**

Day 6				
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)**

Day 7				
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)**

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)

8.2 Appendix B

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

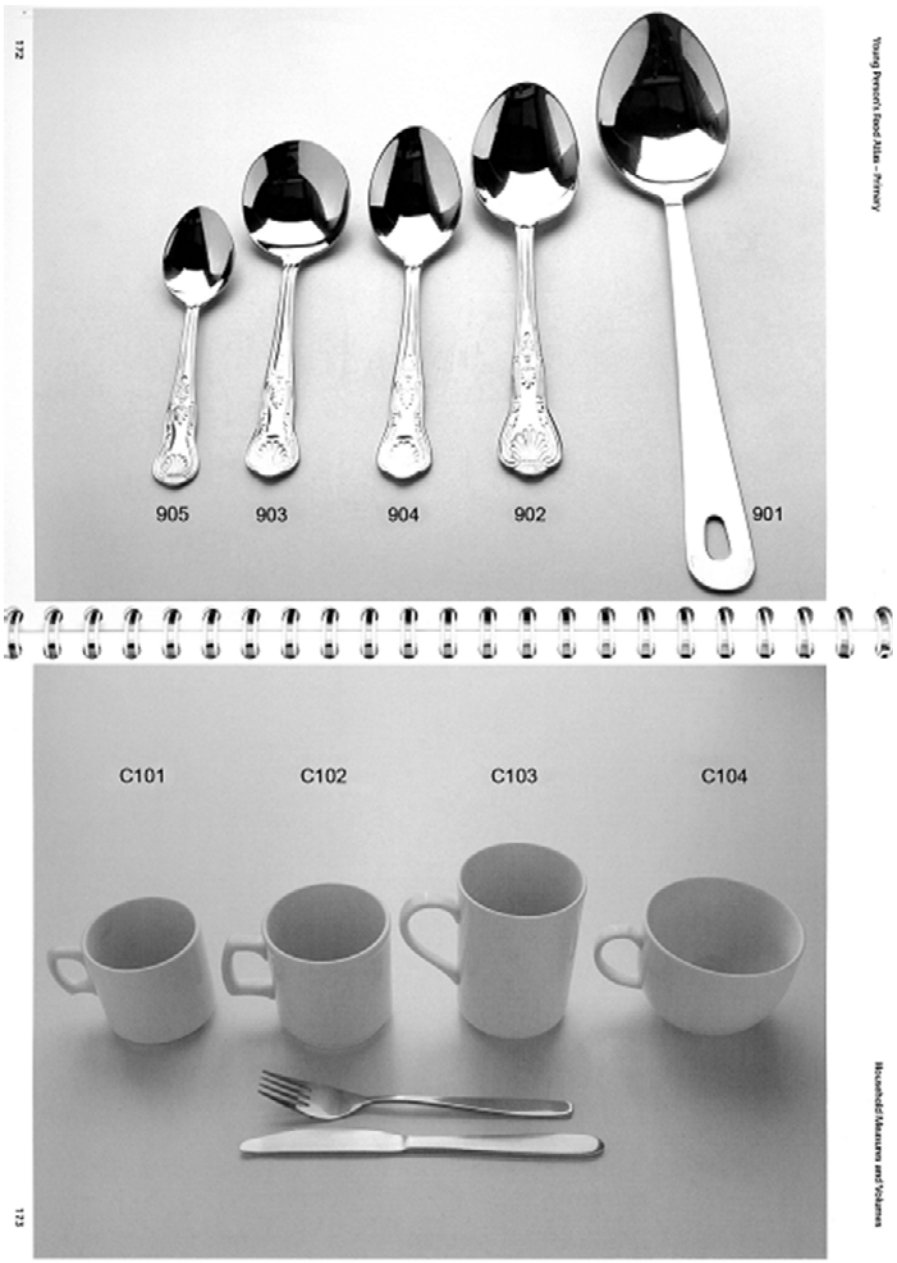
Bad examples

- ✘ **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.**

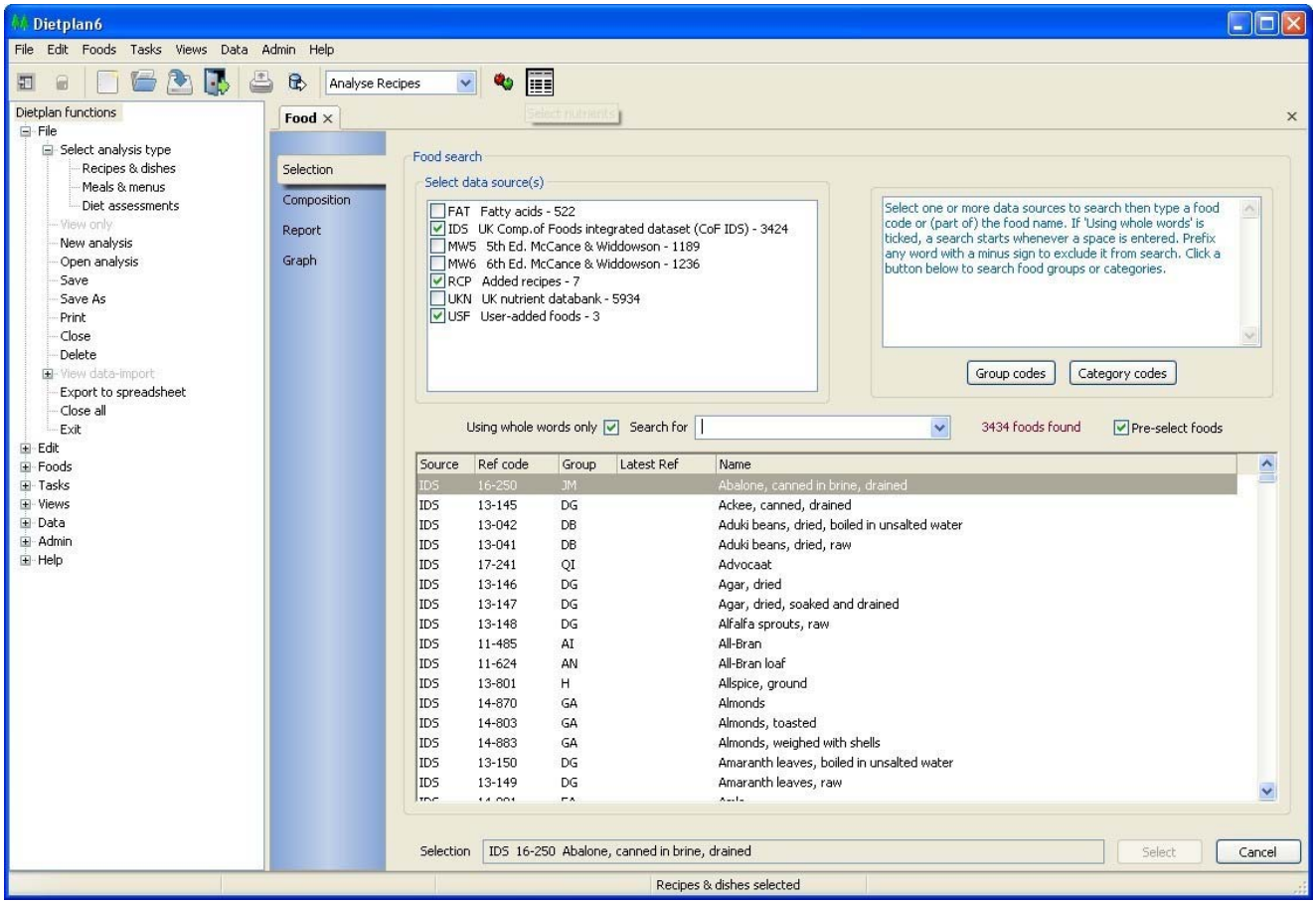
- ✘ **No knife or fork.**
- ✘ **No 'after' picture.**

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

8.3 Appendix C



8.4 Appendix D



8.5 Appendix E

Department of Sports Science and Physical Activity (SSPA)

Bedford Campus

Polhill Avenue

Bedford

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)

Department of Sport and Exercise Sciences,

University of Bedfordshire

Bedford Campus,

Polhill Avenue,

Bedford

8.6 Appendix F

SUBJECT CONSENT FORM

UNIVERSITY of BEDFORDSHIRE

Consent form

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: _____

8.7 Appendix G

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...

8.8 Appendix H

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

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 he 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: _____

8.9 Appendix I

DEPARTMENT OF SPORT &
EXERCISE SCIENCES

Bedford Campus

Polhill Avenue

Bedford

INFORMATION SHEET

Title: The effect of a 7 day imposed intensified exercise on energy balance and appetite-regulating hormones in young, healthy, physically active men

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 14 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 & 14:

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 800 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

8.10 Appendix J

SUBJECT CONSENT FORM

UNIVERSITY of BEDFORDSHIRE

Consent form

The effect of a 7 day imposed intensified exercise on energy balance and appetite-regulating hormones in young, healthy, physically active men

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: _____

8.11 Appendix K

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.