Examining the acute effects of exercise intensity on subsequent appetite, food intake, resting energy expenditure and fat oxidation.

Ghalia Shamlan
PhD Student

March 2015

Supervisor:
Dr. Denise Robertson
Dr. Adam Collins

Department of Nutritional Science

Faculty of Health and Medical Science

University of Surrey
Declaration

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service Turnitin UK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above. The thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement or consent.

Signed: …………………………… Date: 12 November 2015
Dedication

This thesis is lovingly dedicated to my mother, Zenab Seet. Her support, encouragement, and constant love have sustained me throughout my life
Acknowledgment

I wish to send my sincerest gratitude to the following people who have all had invaluable roles to play throughout the duration of my PhD

Firstly I would like to acknowledge the support and the guidance, which my supervisor Dr Denise Roberson and Adam Collins have provided throughout my PhD. They have always been available to offer advice, and to assist me whenever I have required their help throughout my research project. Furthermore, I thank Denise Roberson for given me the opportunity and the approval to undertake the project and has provided me with the drive and resources necessary to see the project through from start to finish. Hopefully, Work together in the future to continue what we have started here and develop exciting research.

I would like to thank my sponsor King Saud University for their trust and funds for the project especially, Dr Abdullah and Dr Muhammad for their guidance and advice.

I thank my family: my lovely mother Omme Zenab for her love and support as she wakes me up every day to work for my study. Also, my brothers especially Suleiman and Tariq for their support and understanding for all the long nights I had to spend working. I would like to thank my colleagues at University of Surrey and friends for all of their support over the past few years especially Modhi, Camila, and my friend Alia and Nora for their guidance and assistance to achieve my goals. Also, the University of Surrey Library and the Student Union for their support over the year I have spent in Surrey.
# Table of content

Declaration ............................................................................................................. i

Dedication ............................................................................................................. ii

Acknowledgment ................................................................................................. iii

Table of content ................................................................................................... iv

List of abbreviation .............................................................................................. xiii

List of tables ........................................................................................................... xvii

List of figures ......................................................................................................... xix

Summary ............................................................................................................... xxiii

Chapter one ............................................................................................................ 1

1 Introduction and literature review .................................................................... 2

1.1 Obesity ........................................................................................................... 2

1.2 Energy balance ............................................................................................... 4

1.2.1 Energy intake (EI) ..................................................................................... 5

1.2.1.1 Appetite and food intake regulations ..................................................... 5

1.2.1.2 The central control of food intake ......................................................... 5

1.2.1.3 Origins of appetite regulation ................................................................. 7

1.2.1.4 The peripheral control of food intake ................................................... 7

1.2.1.5 Adipose tissue ........................................................................................ 7

1.2.1.6 Gastrointestinal hormone and endocrine pancreas .............................. 8

1.2.1.6.1 Glucagon-like peptide-1 (GLP-1) ....................................................... 9
1.2.1.6.2 GLP-1 and the brain...........................................................................................................9
1.2.1.6.2.1 GLP-1 and appetite ........................................................................................................9
1.2.1.6.2.2 GLP-1 and nutrients........................................................................................................10
1.2.1.6.2.3 GLP-1 and obesity .........................................................................................................11
1.2.1.7 Other factors..........................................................................................................................11
1.2.2 Energy Expenditure (EE)................................................................................................ ..........13
1.2.2.1 Physical activity (PA) ...........................................................................................................13
1.2.2.2 Physical activity (PA) and total energy expenditure (TEE). ................................................13
1.2.2.2.1 Exercise and Physical Activity (PA) .....................................................................................14
1.2.2.2.2 Physical activity (PA) and obesity ......................................................................................14
1.2.2.3 Exercise intensity ................................................................................................................15
1.2.2.4 Current Recommendations ..................................................................................................16
1.2.2.5 The role of exercise...............................................................................................................20
1.2.2.5.1 The effects of exercise on appetite and satiety .................................................................20
1.2.2.5.2 The effects of exercise on energy intake (EI) .................................................................22
1.2.2.5.3 The metabolic effect of exercise .......................................................................................23
1.2.2.5.3.1 The effects of an exercise-induced energy deficit .........................................................23
1.2.2.5.3.2 The effects of exercise on gut peptides .........................................................................24
1.2.2.5.3.3 The effects of exercise on GLP-1 ..................................................................................25
1.2.2.5.4 The effect of exercise on utilisation of energy substrates .................................................25
1.2.2.6 The effect of exercise on the other factors............................................................................28
2.2.2.1 Resting Energy Expenditure (REE) and maximum oxygen uptake (VO\textsubscript{2max}) test......46
2.2.2.2 Standardised meal ........................................................................................................................................46
2.2.2.3 Determination of Resting Energy Expenditure (REE).....................................................................................47
2.2.2.4 Maximum oxygen uptake (VO\textsubscript{2max}) test ..........................................................................................48
2.2.3 Third and fourth visits: comparison of matched LI and HI exercise .................................................................49
2.2.3.1 Standardized breakfast ...................................................................................................................................49
2.2.3.2 Exercise Study Visit ..........................................................................................................................................50
2.2.3.3 Exercise intervention: High (HI) and Low intensity (LI) exercises .................................................................51
2.2.3.4 Resting Energy Expenditure (REE) post-exercise ..............................................................................................52
2.2.3.5 Measurement of appetite and food intake ........................................................................................................52
2.2.3.5.1 Assessment of appetite: Visual analogue scales (VAS) .................................................................................52
2.2.3.5.2 Ad libitum (pasta test-meal) .........................................................................................................................53
2.2.3.5.3 48 hour post-exercise energy intake ............................................................................................................55
2.3 Laboratory analysis ..................................................................................................................................................55
2.3.1 Analysis of plasma TAG ......................................................................................................................................55
2.3.2 Analysis of plasma NEFA ......................................................................................................................................56
2.3.3 Analysis of plasma glucose ..................................................................................................................................56
2.3.4 Analysis of plasma insulin ....................................................................................................................................56
2.3.5 Analysis of GLP-1 ..................................................................................................................................................57
2.4 Statistical analyses ..................................................................................................................................................58

Chapter three .................................................................................................................................................................60
3 Validation PRO-Diary against the traditional pen and paper (P&P) method in a laboratory setting using healthy young adults: ................................................................. 61

3.1 Introduction ........................................................................................................ 61

3.2 Aim ...................................................................................................................... 62

3.3 Analysis ............................................................................................................... 62

3.4 Participants ......................................................................................................... 63

3.5 P&P VAS and PRO-Diary .................................................................................. 63

3.6 Study design ....................................................................................................... 64

3.7 Statistical methodology ..................................................................................... 64

3.8 Results ................................................................................................................. 65

3.8.1 Hunger score .................................................................................................. 65

3.8.2 Prospective food consumption score ............................................................... 68

3.8.3 Fullness score ................................................................................................ 71

3.8.4 Discussion ....................................................................................................... 74

Chapter four ............................................................................................................ 78

4 Examining the acute effects of exercise intensity on subsequent appetite, food intake, resting energy expenditure and fat oxidation in lean participants. ........................................ 79

4.1 Introduction ....................................................................................................... 79

4.2 Aim ...................................................................................................................... 79

4.3 Methods .............................................................................................................. 80

4.4 Results ................................................................................................................. 80

4.4.1 Participant characteristics and measurements ............................................... 80
4.4.2 During exercise measurement

4.4.2.1 Energy Expenditure (EE) and work load

4.4.2.2 Substrate Utilisation during exercise

4.4.3 Post-exercise measurements

4.4.3.1 Subjective appetite ratings

4.4.3.2 Plasma metabolites and glucagon-like peptide-1 (GLP-1)

4.4.3.2.1 Triacylglyceride (TAG)

4.4.3.2.2 Non-esterified fatty acid (NEFA)

4.4.3.2.3 Glucagon-like peptide-1 (GLP-1)

4.4.3.2.4 Glucose and Insulin

4.4.3.3 Energy expenditure (EE)

4.4.3.4 Substrate utilisation

4.4.3.5 Fat oxidation

4.4.3.6 Ad Libitum pasta test meal intake and 48 hours post-exercise intake

4.5 Discussion

Chapter five

5 The independent effect of exercise intensity on appetite, energy intake and energy expenditure: is there a gender difference?
6.4.3.1 Subjective appetite ratings ................................................................. 133
6.4.3.2 Plasma metabolites and GLP-1 ......................................................... 135
  6.4.3.2.1 Triacylglyceride (TAG) ............................................................... 135
  6.4.3.2.2 Non-esterified fatty acid (NEFA) .................................................. 136
  6.4.3.2.3 Glucagon-like peptide-1 (GLP-1) .................................................. 136
  6.4.3.2.4 Glucose and Insulin ................................................................... 137
  6.4.3.2.5 Energy expenditure (EE) .............................................................. 139
  6.4.3.2.6 Substrate Utilisation .................................................................... 139
  6.4.3.2.7 Fat oxidation ............................................................................... 140
  6.4.3.2.8 Ad Libitum pasta test meal intake and 48 hours intake. ............... 141
6.5 Discussion .............................................................................................. 144
Chapter seven .............................................................................................. 155
7 Effect of BMI on energetic and physiological responses to exercise intensity. 156
  7.1 Introduction ........................................................................................... 156
  7.2 Aim ........................................................................................................ 157
  7.3 Methods ................................................................................................. 157
  7.4 Statistical analyses ................................................................................. 157
  7.5 Results: .................................................................................................. 158
    7.5.1 Participants ..................................................................................... 158
    7.5.2 During exercise measurements: ....................................................... 159
      7.5.2.1 EE and substrate utilisations: ..................................................... 159
7.5.3 Post-exercise measurements: ................................................................. 160
7.5.3.1 Subjective appetite ratings, plasma metabolites and GLP-1 .................. 160
7.5.3.2 EE and substrate utilisation ............................................................... 162
7.5.3.3 Fat oxidation ................................................................................. 165
7.5.3.3.1 Ad Libitum pasta test meal intake and 48 hours intake ................. 167
7.6 Discussion: ......................................................................................... 168
Chapter eight ......................................................................................... 174
8 General Discussion: ............................................................................. 175
8.1 Strength and Limitations .................................................................... 179
8.2 Future directions for research .............................................................. 180
9 References: .......................................................................................... 182
10 Publications and presentations ............................................................... 203
10.1 Conferences ..................................................................................... 203
10.2 Journals ........................................................................................... 203
11 Appendix ............................................................................................ 204
11.1 Appendix A: ethics letter ................................................................. 204
11.2 Appendix B: Dutch Eating Behaviour Questionnaire (DEBQ) (van strien t; 1987) .... 207
11.3 Appendix C: physical activity questionnaire (par-q screening questionnaire) .... 208
11.4 Appendix D: .................................................................................... 209
11.4.1 TAG ............................................................................................ 209
11.4.2 NEFA ........................................................................................ 209
## List of abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
</tr>
<tr>
<td>AgRP</td>
<td>Agouti-related peptide</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARC</td>
<td>Arcute nucleus</td>
</tr>
<tr>
<td>AT</td>
<td>Anaerobic threshold</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>b-HAD</td>
<td>b-hydroxy-acyl-CoA-dehydrogenase</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BMR</td>
<td>Basal metabolic rate</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine-amphetamine-regulated transcript</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrates</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CPT-1</td>
<td>Carnitine palmitoyltransferase</td>
</tr>
<tr>
<td>CVs</td>
<td>Coefficient of variability</td>
</tr>
<tr>
<td>DMN</td>
<td>Dorsomedial hypothalamus</td>
</tr>
<tr>
<td>DPP-4</td>
<td>Dipeptidyl peptidase-4</td>
</tr>
<tr>
<td>EAT</td>
<td>Exercise activity thermogenesis</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EE</td>
<td>Energy expenditure</td>
</tr>
<tr>
<td>EI</td>
<td>Energy intake</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzymelinked immunosorbent assay</td>
</tr>
<tr>
<td>EPOC</td>
<td>Excess post-exercise oxygen consumption</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat free mass</td>
</tr>
<tr>
<td>GCGR</td>
<td>Glucagon receptor</td>
</tr>
<tr>
<td>GHS</td>
<td>Growth hormone secretagogue receptor</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon like peptide 1</td>
</tr>
<tr>
<td>H+</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HI</td>
<td>High intensity exercise</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>HR_{max}</td>
<td>Maximum heart rate</td>
</tr>
<tr>
<td>HSL</td>
<td>hormone-sensitive lipase</td>
</tr>
<tr>
<td>Kcal</td>
<td>Kilocalorie</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>Km</td>
<td>Kilometers</td>
</tr>
<tr>
<td>LHA</td>
<td>Lateral hypothalamic nucleus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>LI</td>
<td>Low intensity exercise</td>
</tr>
<tr>
<td>MC4R</td>
<td>melanocortin 4 receptor</td>
</tr>
<tr>
<td>METs</td>
<td>Metabolic equivalents = Metabolic cost (energy expenditure or oxygen consumption) of physical activity.</td>
</tr>
<tr>
<td>MI</td>
<td>Milliliters</td>
</tr>
<tr>
<td>NEAT</td>
<td>Non-exercise activity thermogenesis</td>
</tr>
<tr>
<td>NEFA</td>
<td>non-esterified free fatty acids</td>
</tr>
<tr>
<td>Nmol</td>
<td>Nanomol</td>
</tr>
<tr>
<td>NPY</td>
<td>neuropeptides Y</td>
</tr>
<tr>
<td>NR-NCDs</td>
<td>nutrition – related non-communicable diseases</td>
</tr>
<tr>
<td>NTS</td>
<td>nucleus of the solitary tract</td>
</tr>
<tr>
<td>OXM</td>
<td>Oxyntomodulin</td>
</tr>
<tr>
<td>PA</td>
<td>Physical activity</td>
</tr>
<tr>
<td>PAEE</td>
<td>Physical activity energy expenditure</td>
</tr>
<tr>
<td>PAL</td>
<td>Physical activity level</td>
</tr>
<tr>
<td>PARQ</td>
<td>Physical activity readiness questionnaire</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphorylated creatine</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PP</td>
<td>Pancreatic polypeptide</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nuclei</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide YY</td>
</tr>
<tr>
<td>QC s</td>
<td>Quality controls</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Term</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Ra</td>
<td>Rate of appearance</td>
</tr>
<tr>
<td>REE</td>
<td>Resting energy expenditure</td>
</tr>
<tr>
<td>REI</td>
<td>Relative energy intake</td>
</tr>
<tr>
<td>RER</td>
<td>Respiratory exchange ratio</td>
</tr>
<tr>
<td>RMR</td>
<td>Resting metabolic rate</td>
</tr>
<tr>
<td>RQ</td>
<td>Respiratory quotient</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>TAG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>TEE</td>
<td>Total energy expenditure</td>
</tr>
<tr>
<td>TEF</td>
<td>Thermic effect of food</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scales</td>
</tr>
<tr>
<td>VMN</td>
<td>Ventromedial nucleus</td>
</tr>
<tr>
<td>VO2</td>
<td>Oxygen consumption</td>
</tr>
<tr>
<td>VO2max</td>
<td>Maximum oxygen consumption</td>
</tr>
<tr>
<td>W</td>
<td>Watt</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
</tbody>
</table>
List of tables

Table 1.1 The actions of the gut hormones .................................................................8
Table 1.2 Effect of high (HI) vs. low exercise intensity (LI) on appetite, energy intake (EI) and appetite regulation hormones. .................................................................34
Table 2.1 The nutritional composition of the standard evening meal. .........................46
Table 2.2 Nutritional content of the liquid test meal one hour before the exercise ..........49
Table 2.3: Energy and micronutrient composition of the pasta based test meal. ..........54
Table 3.1 Differences of >30 between the P&P and the Pro-Diary for the hunger score.......65
Table 3.2 Score of participants Identified by Bland-Altman procedure of outliers. ....68
Table 3.3 Differences of >30 between the P&P and Pro-Diary. ....................................69
Table 3.4: Score of participants Identified by Bland-Altman procedure of outliers. ....71
Table 3.5 Differences of >30 between the P&P and Pro-Diary. ....................................72
Table 3.6: Score of participants Identified by Bland-Altman procedure of outliers. ....74
Table 4.1 Baseline characteristics of lean participants who completed the exercise study intervention. .................................................................................................82
Table 4.2 Ad libitum Pasta test meal post-exercise and the total two days of energy and macronutrient intake following Low or High exercise in lean participants. ........93
Table 5.1 Baseline characteristics of lean participants (males and females) who completed the exercise study intervention. .................................................................109
Table 5.2 Significant mean difference of ad libitum pasta intake post-exercise and, energy intake with macronutrient intake in the first 24 second of the 24 hour post-exercise at Low and High in each gender. .................................................................118
Table 5.3 The adjusted and expressed relative to resting energy expenditure (REE) in Low and High intensity exercise in each gender. .................................................................119
Table 6.1 Baseline characteristics of overweight participants who completed the exercise study intervention. .......................................................... 131

Table 6.2 *ad libitum* pasta intake post-exercise and energy intake with macronutrient intake in a 48 post-exerciseperiment. .......................................................... 142

Table 7.1 Baseline characteristics of lean and overweight participants who completed the exercise study intervention. .......................................................... 158
List of figures

Figure 1.1 Illustration of the factors that affect energy balance. ...........................................4

Figure 1.2 Schematic of the central and peripheral control of appetite regulation. ..............6

Figure 1.3 Satiety cascade showing the relationship between satiation and satiety, and some psychological and physiological processes.................................................................13

Figure 1.4 The ACSM’s recommendations for exercise..........................................................16

Figure 1.5 Consequences of exercise on energy expenditure .................................................18

Figure 2.1 Study design. .........................................................................................................42

Figure 2.2 Timeline of the study day. ....................................................................................51

Figure 2.3 Exercise protocol. ................................................................................................51

Figure 2.4 An example of visual analogue scale (VAS) used for assessing appetite. ..........53

Figure 3.1 Hunger (HUN) both pen and paper VAS (P&P) and pro-diary watch (Pro-Diary) ..............................................................................................................................67

Figure 3.2 Prospective food consumption (EAT) for both pen and paper VAS (P&P) and pro-diary VAS watch (Pro-Diary) .................................................................................................................................70

Figure 3.3 Fullness (FULL) both pen and paper VAS (P&P) and pro-diary VAS watch (Pro-Diary) .................................................................................................................................73

Figure 4.1 Lean participants flow diagram. ........................................................................81

Figure 4.2 Energy expenditure (EE) during 30 minutes of Low (50% VO2max) and 20 minutes of High (95% VO2max) exercise on each study day in lean participants. .........................83

Figure 4.3 Respiratory exchange ratio (RER) during 30 minutes of Low (50% VO2max) and 20 minutes of High (95% VO2max) exercise on each study day in lean participants.............84

Figure 4.4 The effects of Low versus High intensity exercise on subjective appetite ratings in lean participants. .........................................................................................................................85
Figure 4.5 Plasma triacylglyceride (TAG) concentrations (mmol/L) over time pre, during and post exercise in lean participants. ................................................................. 86

Figure 4.6 Plasma non-esterified acid (NEFA) concentrations (mmol/L) over time pre, during and post exercise in lean participants. ................................................................. 87

Figure 4.7 Plasma GLP-1 concentrations (pmol/L) over time pre, during and post exercise in lean participants. ................................................................. 88

Figure 4.8 Plasma glucose concentrations (mmol/L) (A) and insulin concentrations (pmol/L) (B) over time pre, during and post exercise in lean participants. .......................... 89

Figure 4.9 Energy expenditure (EE) following either Low or High exercise measured at 15, 45 and 75 minutes post exercise by indirect calorimetry in lean participants. .................. 90

Figure 4.10 Mean Respiratory Quotient (RQ) post-exercise at 15, 45 and 75 minutes measured by indirect calorimetry in lean participants. ............................................. 91

Figure 4.11 Mean fat oxidation post-exercise at 15, 45 and 75 minutes in lean participants. 92

Figure 5.1 Rating of hunger pre, during and post-exercise for each gender. ...................... 110

Figure 5.2 Rating of prospective food consumption pre, during and post-exercise for each gender. ........................................................................................................... 111

Figure 5.3 Plasma non-esterified acid (NEFA) concentrations (mmol/L) over time pre, during and post-exercise for each gender. ................................................................. 112

Figure 5.4 Plasma insulin concentrations (pmol/L) over time pre, during and post-exercise for each gender. ........................................................................................................... 113

Figure 5.5 Energy expenditure (EE) at 5, 45 and 75 minutes following exercise intensities measured with indirect calorimetry for each gender. ................................................................. 114

Figure 5.6 Energy expenditure (EE) (KJ/Kg FFM/min) at 5, 45 and 75 minutes exercise intensities measured using indirect calorimetry for each gender. ................................................................. 115
Figure 5.7 Calculated fat oxidation at 5, 45 and 75 minutes following Low and High exercises for each gender.

Figure 5.8 Fat oxidation in (gram /minute/ FFM) at 5, 45 and 75 minutes following Low and High intensity exercises for each gender.

Figure 6.1 Overweight participants’ flow diagram.

Figure 6.2 Measured energy expenditure (EE) during 30 minutes of Low (50% VO\(_2\)max) and 20 minutes of High (95% VO\(_2\)max) exercise on each study day in overweight participants.

Figure 6.3 Respiratory exchange ratio (RER) during 30 minutes of Low (50% VO\(_2\)max) and 20 minutes of High (95% VO\(_2\)max) exercises on each study day in overweight participants.

Figure 6.4 Rating of hunger (A), prospective food consumption (B) and fullness (C) pre, during and post-exercise in overweight participants.

Figure 6.5 Plasma TAG concentrations (mmol/L) over time pre, during and post exercise in overweight participants.

Figure 6.6 Plasma non-esterified acid (NEFA) concentrations (mmol/L) over time pre, during exercise and post exercise in overweight participants.

Figure 6.7 Plasma GLP-1 concentrations (pmol/L) over time pre, during and post-exercise in overweight participants.

Figure 6.8 Plasma glucose concentrations (mmol/L) (A) and insulin concentrations (pmol/L) (B) over time pre, during and post exercise in overweight participants.

Figure 6.9 Energy expenditure (EE) following either Low or High exercise measured at 15, 45 and 75 minutes post-exercise by indirect calorimetry in overweight participants.

Figure 6.10 Mean respiratory quotient (RQ) post-exercise at 15, 45 and 75 minutes in overweight participants.
Figure 6.11 Mean fat oxidation at 15, 45 and 75 minutes post-exercise in overweight participants. ................................................................. 141

Figure 7.1 Energy expenditure (EE) during exercise in lean and overweight participants. .159

Figure 7.2 Energy expeditor (EE) (KJ/FFM Kg/ day) during exercise in lean and overweight participants. ............................................................................................................... 160

Figure 7.3 Plasma glucose concentrations (mmol/L) over time pre, during and post-exercise in lean and overweight participants. ................................................................. 161

Figure 7.4 Plasma GLP-1 concentrations (pmol/L) over time pre, during and post exercise in lean and overweight participants. ............................................................................................................... 162

Figure 7.5. Energy expenditure (EE) over 5, 45 and 75 minutes following exercise in lean and overweight participants. ............................................................................................................... 163

Figure 7.6 Energy expenditure (EE) (KJ/min/FFM) over 5, 45 and 75 minutes following exercise in lean and overweight participants. ............................................................................................................... 164

Figure 7.7 RQ over 5, 45 and 75 minutes following exercise in lean and overweight participants. ............................................................................................................... 165

Figure 7.8 Fat oxidation over 5, 45 and 75 minutes following exercise in lean and overweight participants. ............................................................................................................... 166

Figure 7.9 Fat oxidation (g/min/FFM) over 5, 45 and 75 minutes following exercise in lean and overweight participants. ............................................................................................................... 167
Summary

Energy balance is important for weight maintenance with exercise having documented physiological, behavioural, and appetite effects. Exercise is known to acutely influence appetite but evidence for an independent effect of intensity is lacking. The purpose of this dissertation was to investigate the role of exercise intensity on appetite and energy intake (EI), energy expenditure (EE), and the metabolic effects of exercise intensity per se in lean and overweight individuals and to determine whether there was influence of gender or differences between groups.

Forty healthy volunteers (30 lean and 10 overweight) undertook 2 periods of exercise matched for energy cost, (i) 8 repeated 60 second bouts of cycling at 95% VO₂ max; high intensity exercise (HI) and (ii) 30 minutes of continuous cycling, at a fixed cadence, at 50% VO₂ max; low intensity exercise (LI) in a randomised cross-over design. Satiety to a standard meal was assessed subjectively using visual analogue scales. Ad libitum intake was measured 3-h post-breakfast and for 2 days post-exercise. EE and fat oxidation were measured every 30 mins post-exercise. The results showed that in the lean group relative to LI, HI suppressed prospective food consumption, increased EE (P=0.001), fatty acid (NEFA) utilisation (P=0.004) and fat oxidation (P<0.001), but did not affect appetite, EI, plasma glucose, insulin, GLP-1 or lipid levels post-exercise. There was a differential effect of gender on prospective food consumption and NEFA response post-exercise. HI increased EE and fat oxidation post-exercise for men. In the overweight individuals, HI did not differ from LI in terms of appetite, GLP-1, glucose, insulin, lipid or NEFA levels, with no difference in EI, EE and fat oxidation post-exercise. In conclusion, there are different consequences of exercise intensity in short-term control of energy balance depending on BMI and gender; our results support the need for longer term intervention to test these mechanisms.
Chapter one
1 Introduction and literature review

1.1 Obesity

The medical definition of obesity and overweight people is an increase in body weight, particularly adipose tissue, which leads to multiple health complications including type 2 diabetes (T2DM) and cardiovascular disease (Smith et al., 2014). It has been thought that many specific causes of mortality are associated with obesity (Visscher et al., 2010), especially in elderly and middle aged adults (Park et al., 2006).

The World Health Organization (WHO) recommends that body mass index (BMI, Kg/m²) is used as the default measurement to define the concepts of overweight and obesity in the adult population (WHO, 2000). Obesity is a growing problem as studies have predicted approximately 230 billion adults will be overweight by 2015 and about 700 million will be obese (WHO, 2006) and by 2030 up to 3.3 billion people might be either overweight or obese if recent trends continue (Kelly et al., 2008). In 2008 about 1.5 billion of adults over 20 years old were overweight, and of these more than 200 million men and 300 million women were obese (WHO, 2011). In the year 2007-2008, the UK had the third highest incidence of obesity after the US and Mexico (CDC, 2011).

In 2009, almost a quarter of adults (22 % of men and 24 % of women aged 16 or over) in England were classified as obese (BMI 30kg/m² or over) (The NHS Information Centre Lifestyles Statistics, 2011). In addition, studies suggest that by 2050 this number could reach as high as 60 % of men, 50 % of women and even 25 % of children (Jebb et al., 2007). This trend is not restricted to the UK, even in Arab Gulf areas the trend for increasing obesity and overweight people has shown a similar pattern with 66-75 % of gulf population now classified as overweight or obese (Ng et al., 2011).
Since 1990, the role of nutrition in disease processes has been acknowledged with the new definition of “nutrition-related non-communicable diseases” (NR-NCDs) specifically relating to obesity (Ng et al., 2011). Studies suggest that nutrition alone does not account for the increase in obesity rate; Healy et al. (2008) reported a strong positive correlation between the risk of cardiovascular disease mortality, T2DM and obesity with time spent sitting watching television, which showed that one of the most important factors is “inactivity” with excess food intake (Healy et al., 2008). A common route to obesity is the combination of energy intake (EI) exceeding energy expenditure (EE) with other bio-psychosocial and environmental factors, such as inactivity (NICE, 2006). Inactivity can affect some groups more than others, for example in the UK about 20 % of smokers, 6-9 % of alcoholics, and 24 % of obese are classified as inactive (Chief Medical Officer, 2009). Furthermore, for some Arab countries including Egypt, Iraq, Jordan, Kuwait, Saudi Arabia, Sudan and Syria, WHO statistics have shown that the proportion of inactive people is even higher, with between 33 % to 86 % of adults classified as inactive (Al-Shayji and Akanji, 2004). All this suggests that inactivity is a serious problem and can further contribute to conditions such as obesity.

The effects of inactivity and obesity for the economy and public health are substantial, for example in the UK the average cost of inactivity is nearly £5 million per year in each region of the country (Chief Medical Officer, 2009), therefore the introduction of interventions designed to prevent or reduce the impact of obesity may be of great benefit (Wang et al., 2011). Obesity is recognised as a global epidemic and the treatment of obesity-related diseases becoming the greatest economic burden faced by health care services across the world (Withrow and Alter, 2011).

Despite the slow increase in the prevalence of obesity in previous decades, by 2030, the cost of obesity in the UK is predicted to have increased by £1.9 billion (Dietz, 2011), whereas the cost of obesity and inactivity in gulf state is unknown. Recent studies have indicated potential
treatments for obesity which would also protect against its complications, these include decreasing energy intake and/or increasing energy expenditure (Wyatt, 2013).

1.2 Energy balance

To understand obesity and the effect of physical activity (PA), we first need to understand the concept of energy balance and the interaction between activity and food intake regulation. See Figure 1.1.

Figure 1.1 Illustration of the factors that affect energy balance.

Energy homeostasis is key to controlling body weight and energy balance and has two opposing sides, energy intake (EI) and energy expenditure (EE) each of which have multiple components (Spiegelman and Flier, 2001, Bolborea and Dale, 2013).

EE is composed of PA, resting energy expenditure (REE) and thermogenesis, whereas the control of EI is complex and for example can be affected by gastrointestinal peptide secretion
and gastrointestinal motility, both of which influence subjective feelings of hunger and satiety. Both components (EE and EI) are influenced by the hypothalamus (Spiegelman and Flier, 2001, Morton et al., 2006).

1.2.1 Energy intake (EI)

1.2.1.1 Appetite and food intake regulations

Appetite is the desire to eat food or a specific nutrient (Harris et al., 2008). Food intake regulation is controlled by the interaction between the central nervous system (CNS), the gastrointestinal tract (GI), liver and the adipose tissue (Ahima, 2006, R.James, 1999). These interactions are expressed by the sensation of hunger, satiation (the disappearance of hunger during a meal) and satiety (the sensation of satisfaction after a meal that gradually disappears to make way for hunger), which are balanced in order to regulate appetite. Other factors can play a role in influencing food intake such as: gastric motility, amount of secretions and visceral sensitivity (Delgado-Aros et al., 2003, Goetze et al., 2009).

1.2.1.2 The central control of food intake

Studies have shown that within the CNS, the hypothalamus plays an important role in regulating energy homeostasis (Anand and Brobeck, 1951, Parker and Bloom, 2012). Within the hypothalamus the ventromedial nucleus (VMN) and the lateral hypothalamic nucleus (LHA) are the satiety and hunger centres and therefore play a role in appetite regulation and eating behaviour (Wynne et al., 2005). However, there is evidence that the arcute nucleus (ARC) works with the other neurons to integrate the signals of appetite regulation (Figure 1.2), for example one of these populations of neurons play a role as a food intake inhibitor via pro-opiomelanocortin (POMC) and cocaine-amphetamine-regulated transcript (CART). The other neural circuit, which stimulates food intake and is active in the paraventricular nucleus (PVN), dorsomedial hypothalamus (DMN) and LHA, involves neuropeptides Y (NPY), agouti-related
peptide (AgRP) and the melanocortin 4 receptor (MC4R) (Schwartz et al., 2000, Coll et al., 2007, Cegla and Bloom, 2014).

Figure 1.2 Schematic of the central and peripheral control of appetite regulation.

There are three main areas involved in appetite regulation the hypothalamus (specifically the arcuate nucleus (ARC)), the pancreas, and the gastro-intestinal tract. Upon sensing an oral nutrient load negative feedback is initiated in the appetite regulating areas of the hypothalamus by Peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM). Pancreatic polypeptide (PP) and ghrelin from pancreatic islets are released upon detection of nutrients and enter the ARC via the vagus nerve, where they trigger signalling via central appetite centres in the hypothalamus. Within the ARC the first stage of regulation is the inhibition of firing of pro-optiomeranocortin (POMC) and cocaine-amphetamine-regulated transcript (CART) neurons, which decrease food intake. The second stage of regulation results in the firing of the orexigenic neurons through neuropeptides Y (NPY) and agouti-related peptide (AgRP), which increases appetite. Other regulatory molecules involved are leptin, insulin and PYY can directly diffuse into ARC, where they exert indirect influence on food intake regulation by suppressing expression of NPY and AgRP. Adapted from (Troke et al., 2014).
1.2.1.3 Origins of appetite regulation

There is some evidence that the caudal brainstem nucleus of the solitary tract (NTS) in the CNS is involved during eating, as it can produce signals to restrict the amount of food consumed. This signalling system is activated when it detects nutrient chemicals and gut peptides in the gut via vagal and non-vagal visceral afferent. The system connects with the hypothalamus and limbic system in order to suppress food intake (Berthoud et al., 2001, Chambers et al., 2013). The amygdala is a group of nuclei that play an important part in the limbic system, and it plays an important role in body weight regulation and dietary behaviour (Berthoud et al., 2001, Harrold et al., 2012).

1.2.1.4 The peripheral control of food intake

The hypothalamus receives peripheral signals from the gut, liver, pancreas and adipose tissue stores, which all influence the regulation of appetite and food intake (Berthoud and Levin, 2015).

1.2.1.5 Adipose tissue

Adipose tissue is a metabolically active tissue that secretes a wide range of hormones and adipokines in response to changes in energy balance and inflammatory status (Ahima and Lazar, 2008). The hormone leptin plays an important role in the regulation of feeding behaviour; it activates POMC and melanocortin, and also causes a decrease in AgRP, which stimulates the signalling of MC4R and as a result food intake decreases and EE increases. Furthermore, the leptin signal produced is proportional to adipose tissue mass and it can affect the reward centres of the brain and feeding behaviour (Farooqi et al., 2007). Adiponectin released from adipose tissue also plays a major role in reducing EE and inducing EI (Kadowaki et al., 2008).
1.2.1.6 Gastrointestinal hormone and endocrine pancreas

The peripheral signals from the gut work by stimulating both vagal afferent but also in many cases by crossing the incomplete blood-brain barrier (Gejl et al., 2014). One of the main gastrointestinal signalling hormones is ghrelin; it is an orexigenic hormone which is produced by the P/D1 cells in the human stomach, duodenum, ileum, caecum and colon. It influences appetite by acting on the NPY and AgRP neurons and it has been suggested that a high ghrelin concentration might be as a result of food anticipation (Kojima and Kangawa, 2008).

All other hormones released from the gut in Table 1.1. Inhibit food intake in the GI tract (with the GI tract going from the stomach to the rectum), almost all of which have been implicated in feeding behaviour. One of the key biomarkers for satiety, GLP-1, may have a determining influence on hunger and food consumption (Gibbons et al., 2013).

Table 1.1 The actions of the gut hormones

<table>
<thead>
<tr>
<th>Gut hormone</th>
<th>Effect</th>
<th>Mechanism &amp; receptors</th>
<th>Secretion site</th>
<th>Additional action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin</td>
<td>↑ Hunger</td>
<td>GHS in the brain</td>
<td>Stomach</td>
<td>Growth hormone secretion Long-term effect of EE</td>
</tr>
<tr>
<td>CCK</td>
<td>↑Satiation</td>
<td>CCK1.2 via vagus nerve</td>
<td>I cell of small intestine (duodenum and jejunum)</td>
<td>Delay gastric emptying</td>
</tr>
<tr>
<td>GLP-1</td>
<td>↑ satiety</td>
<td>GLP-1 in brain</td>
<td>L cell in intestine and brain</td>
<td>Incretin (stimulate insulin production), delay gastric emptying and decrease blood glucose</td>
</tr>
<tr>
<td>OMX</td>
<td>↑ satiety</td>
<td>GLP-1 in brain</td>
<td>L cell in intestine and brain</td>
<td>Slow gastric emptying</td>
</tr>
<tr>
<td>PYY(3-36)</td>
<td>↑ satiety</td>
<td>Y2 in brain</td>
<td>L cell in gut (ileum, colon and rectum)</td>
<td>Delay gastric emptying and intestinal transport</td>
</tr>
<tr>
<td>PP</td>
<td>↑ satiety</td>
<td>Y4,Y5 in brain and via vagus nerve</td>
<td>F cell in pancreas</td>
<td></td>
</tr>
<tr>
<td>Amylin</td>
<td>↑ satiety</td>
<td>AMY1-3</td>
<td>Pancreatic β cell</td>
<td>Decrease blood glucose level</td>
</tr>
<tr>
<td>Glucagon</td>
<td>↑ satiety</td>
<td>GCGR</td>
<td>Pancreatic α cell</td>
<td>Increase blood glucose and insulin secretion</td>
</tr>
</tbody>
</table>

GHS, Growth hormone secretagogue receptor; PYY peptide YY; PP pancreatic polypeptide; GLP-1 glucagon-like peptide-1; OXM oxyntomodulin; CCK cholecystokinin; GCGR glucagon receptor; EE energy expenditure. Adapted from (Benelam, 2009, Suzuki et al., 2010).
1.2.1.6.1 Glucagon-like peptide-1 (GLP-1)

Within the ileum and colon GLP-1 (an incretin hormone) is released by the endocrine cells. All GLPs are synthesized from proglucagon in response to intraluminal nutrients by the tissue specific prohormone convertase, which is found in the distal gut in the L-cells (Degen et al., 2006, Dailey et al., 2011). GLP-1 can cause glucose-dependent insulin secretion and also inhibits glycogen release which can alter appetite (Dailey and Moran, 2013).

1.2.1.6.2 GLP-1 and the brain

There are two main hypotheses surrounding the control of GLP-1 secretion, the first is that it is under neural regulation, as GLP-1 receptors are expressed in the brain in regions such as the ARC and hypothalamic regions involved in food intake regulation (Richards et al., 2014). The second hypothesis suggests the control of GLP-1 is hormonal, and is influenced by gut hormones including CCK, as this is where they are synthesized (Deacon, 2005).

The first hypothesis is supported by the fact that GLP-1 can enter areas of the brain that lack a complete blood-brain barrier, such as the postrema area and the subfornical organ in the CNS (Deacon, 2005, Gejl et al., 2014). Further evidence of a direct effect of GLP-1 on the brain comes from the link between increased activation in the hypothalamus and the postprandial plasma concentration of GLP-1, which was highlighted by a recent brain mapping study (Pannacciulli et al., 2007).

1.2.1.6.2.1 GLP-1 and appetite

There is evidence that an exaggerated GLP-1 secretion can directly cause a reduction in appetite and food intake via its effects on glucagon and insulin secretion (Dirksen et al., 2010). Other studies have shown this effect can be re-produced by intravenous infusions of GLP-1, which reduced food intake and weight gain (Torekov et al., 2011), but did not totally suppress appetite (Verdich et al., 2001a). GLP-1 can also affect appetite as it is involved in the ‘ileal brake’
mechanism; this mechanism is part of a feedback loop that regulates the flow of nutrients entering the small intestine to keep it at a digestible flow rate, which allows efficient nutrient uptake (Degen et al., 2006). The first secretion peak for GLP-1 is observed about 15–30 min after digestion, which is before the nutrients enter the colon and distal ileum, and may be involved in meal termination (Marathe et al., 2013, Bowen et al., 2006). The intensity of the first peak is affected by the concentration of carbohydrates (CHO) and protein (equivalent calories from fat) in the ingested meal (Pannacciulli et al., 2007). The second peak of GLP-1 secretion is probably triggered by nutrients in the large intestinal lumen, and therefore the extent of secretion may be dependent on metabolite production by gut microbiota, including short chain fatty acids such as propionate, acetate and butyrate (Delzenne et al., 2010). The second peak occurs several hours after ingestion, which makes it difficult to observe as it would require the blood sampling to be taken at the correct time (Pannacciulli et al., 2007, Delzenne et al., 2010).

1.2.1.6.2.2 GLP-1 and nutrients

The main meal component that acts as a potent GLP-1 secretion stimulant is glucose, but other sugars such as fructose and also some proteins are effective at stimulating secretion (Tolhurst et al., 2009, Wu et al., 2010). Studies suggest GLP-1 secretion is mainly nutrient-related; when compared to an iso-energetic high-protein lunch the highest GLP-1 release occurs after a high CHO lunch, but GLP-1 release may not be quantitatively synchronized with satiety as high-protein is more satiating than the CHO lunch (Smeets et al., 2008). The GLP-1 level can also be increased after consumption of indigestible CHO or different types of fibre, suggesting the nutrient type is important in GLP-1 response (Juntunen et al., 2002).

There is evidence that gut absorption is vital in stimulating GLP-1 secretion, there is only a response if glucose is administered orally and not intravenously (Nauck et al., 2004). During
fasting, adults generally have a plasma concentration of amidated GLP-1 between 5–10 pmol/l and after consumption of a mixed meal, this can rise to 50 pmol/l, showing GLP-1 secretion can increase 10-fold after ingestion (Padidela et al., 2009).

1.2.1.6.2.3 GLP-1 and obesity

GLP-1 may have a role in the development of obesity as GLP-1 secretion can be reduced in obese patients, which reduces the satiety signal and allows an increase in food consumption (Torekov et al., 2011). The metabolic effects of GLP-1 is conserved in diabetes and obese patients (Zander et al., 2002, Cegla and Bloom, 2014), but there is a decreased response in obesity (compared to lean individuals), which can be restored following weight-loss (Verdich et al., 2001b, Torekov et al., 2011). Further evidence of the important role that GLP-1 (and also PYY) plays in appetite regulation can be found by studying patients after gastric bypass surgery; the results show elevated postprandial secretion of both hormones, which can cause reduced appetite and food intake and result in weight loss (Morinigo et al., 2006).

A potential treatment for obesity (currently only licenced in the UK for the treatment of T2DM) is liraglutide, a GLP-1 agonist that can delay gastric emptying and reduces glucagon secretion (Raun et al., 2007). It is long acting as it has a plasma half-life of 13 hours, and can act via the brain and also the gastrointestinal tract as shown by the investigation in minipigs, where liraglutide reduced meal size and feeding frequency, which resulted in weight loss (Raun et al., 2007).

1.2.1.7 Other factors

There are various processes controlling meal inhibition and meal termination, such as meal size, meal frequency and meal composition, as well as food intake regulation related to energy requirements showed in Figure 1.3 (Schwartz and Zeltser, 2013, Blundell, 2010). Moreover, food intake behaviour, particularly appetite, is dependent on the energy density and palatability
of food, the social situation, culture, environment and emotional cues (Cruwys et al., 2014). The CNS especially the hypothalamus, plays a role and this information is combined with sensory information such as sight, taste and smell, which also interacts with the internal physiological signals and with the nutrient and energy status in the body to determine the meal composition, duration and size (Rolls, 2007, Rolls, 2012). Moreover, the smell and the sight of the meal, which also generates the physiological signals, stimulate appetite. Nevertheless, the signals that provide information about the amount and composition of ingested nutrition in the body are the gastric and intestinal signals (Chaudhri et al., 2008). The gut peptides and hormones are stimulated by the intra-luminal food and nutrient content (Chaudhri et al., 2008), and food can influence appetite and food intake by some psychological and physiological factors, which interact to switch between hunger and satiety (Blundell, 2010). Besides hormones, various physiological (gender, body weight), and psychological factors (cognitive factor, motivation to eat, dietary restraint, and palatability) collectively determine overall appetite and amount of EI (King, 1999, Martins et al., 2008a).
The satiety cascade shows psychological and physiological factors that modulate the structure and pattern of meals from the consumption of a food, which also affects the appetite sensations (Blundell, 2010).

1.2.2 Energy Expenditure (EE)

1.2.2.1 Physical activity (PA)

Physical activity (PA) is the most variable component that plays a role in the energy balance between food intake and EE. This balance is regulated by the peripheral nervous system (Stanley S, 2005, Hall et al., 2012), and the combination of increasing energy-dense foods with a decrease in PA, either in the quantity or the intensity of the PA, causes a change in lifestyle toward a positive energy balance (Caballero, 2007, Hall et al., 2012).

1.2.2.2 Physical activity (PA) and total energy expenditure (TEE)

The brief definition of the total energy expenditure (TEE) is the sum of energy expended in PA, resting energy expenditure (REE) and the thermic effect of food (TEF) (Coulston et al.,
REE has been defined as the amount of energy expended measured in the fasting and resting state in a temperate and thermo-neutral environment (Berdanier et al., 2007). REE is dependent on age, gender, body mass and composition. Likewise, TEF is defined as the dietary-induced thermogenesis and refers to the energy cost of digesting and absorbing food, which is difficult to assess (Thearle et al., 2013).

1.2.2.2.1 Exercise and Physical Activity (PA)

There is a difference between PA and exercise, PA is any bodily movement produced by skeletal muscles that results in EE and is usually measured in kilocalories (Kcal) per unit of time (Caspersen et al., 1985, Butte et al., 2012). However, exercise is a subset of PA that is planned, structured, and repetitive and has as a final or an intermediate objective, the improvement or maintenance of physical fitness (Vainio et al., 2002). Exercise EE can be referred to as exercise activity thermogenesis (EAT). The definition of PA encompasses the full range of human movement from competitive sport and exercise to hobbies or activities involved in daily living i.e. any activity above a resting state (described as ‘a state in which bodily movement is minimal and EE approximates the resting metabolic rate’ (Vainio et al., 2002)). Another definition for PA views it as a complex, multi-dimensional behaviour, with many different modes of activity contributing to total PA; these include occupational, household (e.g. caregiving, domestic cleaning), transport (e.g. walking or cycling to work) and leisure-time activities (e.g. dancing, swimming), which can be called non-exercise activity thermogenesis (NEAT) (Levine, 2004).

1.2.2.2.2 Physical activity (PA) and obesity

According to the WHO assessment of the observational and trial evidence, the risk of weight gain and obesity is increased by sedentary lifestyles and is decreased by regular PA (WHO, 2003).
In 1980, the number of people who could not control their energy balance reached the critical point, when measured by the average daily physical activity level (PAL) (TEE/REE) (Hill and Wyatt, 2005). This might be due to an increase in technology, which includes computers and telecoms, as in a sedentary lifestyle the body starts to store an excessive amount of energy and the prevalence of obesity is increased (Hill and Wyatt, 2005). Epidemiological data suggests that PAL should be 1.6 to avoid weight gain (SACN, 2012). The link between physical inactivity and risk of weight gain and obesity has been observed by several reports (WHO, 2003, Department of Health, 2004), and exercise chronically influences individuals either by changing their behaviour or by improving their fitness or both, and it also acutely stimulates NEAT as a contributor to TEE (Speakman and Selman, 2003).

1.2.2.3 Exercise intensity

The metabolic equivalent (MET) is a unit used to estimate the metabolic cost of EE or oxygen consumption of PA (Black, 2000). One MET is a person's metabolic rate when at rest, which is set as REE, approximately 3.5 ml of oxygen consumed per kilogram of body mass per minute (Hills et al., 2014). It is used to define categories of intensity of PA, for example, as light, moderate and heavy intensity of exercise (Westerterp and Plasqui, 2004).

Differences in body size influence exercise EE of the same mode and intensity, which means in the same task of exercise, a heavier person is expending more energy than a lighter person (Vainio et al., 2002). MET values are given in multiples of REE and are assigned to activities to denote their intensity. There is another expression for intensity of PA which is measured as a percentage of a person's maximal oxygen consumption (VO$_{2\text{max}}$). As oxygen consumption and heart rate (HR) during maximal oxygen consumption are well correlated, the percentage of maximal HR is often used to reflect the relative effect on VO$_{2\text{max}}$ (Vainio et al., 2002). Other categories can be used to define PA such as frequency, duration and intensity. The first and
second terms are linked with amount and number of tasks in particular time, whereas intensity refers to a performer's workload or it is associated with physical work and EE rates (King et al., 1994, Warren et al., 2009). This lack of consistency is probably due to an exercise and an excess post-exercise oxygen consumption: differences in methodology and study protocol, namely the intensity of the exercise (Thompson et al., 2012).

1.2.2.4 Current Recommendations

In 2011, the American College of Sports Medicine's (ACSM) overall recommendation was for most adults to engage in at least 150 minutes of moderate-intensity exercise each week as shown in Figure 1.4 (Garber et al., 2011).

![Figure 1.4](image)

**Figure 1.4** The ACSM’s recommendations for exercise.

- **Cardiorespiratory Exercise:**
  
  - at least 150 minutes of moderate-intensity exercise per week.
  - 30 to 60 minutes of moderate-intensity exercise five days per week.
  - 20 to 60 minutes of vigorous-intensity exercise three days per week.
  - One continuous session and multiple shorter sessions of at least 10 minutes are both acceptable.

- **Resistance Exercise:**
  
  Adults train each major muscle group two or three days each week.

- **Flexibility Exercise:**
  
  Flexibility exercises at least two or three days each week to improve range of motion.

- **Neuromotor Exercise:**
  
  Neuromotor exercise, also referred to as "functional fitness training," is recommended two or three days per week. Exercises should involve motor skills (balance, agility, coordination and gait), proprioceptive exercise training, and multifaceted activities.

Exercise is classified into categorize cardiorespiratory exercise, resistance exercise, flexibility exercise and neuro-motor exercise (Garber et al., 2011)

The WHO recommendation for adults aged 18-64 is at least about 150 minutes of moderate intensity aerobic, or 75 minutes of vigorous-intensity aerobic physical activity, or a combination of moderate and vigorous-intensity activity should be performed per week, which
is in agreement with the ACSM guidelines (WHO, 2010). This should be increased to 300 minutes of moderate-intensity aerobic or 150 minutes of vigorous-intensity aerobic, or an equivalent combination of moderate- and vigorous-intensity activity, to produce more health benefits. Aerobic activity should be performed in bouts of at least 10 minutes duration, and muscle-strengthening activities should be undertaken involving major muscle groups on two or more days a week. These recommendations aim to improve cardio-respiratory and muscular fitness, bone health, reduce the risk of non-communicable diseases (NCDs) and depression (WHO, 2010).

When considering the contribution of activity to TEE, you need to consider that the energy cost of activity itself is equal to physical activity energy expenditure (PAEE) combined with excess post-exercise oxygen consumption (EPOC) and active (EPOC). See Figure 1.5 (Speakman and Selman, 2003).
EPOC has two phases, the first is less than two hours and the second phase can extend up to 48 hours (Speakman and Selman, 2003). EPOC depends on the duration and intensity of exercise and the influence of TEF (Børsheim and Bahr, 2003), and it can increase with interval-type exercise (Laforgia et al., 2006, Laforgia et al., 1997). If the exercise intensity is between 50–60 % \( \dot{V}O_2\text{max} \), this can lead to induction of EPOC for several hours (Børsheim and Bahr, 2003). Indeed, it has been thought that EPOC leads to an increase in the REE following acute exercise and can lead to an increase in fat oxidation for many hours post-exercise (Henderson et al., 2007). Studies that estimate changes in REE after exercise often have a limitation mainly due to the difficulty of measuring the REE accurately for up to in 48 hours post-exercise, as it is time-consuming, costly and it is often unavailable (Malavolti et al., 2007).
When exercising over long time periods obese people mismatch their EE and EI, whereas lean subjects can increase their EI and have no significant change in body mass (Melzer et al., 2005). This may be due to the adipose in obese people acting as an energy buffer, which means a change in EI in response to activity is not effective until the excess stored energy is depleted and the risk to energy homeostasis is removed (Melzer et al., 2005). This is in contrast to well-trained athletes who expend vast quantities of energy during training and can match EE with EI very well, which allows them to maintain an energy balance and avoid weight loss resulting in a poor performance (Melzer et al., 2005).

Studies so far are inconclusive as to whether there is a potential effect of exercise mode, gender and training status on EPOC (Børsheim and Bahr, 2003).

EPOC occurs post-exercise and during recovery to help return metabolic processes to the previous conditions, and has been associated with an increased body temperature, an increase in pulmonary and cardiac function, H+ and lactate removal, glycogen resynthesis and catecholamine effects (Tomlin and Wenger, 2001).

During exercise, the concentration of catecholamines undergoe a linear increase which correlates to the exercise duration and intensity, which is similar to the linear relationship observed between exercise duration and intensity and EPOC level (Børsheim and Bahr, 2003). Catecholamines are one of the main regulators of triglyceride (TAG) and fatty acid (FA) synthesis and oxidation, and they work via the stimulation of lipolysis; these processes are increased after exercise and may significantly contribute to EPOC, as will the influence of catecholamines on processes during exercise (Børsheim and Bahr, 2003). Studies suggest that EE during exercise has the largest impact on body mass. How this is combined with the effect of EPOC and results in fat loss needs to be investigated fully in future research (Laforgia et al., 2006).
1.2.2.5 The role of exercise

1.2.2.5.1 The effects of exercise on appetite and satiety

An exercise and appetite study in lean and obese participants has suggested that an acute bout of exercise can lead to a large energy deficit, but it does not necessarily lead to an increase in hunger as a compensatory response (Blundell et al., 2003). In contrast, Hubert et al. (1998) found that an increased appetite and EI occurs as a result of negative energy balance and low EI. In fact, EI may be inhibited during and after short-term exercise, and also as a result of an increase in fuel mobilisation in the blood such as glucose, non-esterified free fatty acids (NEFA) and plasma lactate (Melzer et al., 2005). In addition, Long et al. (2002) showed that there were no significant differences between the hunger score post high energy or low energy preload for active and sedentary groups (Long et al., 2002). However, they also showed a better compensatory response to previous preload energy was found in active men compared with inactive men (Long et al., 2002). The effect of exercise can be extended to modulate EI in addition to its effect on negative energy balance (King et al., 1997b), and the role of exercise has an indirect effect by leading to more sensitive behaviour in response to previous EI (Martins et al., 2007b).

Exercise can potentially effect appetite by reducing hunger, which is caused by an elevated body temperature and lactic acid level in the blood (King et al., 1997b). Overall, feelings of hunger are suppressed by intense exercise within one day, but there is no evidence for exercise induced anorexia after moderate-or low intensity exercise (LI) (Westerterp-Plantenga et al.,
This may be due to the distribution of blood after exercise, which is directed away from the gut to the muscles (Blundell et al., 2003).

In the case of high intensity exercise (HI), when EI cannot match body demand, the body tends to use stored adipose tissue to balance body energy, and therefore EI is not going to increase unless lean body mass is threatened (Blundell et al., 2003).

The majority of studies using human subjects have found that exercise caused non-significant changes to hunger, fullness or appetite (Thompson et al., 1988, Reger and Allison, 1987, Borer et al., 2005). However, some studies have found that for healthy lean female subjects, EI was lowered only after HI exercise (Kissileff et al., 1990). Therefore it remains important to fully understand the influence of exercise on resting appetite and EI response in the hours post-exercise (Deighton and Stensel, 2014).

After acute bouts of HI exercise there is a large energy deficit, but EI and appetite may not respond to compensate manner (King et al., 2011). In the short term there is no evidence for exercise increasing hunger, appetite or EI, but there is some evidence that HI exercise can acutely suppress hunger (exercise-induced anorexia) (King et al., 1994, Thompson et al., 1988). It does this by modulating peripheral appetite hormones to enhance satiety during and post-exercise (Broom et al., 2007, Broom et al., 2009, King et al., 2010a, King et al., 2010b).

Other studies have shown that HI exercise increased subsequent EI and appetite sensations, suggesting further study is required (Pomerleau et al., 2004, Maraki et al., 2005).

There are different mechanisms that can have an impact on the effect that exercise has on appetite regulation, these mechanisms can either improve the sensitivity of the satiety cascade by regulating macro-nutrient preference and food choices, or they can alter the hedonic
response to food (Blundell et al., 2003), although appetite may not always reflect actual EI or food intake (Mattes, 1990).

1.2.2.5.2 The effects of exercise on energy intake (EI)

The effect of exercise is to induce a negative energy balance, and the extent to which this occurs depends on the impact it has on EE, and the effect is linked to changes in post-exercise EI and also on the extent of compensatory responses (Tremblay et al., 1994, Martins et al., 2010). Research over the past two decades has highlighted the importance of the intensity of the exercise, as this can be used as a way to regulate the energy balance (King et al., 1997a). Regular exercise has been shown to have a positive psychological effect, with improvements observed in the sense of well-being, self-confidence, self-image and reduced anxiety, all of which can effect EI (King and Tribble, 1991, Durrant et al., 1982). There is a general trend that people usually overestimate exercise induced EE and underestimate the energy value of food, especially high energy dense food, which leads to excessive food consumption as a reward for exercise (Willbond et al., 2010, Allcott, 2011). This can lead to people failing to properly adjust their eating behaviour, resulting in exercise being an unsuccessful approach to weight loss (King, 1999). Studies suggest that acute exercise decreases EI and can cause a significant suppression in hunger, for example after 2 h of cycling at 60 % VO$_{2\text{max}}$ workload there was a reduction in hunger for both normal-weight and obese men (Westerterp-Plantenga et al., 1997).

In contrast, other studies have reported that exercise increased subsequent EI on the same day (Pomerleau et al., 2004), and that men failed to compensate for exercise-induced EE by increasing their EI after exercise (Imbeault et al., 1997, King et al., 1997a). Problems can also occur when men performed high levels of exercise on seven consecutive days without compensating their EI (King et al., 1994, Stubbs et al., 2002). The term ‘relative energy intake’ (REI) can be used to account for the energy cost of exercise, as this variable can be significantly modified by a long duration HI session, and some studies have reported that intense exercise
of long duration reduces the relative EI (post-exercise EI corrected for the energy cost of exercise above the resting level) (Westerterp-Plantenga et al., 1997).

The effect of diet composition on EI needs to be considered when examining the potential effect of exercise on food preferences and relative macro-nutrient intake, for example studies have shown exercise can induce a substantial increase in CHO intake or protein intake, but this is not universal across all studies (Thompson et al., 1988, Verger et al., 1994, King et al., 1994).

Experimental evidence suggests that the type of substrate oxidized during exercise also influenced EI post-exercise, for example high-fat oxidation during exercise decreased EI post-exercise (adjusted for the energy cost of exercise) when compared with subject who has a less energy from fat oxidation during exercise (Alméras et al., 1995). This also suggests that exercise might alter the effect of the high fat diet on EI post-exercise via its influence on fat oxidation (Tremblay et al., 1989).

1.2.2.5.3 The metabolic effect of exercise

1.2.2.5.3.1 The effects of an exercise-induced energy deficit

Exercise is often recommended to individuals who are attempting to reduce their body weight, as exercise can increase EE (Donnelly et al., 2009), this can create an energy deficit which may be compensated through an increase in EI thereby restoring energy balance, but if this is incorrectly managed it can create a positive energy balance resulting in weight gain not loss (Blundell and King, 2000).

It has been shown that both lean males and females can tolerate an energy deficit caused by increased EE for about 14 days without an increase in EI (Whybrow et al., 2008). After this time the body starts to compensate for about 30 % of the deficit by increasing EI. This
compensation occurs over a relatively long duration of about a couple of weeks, and there is an inter-individual difference in this adjusted EI response, but the mechanism is still unclear (Whybrow et al., 2008).

It is has been reported that post-exercise EE increases proportionally with exercise intensity (Smith and Mc Naughton, 1993, Malatesta et al., 2009). By comparing studies that were matched for EE, the results show conflicting findings; some suggest that after the exercise intensity is increased post-EE increases and RQ decreases during the post-exercise period (Phelain et al., 1997), but many other studies report no effect of intensity on post-exercise REE, especially when exercise bouts are tightly matched for EE (Mulla et al., 2000, Kuo et al., 2005).

1.2.2.5.3.2 The effects of exercise on gut peptides

Studies have demonstrated that exercise has beneficial effects in reducing appetite, modifying EI and allowing better energy compensation for high-energy loads (Martins et al., 2010). This acute suppression of hunger has often been called “exercise-induced anorexia”, and it has been shown to operate partly through modulation of gut hormone secretion (Martins et al., 2010), and has been linked to the increase in satiety hormones, such as PYY, GLP-1 and PP, during exercise (Martins et al., 2007a). This increase was in contrast with hunger score, which was significantly decreased during exercise; however exercise did not have the same effect on hunger score post-exercise (Martins et al., 2007a).

Studies have also shown there was no significant change in postprandial ghrelin plasma level during exercise, and that fasting ghrelin level was not changed as a response to acute exercise in normal-weight subjects (Schmidt et al., 2004, Martins et al., 2007b). Therefore further studies are needed to determine the optimal intensity, duration and frequency of exercise to improve short-term appetite control, and the mechanism behind it also needs to be elucidated (Martins et al., 2008b).
1.2.2.5.3.3 The effects of exercise on GLP-1

Studies have demonstrated that in obese and lean individuals, a single session of aerobic exercise could cause a significant increase in GLP-1 and PYY plasma levels and cause a decrease in the following EI, but the different effects on these gut hormones and EI need to be determined (Ueda et al., 2009b). GLP-1 and PYY have similarities, they have the same release sites and inactivated by the same enzyme (Dipeptidyl peptidase-4 (DPP-4)) but at different points in each system; PYY1–36 is converted to PYY3–36 (Medeiros and Turner, 1994), and GLP-1 uses this enzyme at a later stage by converting GLP-17–36 amide to GLP-19–36 amide (Deacon et al., 1995). As this enzyme is used by both hormones it has the potential to be altered post-exercise, and therefore studying its levels could provide information about how these hormones are altered by exercise (Ueda et al., 2009a). It has been reported that in normal-weight and obese men there is no change in orexigenic hormones and there is a significant increase in GLP-1 and PYY plasma levels during exercise of moderate-intensity (compared with rest) (Ueda et al., 2009a). Other studies have also reported that in athletes and normal weight people, there is a short term increase in the GLP-1 plasma levels following acute exercise (O’connor et al., 1995, O’Connor et al., 2006, Martins et al., 2007a). By combining these results with the observation that hormone secretion is affected by the intensity and type of nutrient stimuli, this data suggests hormone secretion control is multi-faceted and can be altered by different intensities of exercise (Huda et al., 2006).

1.2.2.5.4 The effect of exercise on utilisation of energy substrates

After consumption, CHO and fats are immediately oxidized and their contribution to energy levels are dependent on a number of different factors; for example at rest healthy subjects use CHO preferentially and after consumption glucose availability affects the utilisation of fat and CHO (Melzer, 2011). Exercise intensity and duration can alter substrate use, as duration increases, so too does fat utilisation (Melzer, 2011). The ability to oxidise fatty acids such as:
NEFA is related to improved performance, and these changes are likely to be the result of an overall increased aerobic capacity (Achten et al., 2002). Studies of VO$_{2\text{max}}$ show that fat rather than glycogen is utilised following exercise in well trained people, and exercise increases fat oxidation (24 hours post exercise) in response to increased intake of dietary fat (Van Baak, 1999). For people with a PAL of 1.4, 1.6 or 1.8, after the transition from a low-fat controlled diet to a high-fat diet, fat oxidation at 2 hours can be increased by increasing the level of exercise undertaken (Van Baak, 1999).

HI favours a lower body fat deposition as it acutely increases post-exercise energy metabolism-related variables, an effect which can be observed by increased exercise intensity in test subjects (Yoshioka et al., 2001). A HI training programme has been shown to induce a greater reduction in subcutaneous fat (compared to moderate intensity), possibly due to the increase in lipid oxidation occurring in the skeletal muscle (Tremblay et al., 1994). This suggests that for a specific EE, HI could produce a larger negative energy balance and increased fat loss, however, other studies suggest that HI aerobic exercise, matched with LI for energy expended during exercise, has a similar effect on the 24 hour nutrient oxidation (Henderson et al., 2007).

A study to determine the effect of a 12 week regime of 3 sessions of 20 min of HI a week on body composition of young overweight males, showed the exercise group had a significantly higher (13 %) fat oxidation and a lower CHO oxidation (compared to the controls), and that the HI significantly reduced abdominal, visceral, trunk, and total fat for the test subjects (Heydari et al., 2012). Other studies have shown that 6 to 7 sessions of HI could lead to a significant increase in fatty acid oxidation in skeletal muscle and the whole body (Burgomaster et al., 2005). In addition it is feasible that the significant levels of catecholamines generated during acute HI could increase post-exercise fat oxidation (Trapp et al., 2007), and may also alter the TAG/FA cycling/re-esterification during the post-exercise recovery period, at a time when the intracellular FA pool size might be changing (Henderson et al., 2007).
Furthermore, there may be an increased fat oxidation after HI due to the need to remove lactate and hydrogen (H+) and to resynthesize glycogen (Stiegler and Cunliffe, 2006). Other factors that can contribute to increased EE and fat oxidation after exercise are uncoupled respiration, protein turnover, and sympathetic nervous system activity (Stiegler and Cunliffe, 2006).

After a bout of HI, the acute responses observed include a significant increase in heart rate, catecholamines, cortisol, growth hormone, glycerol, lactate and glucose plasma levels, a significant decrease in parasympathetic reactivation, and also a depletion of ATP, phosphorylated creatine (PCr), and glycogen stores (Boutcher, 2010). There has been significantly less research on the characteristics of an exercise that affect post-exercise metabolism, particularly fat oxidation when compared to studies on the effects of substrate oxidation during exercise (Kuo et al., 2005), but it has been suggested that post-exercise EE and fat oxidation can be augmented by increasing exercise intensity (Warren et al., 2009).

To maximize fat oxidation the focus should be on the exercise bout rather than the post-exercise period (Laforgia et al., 2006). For example, the ability of HI training to promote a higher fat loss depends in part on the acute effect of exercise on EI; long term in the regular exerciser this may represent a substantial negative energy balance (Imbeault et al., 1997).

Stubbs and colleagues showed that a reduction in exercise can lead to a significant decrease in EE (Stubbs et al., 2004). However, it does not necessarily lead to a reduction in appetite and EI. In the study of lean men EI and appetite were rated over nine days of a sedentary routine and compared with their result over moderate activity routines, and overall the sedentary routines led to a positive energy balance and weight gain (Stubbs et al., 2004). This continuation of EI despite lower EE has been confirmed by other studies which also found that when subjects were obliged to become sedentary, their eating behaviours continued in their habitual form (Blundell and King, 2000).
1.2.2.6 The effect of exercise on the other factors

1.2.2.6.1 Gender difference in the effect of exercise

The comparison of energy substrate oxidation in exercising men and women have shown that women derive a larger proportion of EE from lipid than men (Carter et al., 2001). There appears to be no direct correlation between activity-induced EE and EI, but it is noticeable that women show much less weight loss and are less successful in achieving the goal of fat loss in response to exercise interventions than men (Westerterp et al., 1992, Donnelly and Smith, 2005). However, this may be due to the lack of similarity between the studies used rather than differences due to gender (Imbeault et al., 1997).

There is some evidence that a group of women (with a gynoid fat distribution pattern) may even gain weight upon exercising rather than losing (Blundell and King, 2000). It has also been shown that some women exhibit a stronger (although still incomplete) compensatory increase in EI in response to severe exercise, when compared to men (Blundell and King, 2000). A possible reason for this may be due to the fact that women possess biological mechanisms designed to preserve their fat stores and to protect their physical integrity for purposes of child rearing (Blundell and King, 2000).

Studies suggest that women oxidize more lipid than men during exercise, but lipid mobilization and metabolism are increased to a greater extent in men than women during the first 3 h of the post-exercise recovery period (Henderson et al., 2007). This difference may partly be due to the significantly greater fat stores found in women, this is supported by the fact that when the values are corrected for mass of adipose tissue, there is no such gender-related difference (Bülow et al., 2006). Despite having lower concentrations of catecholamines in arterial blood, women show evidence of greater lipolysis during exercise at the same relative intensity compared to men (Davis et al., 2000). This suggest that at least at the whole body
level, the gender-related differences observed during exercise are reversed in the hours after exercise (Thompson et al., 2012).

1.2.2.6.2 Body mass index (BMI) difference and the effect of exercise

In overweight/obese individuals, iso-caloric bouts of moderate or HI exercise can lead to a similar appetite response (Martins et al., 2014, Alkahtani et al., 2014). This result correlates with previous findings that in lean individuals acute exercise, even at HI, does not induce any known physiological adaptation that could lead to increased EI (Martins et al., 2014). By altering the type and intensity of exercise it may be possible to alter appetite-related factors, for example a recent study reported that for obese adolescents after a session of HI (75 % VO_{2max}) stationary cycling there was a reduced ad-libitum EI at the following lunch and dinner when compared with an iso-caloric session of LI exercise (40 % VO_{2max}) (Thivel et al., 2012).

The effect of different intensities of exercise on appetite in overweight men was investigated using three levels of exercise: continuous exercise (cycling at 60 % VO_{2max} for 30 min), HI intermittent exercise (cycling at 100 % VO_{2max}, for 1 min, then recovery at 50 % VO_{2max} for 4 min, repeated for 30 min), and very-HI (cycling at 170 % VO_{2max}, for 15 s, then recovery at 32 % VO_{2max} for 60 s, repeated for 30 min), all of which were compared to controls at rest (Sim et al., 2013). Immediately after exercise a standardised liquid meal was provided to allow measurements of EI to be taken at 70 minutes then for the total of 38 hours. before and after exercise there were no changes in subjective appetite score for any of the investigated regimes, but there was a reduction in ad libitum EI 70 minutes after the liquid meal post-exercise for all conditions except continuous exercise and rest (Sim et al., 2013). During the 38 hours after exercise there was a significant reduction in ad libitum EI for the group subjected to very-HI exercise (compared to rest or continuous exercise), which suggests if there is a high enough intensity to the exercise undertaken then there may be an influence on food intake even if there
is no change in subjective appetite (Sim et al., 2013). These results are in agreement with previous studies that found a HI exercise training programme can result in a greater subcutaneous fat loss when compared to moderate intensity exercise (Tremblay et al., 1994), but disagree with other studies conducted in moderately overweight women where they found that fat loss was dependent on TEE instead of exercise intensity (Grediggin et al., 1995), and studies that found weight and fat loss varied on an individual basis depending on duration, intensity and frequency of the exercise carried out (Church et al., 2009). Overall, these results suggest that further study is required to determine the effect of regular HI exercise on EI and fat loss in the long-term, in the obese and overweight population (Imbeault et al., 1997).

1.3 High intensity exercise (HI) vs. low intensity exercise (LI) studies

1.3.1 Appetite and energy intake (EI)

Studies into the effect of HI on EI are contradictory; several studies have shown that HI consistently suppresses appetite and EI (Kissileff et al., 1990, Imbeault et al., 1997, King et al., 1997a), whereas other studies have reported that acute bouts of HI either increase EI (Pomerleau et al., 2004) or have no effect on appetite and feeding behaviour (George and Morganstein, 2003, Tsolliou et al., 2003, Imbeault et al., 1997).

One of the ways HI has been shown to influence appetite is by altering the levels of peripheral appetite regulating hormones, as changes in the circulation of these hormones can modulate central appetite regulation and enhance satiety (Deighton et al., 2013b, Malik et al., 2008, Broom et al., 2007). However, moderate intensity exercise has been shown to have limited influence on these hormones, and may not affect central appetite regulation either (Unick et al., 2010, Cornier et al., 2012).

A study on the differences in post-exercise ad libitum food consumption and post-meal appetite sensations in obese and lean women examined the effects of 40 min of HI (90 W) and LI (30
W) intensity cycling, found there was a reduction in the food intake of lean women following HI (compared to LI), but exercise intensity did not affect the post-exercise EI of obese women; however they did report an increase in hunger following the test meal during the LI trials compared with the HI trials (Kissileff et al., 1981). There are however limitations in this study design; the intensity of the exercise bouts were absolute and not calculated relative to each participant’s individual fitness level, and the intensity of the HI bout (90 W) is quite mild for most individuals.

Current studies suggest that the effect on appetite and EI has only been investigated in a small range of HI (35 to 75 % of VO$_2$$_{\text{max}}$), and the results are equivocal (Deighton et al., 2013a). Of the studies in this area, Thompson et al. (1999) demonstrated there was a greater appetite suppression during exercise as intensity increased, but there was no difference in EI after exercise; (Imbeault et al., 1997) compared the effects in young males of HI (75 % VO$_2$$_{\text{max}}$) and LI (35 % VO$_2$$_{\text{max}}$) treadmill exercise on appetite, and found no significant differences in appetite, feelings of hunger or fullness, but post-exercise EI tended to be low after HI exercise, and REI was lower during HI versus LI trials. Conversely, Pomerleau et al. (2004) found that in active women both LI (40 % VO$_2$$_{\text{max}}$) and HI (70 % VO$_2$$_{\text{max}}$) walking stimulated food intake 1 hour post-exercise, however, there was no difference in daily EI between trials over the subsequent 3 days, suggesting the exercise-induced appetite stimulation was short-lived. Overall these findings conflict with those of Ueda et al. (2009a) where a transient suppression in feelings of hunger and a reduced EI in healthy males was observed for 1 hour after a 30 min bout of LI (50 % VO$_2$$_{\text{max}}$) and HI (70 % VO$_2$$_{\text{max}}$) cycling (when compared with at rest results), and there were no differences in the appetite or EI responses to different intensity exercise bouts (Ueda et al., 2009a).
1.3.2 Recent studies and limitations

The study conducted by Deighton et al. (2013b), which compared HI versus LI with a control with the same energy cost, concluded that an acute bout of energy matched LI and HI were equally effective at inducing an energy deficit without stimulation of compensatory increase in appetite, they also found no difference in EI after a bout of HI consisting of six 30-s sprints over 30 min compared with 60 min of endurance exercise and rest (Deighton et al., 2013a). This study was limited due to the small sample size, the fact that the participants were only lean males and that the exercise protocols were not matched for workload. This suggests the findings may not be generalise to other populations, such as the elderly or obese, therefore an investigation of the exercise effect for overweight and obese people needs to be conducted.

The conclusions of a recent meta-analysis showed there is little or no evidence that acute exercise can affect energy or macro-nutrient intake, which would produce a short-term negative energy deficit, but acute exercise was associated with positive changes in appetite-related hormones; it suppressed levels of orexigenic hormones, and simultaneously increased the levels of satiety peptides (PYY, GLP-1 and PP) (Schubert et al., 2013).

Another example of a study investigating the effect of different levels of exercise intensity on appetite comes from Sim and colleagues who randomly assigned overweight sedentary men to iso-caloric bouts of moderate intensity exercise (60 % VO$_{2\text{max}}$), HI (60 s at 100 % VO$_{2\text{max}}$: 240 s at 50 % VO$_{2\text{max}}$), very-HI (15 s at 170 % VO$_{2\text{max}}$: 60 s at 32 % VO$_{2\text{max}}$) and a control group. The results showed no differences in subjective appetite among all the conditions, but there was a lower ad libitum EI after HI and very-HI exercise (compared with control) and a significantly lower active ghrelin plasma level after very-HI exercise (compared with the other conditions), which suggests that exercise intensity does not change appetite sensation in overweight males (Sim et al., 2013). One of the key problems with this type of study is the fact
they included overweight males only, and exercise was performed fasted, which limits the generalization of the results to other populations and in conditions where exercise is performed in the postprandial state, which is the case in the majority of the situations (Martins et al., 2014). Some studies do include females and an obese/overweight group, but the number of participants in these groups is still small to compared the male subjects (Martins et al., 2014). Another study on overweight/obese men investigated the effect of HI vs LI showed no difference in appetite, between intensities (Alkahtani et al., 2014). The use of HI in studies has been questioned due to the high risk of nausea and increased risk of acute cardiovascular events during the intense exercise (Gibala et al., 2012). However, the protocol of HI proposed by Little et al., (2010) has demonstrated that a practical low-volume HI programme is effective for improving muscle metabolic capacity and functional performance, which has also shed light on the potential mechanisms by which exercise training promotes mitochondrial adaptations in skeletal muscle (Little et al., 2010).

Understanding the appetite response to popular exercise protocols is important as it will allow the most effective method of inducing negative energy balance without stimulating compensatory increase in appetite to be determined, as this is inversely associated with exercise induced weight loss (King et al., 2007). Overall it would be beneficial to conduct more randomised trials which include overweight, obese and sedentary people to allow a better comparison of the effects of exercise (Donnelly et al., 2014). More research is needed to identify the minimal dose of HI for the maximal health benefit and to identify the optimal length and intensity of the HI protocol for achieving varying health outcomes. See Table 1.2
Table 1.2 Effect of high (HI) vs. low exercise intensity (LI) on appetite, energy intake (EI) and appetite regulation hormones.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Participants</th>
<th>Trials</th>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Kissileff et al., 1990)</td>
<td>18 female</td>
<td>40 min cycle at 90W + 30W</td>
<td>Ad libitum EI</td>
<td>EI ↓ after high vs. LI cycling in lean group</td>
</tr>
<tr>
<td>(9 lean, 9 OB)</td>
<td></td>
<td>40 min rest</td>
<td>Appetite VAS</td>
<td>Hunger ↑ after LI vs. HI and rest Cycling in OB group</td>
</tr>
<tr>
<td>(Imbeau et al., 1997)</td>
<td>11 young males</td>
<td>~ 72 min walk (35 % VO\textsubscript{2max})</td>
<td>Ad libitum EI</td>
<td>No effect of exercise on EI, hunger, or fullness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~ 34 min run (75 % VO\textsubscript{2max})</td>
<td>Appetite VAS</td>
<td>REI ↓ after run vs. walk and rest</td>
</tr>
<tr>
<td>(Pomerleau et al., 2004)</td>
<td>13 active female</td>
<td>~40 min walk (70 % VO\textsubscript{2max})</td>
<td>Ad libitum EI</td>
<td>EI ↑ after high intensity walk vs rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~65 min walk (40 % VO\textsubscript{2max})</td>
<td>Appetite VAS</td>
<td>REI ↓ post-HI + LI vs. rest</td>
</tr>
<tr>
<td>(Erdmann et al., 2007)</td>
<td>6 males + 8 females</td>
<td>Cycling (30 min at 100 W + 50 W)</td>
<td>Ad libitum EI</td>
<td>Total ghrelin AUC ↑ during 50 W cycle vs rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycling (30, 60 +120 min at 50 W)</td>
<td>Appetite VAS</td>
<td>Total ghrelin unaffected by duration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45 min rest</td>
<td>Plasma total ghrelin</td>
<td>EI ↑ in longer duration, no difference between HI and LI</td>
</tr>
<tr>
<td>(Jurimäe et al., 2007)</td>
<td>9 males rowers</td>
<td>6.5 km row (above AT)</td>
<td>Ad libitum EI</td>
<td>Total ghrelin unaffected by exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.5 km row (below AT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ueda et al., 2009a)</td>
<td>10 healthy males</td>
<td>30 min cycle (75 % VO\textsubscript{2max})</td>
<td>Ad libitum EI</td>
<td>EI and hunger ↓ after HI and LI vs. rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min cycle (50 % VO\textsubscript{2max})</td>
<td>Appetite VAS</td>
<td>No change in GLP-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min rest</td>
<td>Plasma PY\textsubscript{Y}\textsubscript{3-36}</td>
<td>PYY\textsubscript{3-36} ↑ after HI vs LI.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Deighton et al., 2013b)</td>
<td>12 healthy males</td>
<td>4 min cycle (85 % VO\textsubscript{2max})*10 times+2 min rest</td>
<td>Ad libitum EI</td>
<td>No difference in EI. REI ↓ in HI and LI vs. control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 min cycle (60 % VO\textsubscript{2max})</td>
<td>Overall Appetite</td>
<td>Appetite ↓ during HI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma PY\textsubscript{Y}\textsubscript{3-36}</td>
<td>PYY\textsubscript{3-36} ↑ after HI and LI vs. control</td>
</tr>
<tr>
<td>(Sim et al., 2013)</td>
<td>17 overweight males</td>
<td>30 min cycle (60 % VO\textsubscript{2max})</td>
<td>Ad libitum EI</td>
<td>EI ↓ after HI and v HI vs. rest, EI ↓ after v HI vs LI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min cycle (60 s at 100 % VO\textsubscript{2max} + 240 s at 50 % at</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VO\textsubscript{2max}) : 1:4</td>
<td>Appetite VAS</td>
<td>No difference in appetite and VAS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min cycle (15 s at 170 % VO\textsubscript{2max} + 60 s at 32 % at</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VO\textsubscript{2max}) : 1:4</td>
<td>38h subsequent EI</td>
<td>Subsequent EI ↓ after HI vs LI and rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min rest</td>
<td>Ghrelin</td>
<td>Active ghrelin ↓ after v HI vs LI and rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glucose ↑ after v HI vs HI, LI and rest.</td>
<td></td>
</tr>
<tr>
<td>(Deighton et al., 2013a)</td>
<td>12 healthy males</td>
<td>30 min (6* 30 s sprints)</td>
<td>Ad libitum EI</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 min cycle (65 % VO\textsubscript{2max})</td>
<td>Appetite VAS</td>
<td>Appetite ↓ after HI vs rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rest condition</td>
<td>Plasma PY\textsubscript{Y}\textsubscript{3-36}</td>
<td>PYY\textsubscript{3-36} EI ↑ after HI vs rest</td>
</tr>
<tr>
<td>Study</td>
<td>Group</td>
<td>Intensity</td>
<td>Plasma ghrelin</td>
<td>Appetite VAS</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
<td>----------------</td>
<td>--------------</td>
</tr>
<tr>
<td>(Martins et al., 2014)</td>
<td>5 males + 7 females</td>
<td>250 Kcal (70% HR&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>Ad libitum EI</td>
<td>No difference in EI</td>
</tr>
<tr>
<td>Overweight/Obese</td>
<td>250 Kcal (85-90% HR&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>Appetite VAS</td>
<td>Ghrelin ↓ in 250 kcal HI and LI vs. rest.</td>
<td></td>
</tr>
<tr>
<td>Rest condition</td>
<td>150 Kcal (85-90% HR&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>Acrylate ghrelin</td>
<td>No difference in PYY&lt;sub&gt;3-36&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>(Alkahtani et al., 2014)</td>
<td>12 Overweight/Obese</td>
<td>(±20% FAT&lt;sub&gt;max&lt;/sub&gt;) for 30-min.</td>
<td>Appetite VAS</td>
<td>No difference in appetite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 sec of (85% VO&lt;sub&gt;2max&lt;/sub&gt;) + 15 sec (25% VO&lt;sub&gt;2max&lt;/sub&gt;) ~ 18 min</td>
<td>Liking and Wanting</td>
<td>No difference in Liking and Wanting between intensities</td>
</tr>
<tr>
<td>(Thompson et al., 1988)</td>
<td>15 young males</td>
<td>4.1 kcal/kg/body weight</td>
<td>EI,</td>
<td>No difference in EI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LI cycle (35% VO&lt;sub&gt;2max&lt;/sub&gt;)</td>
<td>appetite VAS</td>
<td>Hunger ↓ in HI v LI and rest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HI cycle (68% VO&lt;sub&gt;2max&lt;/sub&gt;)</td>
<td></td>
<td>Caloric intake ↑ post-exercise vs rest</td>
</tr>
</tbody>
</table>

AT = anaerobic threshold; EI = energy intake; OB = obese; REI = relative energy intake; VAS = visual analogue scale; GLP-1 = Glucagon-like peptide-1; PYY<sub>3-36</sub> = Peptide YY<sub>3-36</sub>.
1.4 Conclusion

This review has illustrated that exercise has an important role on appetite, food intake and EE. Moreover, there is evidence that supports the idea that exercise decreases adipose tissue and plays an important role in influencing hormonal concentrations (Jan Bilski, 2009).

As there is an increasing prevalence of obesity-related morbidity, and also a decline in the rates of exercise participation, there is growing interest in strategies to optimize the metabolic benefits of a specific exercise bout. In particular there is a desire to create an exercise strategy that optimizes fat utilization both during and after exercise which takes into account the recommendations of expert committees. The optimal exercise protocol must be a compromise between what is safe, achievable, and physiologically the most advantageous to all groups (Warren et al., 2009).

In general, various factors can modulate the motivation to eat in response to acute exercise, such as gender, body weight, and eating behaviour. In women it has been observed that food intake increases as a result of acute exercise, impacting on energy balance, whereas EI was not changed in men. In addition, palatability of foods was increased by exercise in normal weight women and hunger suppression was not observed (Jan Bilski, 2009). Exercise is a main influence on energy balance, but research in the area of how gut-related hormones respond to exercise are still limited (O’Connor et al., 2006). To identify any true exercise intensity effect, it will be necessary to match the EE between two intensity sessions (Ueda et al., 2009a), as this will quantify and standardise energy expanded during the exercise intervention.

To date, most studies examined the effect of HI vs LI on appetite and EI for lean and obese individuals, with only a few studies examined this effect on post-exercise EE and fat oxidation.
There is very limited data on comparison between HI vs LI in terms of its effect on appetite and fat oxidation together especially for obese population, which is an area that requires further study.

In conclusion, these results suggest future research should emphasize physical exercise, especially for obese people. Currently, there are mechanisms and consequences of exercise that can be seen in short and long-term appetite control; however, these mechanisms need to be explained. Overall appetite control (i.e. control of EI) is important for weight maintenance. Exercise is the most variable component of EE yet the impact is not just the energy cost of the exercise itself but also the physiological, behavioural, and appetite related effects as well. Exercise is known to influence appetite; yet the independent the effects of intensity are unclear.

There are different mechanisms and consequences of exercise in the short-term control of energy balance between groups (males and females, lean and overweight/obese); however, these mechanisms warrant further explanation.
1.5 Hypothesis

- HI will exhibit different “anorexic” effects on post-exercise ad libitum food intake compared to LI of the same energy cost in healthy lean individuals (young, non-athlete males and females).

- Measurement of post-exercise gut peptides and subjective hunger sensations (via VAS) will be significantly different in LI versus HI in healthy lean and overweight people.

- Post-exercise EE/substrate utilisation will be significantly different between LI and HI exercise of the same energy cost in lean and overweight people.

1.6 Aims and Objectives

To investigate the effects of high versus low intensity exercise on: short-term appetite, food intake, gut peptide secretion, EE and substrate utilisation in lean and overweight people and to investigate whether there is an impact of gender on these variables. The aims is divided to tow parts as follows:

The primary objective: to investigate whether the independent effect of HI exercise can influence the EE and substrate utilisation when compared to LI in sedentary, normal and overweight volunteers, using a cross-over study.

The Secondary objective: to determine if there was any suppression in appetite and food intake observed as a result of the difference in HI and LI, which was associated with gut peptide secretion.
1.7 Scope of thesis

The primary aim of this study was to investigate whether the independent effect of HI exercise can influence the EE and substrate utilisation when compared to LI in sedentary, normal and overweight volunteers, using a cross-over study. Secondary aims were to determine if there was any suppression in appetite and food intake observed as a result of the difference in HI and LI, which was associated with gut peptide secretion. It is suggested that a HI exercise protocol will exhibit different “anorexic” effects on post-exercise ad libitum food intake compared to lower intensity exercise of the same energy cost. Furthermore, it is hypothesised that the measurement of post-exercise gut peptides, subjective hunger sensations, VAS and post-exercise EE/substrate utilisation will be significantly different in LI versus HI exercise. This thesis contains 5 chapters that examine effect of acute bout of HI on EI, EE, and appetite and appetite hormones. The third chapter examines the validity of the Pro-Diary as an electronic appetite measuring method in comparison with a manual pen and paper method (P&P). Chapter 2 and 4 of this thesis describe the effect of HI versus LI on EE, EI post-exercise, appetite and an appetite hormone (GLP-1) in lean and overweight subjects, respectively. Chapter 5 and 7 compare the effect of exercise, for male versus female and lean versus overweight. Finally, chapter 8 discusses the findings from all chapters with possible ideas to develop this work in the future.
Chapter two
2 Materials and methods

This chapter describes the materials and methods used in the clinical studies presented in this thesis.

2.1 Materials

2.1.1 Ethical approval and basic protocol

This study investigated the role of exercise intensity on appetite, energy intake (EI), appetite related hormones, energy expenditure (EE), fat utilisation and subjective measures of appetite. The research was an interventional cross-over study with participants undertaking clinical sessions involving both high intensity (HI) and low intensity (LI) exercise sessions matched for overall workload.

Ethical approval was obtained from the Surrey University Research Ethics Committee (EC/2011/20/FHMS) in 2011 with notices of major amendment submitted in 2012 for the follow-on studies (Appendix A), and written informative consent was taken from study participants before taking part in this investigation.

All subjects were required to complete 4 visits, as depicted in (Figure 2.1).

Due to the nature of the study and the potential safety concerns for performing exercise in sedentary individuals, the first visit was aimed at screening and performing a safety electrocardiogram ECG scan. Upon successful completion of the screening test, a VO$_{2\text{max}}$ test on a cycle ergometer was carried out during the second visit, to determine the workload (watts) for (HI) (95% of maximum workload) and (LI) (50% of maximum workload) exercise bouts. During this visit, a measurement of resting energy expenditure (REE) and substrate utilisation were also performed. The third and fourth clinical visits focused on the clinical tests, where
volunteers undertook identical measurements following low and high intensity exercise (which were matched), each separated by a 5 day (minimum) washout.

Figure 2.1 study design.

Cross-over study with participants undertaking clinical sessions involving both high intensity (HI) and low intensity (LI) exercise sessions matched for overall workload. REE= resting energy expenditure.

2.1.2 Participants

All participants were aware of the real purpose of the study, which was to investigate the effect of exercise intensity on energy intake, expenditure, appetite and appetite related hormone. Participants were recruited from Surrey University and the surrounding areas, through a number of means such as: word of mouth, e-mail and poster advertisements. All participants were given a subject information sheet that described the aim of the study with the associated assessment risks and discomforts. Subjects completed the general health questionnaire prior to taking part in the study. Volunteers also completed the Dutch Eating Behaviour Questionnaire (DEBQ) (Appendix B) (van Strien et al., 1986) with a maximum of 4 points used as a cut-off for inclusion to the study. The Physical Activity Readiness questionnaire (PAR-Q screening) (Appendix C) was completed in order to exclude and identify volunteers who would potentially be harmed by exercise during the course of the study. Most of the volunteers were students at Surrey University and were physically sedentary. This term is applied for individuals who
engaged in less than 20 minutes of continuous exercise per week or for those who exercised less than 3 times a week.

The general inclusion criteria for the recruitment of participants for all studies were as follow:

- Young aged 18-35 years.
- Caucasian (white).
- Non-smokers.
- Exercised less than 3 times a week.
- No evidence of depression, physiological disorder or eating disorders.
- Not taking prescription medication likely to interfere with the endpoints of the study.
- Normotensive.
- Willingness to complete the exercise protocol for HI and LI.
- No history of coronary heart disease, metabolic disease, type 1 or 2 diabetes and/or anaemia.
- No history of drug or alcohol abuse.
- BMI for healthy between 18 and 25 and >25 for overweight.
- Acceptance and tolerance of ingredients presented within the ad libitum pasta test meal.
- Female participants should be on hormonal contraception.
2.2 Methods

2.2.1 First visit

2.2.1.1 Screening and safety

At the first visit and before taking part in the trial, participants visited the Clinical Research Centre (CRC) in order to familiarise themselves with the environment. Fasted blood was taken for the assessment of both glucose and haemoglobin levels and anthropometry and body composition data were collected. All participants were asked to complete (DEBQ).

2.2.1.2 Anthropometry and body composition

Heights were recorded to the nearest millimetres taken with a portable stadiometer, with the participant standing erect without shoes, looking straight in the horizontal plane with the feet together and knees. The heels, buttocks, shoulder blades and the back of the head had to touch against the wall. Following voiding, body weights were measured with the participants dressed in light clothes and without shoes, using a digital weighing scale and taking records to the nearest 0.1kg. Whole body and regional body compositions were measured by a multi frequency bioelectrical impedance using the Tanita MC180A segmental monitor (Tanita Corp, Japan). Body mass index (BMI) and body fat percentage were calculated automatically using a digital scale. Questionnaires were used to measure different components of eating behaviours.

2.2.1.3 Dutch Eating Behaviour Questionnaire (DEBQ) for the assessment of eating structure

All participants were asked to complete the DEBQ. This questionnaire is comprised of 33 items which require the same response to be for use with the general population. The DEBQ aims to understand and analyse the complexity of eating behaviour. This measurement has been validated and gained a good reliability in measuring the emotional and external components of eating behaviour (van Strien et al., 1986) (Wardle, 1987). The questionnaire has three types of
eating style (Braet et al., 2008): restrained eating (10 questions), external eating (10 questions) and emotional eating (13 questions). Each question has a five-response format. In other words, each question is answered using a 5 point scale (1-5); never (1), seldom (2), sometimes (3), often (4) and very often (5), except for item number 26, which has opposite scores.

After the questionnaires were completed, the scores were calculated for each section (restrained, emotional and external eating behaviours). The final score was calculated by dividing the total score by the number of questions (see Appendix B). If ‘not relevant’ was selected, the choice scores 0 and the equation reduces by 1 for this particular part. All participants filled the DEBQ, wherein only those scoring <4 in any subscale took part in the experimental study (van Strien et al., 1986). The selected participants then underwent an ECG scan for safety purposes.

2.2.1.4 The electrocardiogram (ECG)

The electrocardiogram (ECG) can represent the electrical signals during a cardiac cycle. It was therefore used in this study, to detect any contraindication to exercise such as hypotrophy, ischemic S-T segment changes, any lack of heart function and any myocardial infection (McArdle et al., 2010). The ECG is important to exclude any subject who has an abnormal heart function and to ensure that participants do not have any cardiovascular contraindications out the outset of the study (McArdle et al., 2010).

A three-lead electrocardiogram (ECG) was undertaken and checked by a physician at the CRC. This led to some participants being excluded from the study as a result of an irregular ECG result. They were informed about the findings, provided further details and instructed to visit their GPs for referral and treatment.
2.2.2 Second visit

2.2.2.1 Resting Energy Expenditure (REE) and maximum oxygen uptake (VO\textsubscript{2max}) test

All subjects were given a standardised meal the night before the measurements to establish undertaking resting energy expenditure and a VO\textsubscript{2max} test on a cycle ergometer to determine the workload (watts) to be used in the HI and LI exercise bouts.

2.2.2.2 Standardised meal

Participants were provided with standardised commercial ready meals: Tesco Bolognese Pasta Bake, a pudding and 2 Tesco Chocolate Sponges. The energy and macronutrient contents of this meal were taken from the manufacturers labelling. It was necessary to provide a standardised meal to eat on the evening of the pre-study day and the clinical study days in order to avoid any influence on appetite, gut hormone concentrations, or feeding behaviours on a subsequent morning (Chandarana et al., 2009). The nutritional content of the standardised meal provided to participants is shown in Table 2.1.

Table 2.1 The nutritional composition of the standard evening meal.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>KJ</th>
<th>Kcal</th>
<th>CHO (g)</th>
<th>Sugar (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Saturated Fat (g)</th>
<th>Salt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal</td>
<td>2410</td>
<td>575</td>
<td>55.2</td>
<td>4</td>
<td>32.4</td>
<td>24.8</td>
<td>13.2</td>
<td>2</td>
</tr>
<tr>
<td>Dessert</td>
<td>1345</td>
<td>320</td>
<td>64.6</td>
<td>46.0</td>
<td>4.0</td>
<td>5.2</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>3755</td>
<td>895</td>
<td>119.8</td>
<td>50</td>
<td>36.4</td>
<td>30</td>
<td>14.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Participants were provided with exactly the same meal, prior to each visit, and were instructed to consume the standard meal twelve hours prior to attending the pre-study and both study days. Participants were instructed to fast after the meal but were allowed to consume water.
2.2.2.3 Determination of Resting Energy Expenditure (REE)

Participants arrived at CRC for their second visit (post-screen) having fasted overnight (for 12 hours). EE was estimated by measuring oxygen consumption during rest (McArdle et al., 2010). Subjects were laid supine in a comfortable position for 20 minutes to ensure they were rested. Participants were instructed to remain awake in a complete state of rest. The room temperature was kept constant at 25°C. The respiratory CO₂ production and O₂ consumption were measured for 20 minutes immediately following the rest period by indirect calorimetry (GEM gaseous exchange monitor, GEM Nutrition Ltd, Leipzig, Germany). The gas analyser was calibrated one hour before each test, and the volume was also calibrated before each test using standard cylinders. The procedure required the subject to lie supine whilst a ventilated hood was placed over the subject's head and shoulders. The ventilated hood acted to draw air across the face (to ascertain the total volume of air exchanged with the lungs) whilst sampling the expired air for O₂ and CO₂. To estimate energy expenditure, CHO and fat oxidation were calculated as they are the major substrates of energy, with protein oxidation estimated. Hence, the resting energy expenditure and the respiratory quotient (RQ) were calculated from the measured volumes of expired gases. Substrate utilisation was measured from the RQ assuming that an RQ of 0.7 = 100% of fat oxidation; an RQ of 1.0 = 100% of CHO oxidation. EE was calculated using accepted formula (Weir, 1949):

\[
EE (KJ/Day) = 16.318 \times (VO_2) (L) + 4.602 \times (VCO_2) (L) * 1440 \ (Min/Day)
\]

Fat and CHO oxidation rates at rest were calculated from the RQ using the non-protein stoichiometric equations, from Frayn (Frayn, 1983), as follows:

\[
RQ = \frac{CO_2}{O_2} \ (moles).
\]

In case of CHO, RQ =1 while fat has an RQ of 0.7. Protein is about 0.8, which is between CHO and fat.
2.2.2.4 Maximum oxygen uptake (VO$_{2\text{max}}$) test

According to the American College of Sports Medicine recommendations, participants were instructed to drink water in order to avoid dehydration, sit correctly on the cycle ergometer, wear comfortable clothing suitable for exercise, avoid alcohol, caffeine and any PA 24hrs before the pre-exercise test (Medicine, 1990).

Following the measurements of REE, participants underwent a VO$_{2\text{max}}$ test which involved being fitted with a Hans-Rudolph facemask attached to the Cortex Metalyzer 3B for the measurement of inspired VO$_2$ throughout both rest and exercise periods. The incremental exercise test took 10-12 minutes to complete including a 2-3 minute resting period.

Subjects first exercised using an electronically controlled cycle ergometer at 0W at a fixed cadence of 60 rpm. Participants were requested to maintain 60 rpm throughout the test. The workload of the cycle increased incrementally by 25W every 60seconds. The cycle was programmed and participants reached volitional fatigue within 10-12 minutes (Taylor et al., 1955).

Participants were encouraged to continue exercising during the test and were asked to subjectively assess the relative effort levels imposed on them by the exercise, using the Borg scale (Borg, 1998). These are collected at rest and every two minutes during the exercise. The VO$_{2\text{max}}$ outcome was taken when participants raised the hand to indicate that they were to stop for the current min. The criteria used to ensure that participants reached the appropriate maximum oxygen uptake included rating of perceived exertion scale of 19-20, indicating a plateau in O$_2$ consumption, showing an RQ ≥1.15 and maximum heart rate (HR$_{\text{max}}$) of age as predicted (220-age) (Eston and Reilly, 2008).

The exercise test was terminated for any of the following reasons:

1. Participant was fatigued and requested to stop the test.
2. Chest pain, shortness of breath, dizziness and light headedness.

3. Achieving physiological VO$_{2\text{max}}$ as determined by the measured VO$_2$, since VO$_2$ remains constant (plateaus) despite increasing workload.

4. Heart rate fails to increase with exercise.

2.2.3 Third and fourth visits: comparison of matched LI and HI exercise

This was a randomised cross-over design, wherein all subjects were required to complete two intervention visits, separated by a minimum of 5 day washout. At each visit, subjects completed an exercise bout of either LI or HI of identical overall energy cost of 750 KJ (Little et al., 2010). The order of the visits were randomly assigned to an order of either HI followed by LI or LI followed by HI. A standardised breakfast was given 1 hour prior to exercise and REE and substrate utilisation measurements were taken 90 minutes after each exercise session.

2.2.3.1 Standardized breakfast

Participants commenced exercise one hour after the consumption of a standardised liquid test meal (Dunn’s River Nourishment Milk Drink, Hertfordshire, UK). Participants could choose between two flavours; chocolate or vanilla, having the same flavour on both visits. The nutritional content of the milkshake is shown in Table 2.2.

Table 2.2 Nutritional content of the liquid test meal one hour before the exercise.

<table>
<thead>
<tr>
<th>All Nutrient</th>
<th>Content of milkshake (per-can)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KJ</td>
<td>1668</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>54</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>54</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>20</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>12</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>6.8</td>
</tr>
</tbody>
</table>
Participants were asked to consume the test meal within 5 minutes, after which only water was permissible, until the end of the study.

2.2.3.2 Exercise Study Visit

All subjects had weight and body composition re-measured on arrival. At each visit on arrival at the CRC, a cannula was inserted by a qualified clinician and was in place throughout the study day to enable blood samples to be taken every 30 minutes for 3 hours. Appetite was rated on a visual analogue scale (VAS) on arrival at the CRC and every 30 minutes during the study.

Blood was collected into sodium fluro-oxalate for glucose, potassium EDTA for insulin, NEFA and TAG, potassium EDTA containing 200 kIU aprotinin /ml for the analysis of total GLP-1. Blood samples were taken for 3 hours post-breakfast, centrifuged and plasma frozen for later analysis. Samples were analysed for glucose, insulin, TAG, NEFA and GLP-1.

The standard liquid breakfast was given immediately after the first blood sample. Sixty minutes post-meal subjects underwent an exercise bout using an electronically controlled cycle ergometer (Cortex Leipzing, Germany). The timeline followed during the study day is depicted in (Figure 2.2).

Subjects’ heart rates and blood pressures were also measured at the same aforementioned 30 minute intervals. In-between all measurements, subjects remained supine but were allowed to read, listen to music, or watch television.
Participants were asked to record in a food diary all they consume after the pasta lunch until breakfast of the third day in order to estimate their 48 hour energy intakes.

### 2.2.3.3 Exercise intervention: High (HI) and Low intensity (LI) exercises

As it showed in (Figure 2.3), (LI) exercise comprised of a warm up that started from 0-50W over 3 minutes. This was then followed by 30 minutes of continuous cycling, at a fixed cadence, equivalent to 50% of the participants VO$_{2\text{max}}$ (typically 75-150W). Subsequently, this was followed by a cool down from 50-0W over 3 minutes. (HI) exercise was in accordance with the protocol of (Little et al., 2010). It comprised of a warm up which started from 0 - 50W over 3 minutes, and was followed by 8 repeated 60 second bouts of cycling at 95% VO$_{2\text{max}}$. 

---

**Figure 2.2**: Timeline of the study day.

**Figure 2.3**: Exercise protocol.
(typically 150 – 300 W), with each bout being followed by a 90 second active rest at 50W. At the end of 8 bouts, subjects then completed a cool down from 50-0W over 3 minutes. This process was controlled by a PC programme called Cortex MetaSoft 3. Respiratory CO₂, O₂ and heart rates were observed throughout the exercise components of the study using the Cortex Metalyser 3B (Cortex biophysik GmbH). The MetaLyser3B is a stationary metabolic stress test system for pulmonary gas exchange measurements during ergometer or treadmill exercise. The subject wears a mask attached to a volume transducer and oxygen and carbon dioxide analysis cells. Direct measures of gaseous exchange, including O₂ and CO₂ concentration of the expired/inspired air were taken breath by breath via a sampling tube from the facemask. The total volumes of inspired and expired air were also measured via the volume transducer attached to the facemask. From these measurements the respiratory exchange ratio or RER (ratio of CO₂ expired to O₂ consumed) was used to determine substrate utilisation via the designated software of the Cortex. It assumed a RER of 0.7 representing 100% fat oxidation and a RER ≥ 1 representing 100% CHO oxidation (assuming a 2% fixed contribution of protein oxidation during exercise). The approximate energy cost of this protocol was 750KJ (Little et al., 2010).

2.2.3.4 Resting Energy Expenditure (REE) post-exercise

Subjects were laid supine for 90 min post-exercise with measurements taken for 15 minutes every 30 minutes. Respiratory CO₂ and O₂ were measured during the post-exercise rest component of the study via the GEM.

2.2.3.5 Measurement of appetite and food intake

2.2.3.5.1 Assessment of appetite: Visual analogue scales (VAS)

In this research, perceptions of appetite (hunger, fullness and satisfaction and prospective food consumption) were measured by self-rated and validated by Visual Analogue Scales (VAS).
VAS is the most common method used as a simple and subjective technique to measure and record hunger, satiety, fullness, the desire to eat something fatty, salty, sweet or savoury, and the palatability of the meals (Flint et al., 2000). During the study day, participants were presented with a line and were asked to rate their feelings by marking on 100mm printed on paper. Appetite and pre and post-exercise were assessed by both paper-based validated VAS and electronic VAS (PRO-Diary CamNtech, Cambridge, UK) for comparison.

All participants were asked to rate their feelings for each question by placing a mark on the line at a particular moment during the study. Each question was in separate page and it was important not to let the participants look at their previous responses during the test.

Scores were calculated by measuring the distance from the left hand side to the marked point indicated by participant as shown in (Figure 2.4). After that, they were converted to centimetres for statistical analysis.

**Figure 2.4**: An example of visual analogue scale (VAS) used for assessing appetite.

\[
\begin{array}{c}
\text{I am not hungry at all} \\
\text{How hungry do you feel?} \\
\text{I have never been more hungry}
\end{array}
\]

VAS and were taken pre, during and post exercise every 30 min during the appetite studies.

At each visit, post-exercise subjects were provided with a pasta style meal to determine *ad libitum* intake. Food/drink intakes were measured for 48 hours post-exercise following each visit.

**2.2.3.5.2 Ad libitum (pasta test-meal)**

The *ad libitum* test meal was used to determine the amount of EI and macronutrients consumed as a response to the exercise intervention. The amount of food placed on their plates was determined by the participants themselves (Bell et al., 1998). The contents of the *ad libitum*
test meal were weighed before and after consumption, and the total energy intake and macronutrient composition were calculated (Farooqi et al., 2002). Subjects were instructed to eat as much as they wanted (Rolls et al., 1999). This means that participants were instructed to eat until they felt comfortably full and as much as they wanted until satisfied. The same amount of pasta was present to same participant on both test days. In order to avoid overconsumption, participants were told they could take any pasta left after the test meal.

The *ad libitum* pasta meal was served 110 min post-exercise. During the consumption of the meal, each participant ate separately, where in good light was provided and social influences or disturbing factors such as odours or noise were prevented.

Prior to the study, participants were informed of the *Ad libitum* meal contents in the participants’ information sheet to ensure that the pasta presented would be acceptable. The meal was cooked prior to serving and placed in a large bowl. A glass of water (250ml) was provided and more given if required. Each tray also contained a knife, a fork, a spoon, a plate and a napkin. *Ad libitum* intake of a homogenous lunch (pasta and tomato sauce) was measured for energy content of the food consumed, total dish supplied 2325 Kcal. The nutritional composition of the pasta based test meal is shown in Table 2.3.

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Amount</th>
<th>KJ</th>
<th>Kcal</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Saturated Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncooked Tesco (Fusilli) Pasta</td>
<td>400g</td>
<td>6020</td>
<td>1420</td>
<td>292</td>
<td>50</td>
<td>5.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Ragu Original Pasta Sauce</td>
<td>500g</td>
<td>905</td>
<td>215</td>
<td>43.5</td>
<td>8</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Tesco Cheddar Cheese (Mild)</td>
<td>100g</td>
<td>1725</td>
<td>420</td>
<td>0.1</td>
<td>25.4</td>
<td>34.9</td>
<td>21.7</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>30g</td>
<td>1110</td>
<td>270</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>2.2</td>
</tr>
<tr>
<td>Total</td>
<td>9760</td>
<td>2325</td>
<td>335.6</td>
<td>83.4</td>
<td>71</td>
<td>25.1</td>
<td></td>
</tr>
</tbody>
</table>

*Table 2.3: Energy and micronutrient composition of the pasta based test meal.*
2.2.3.5.3 48 hour post-exercise energy intake

Participants completed a food diary for 2 days following the *ad libitum* meal. Suitable time and location were arranged to collect the food diary. The information from the diet diaries was analysed using the dietary analysis software DietPlan 6.60 (Foresfield software Ltd, Horsham, UK) in order to calculate the amount of macronutrients and the energy consumed on each day.

2.3 Laboratory analysis

Blood samples were kept on ice before centrifugation at 3000 rpm for 10 minutes in a refrigerated centrifuge at 4°C. The plasma was then separated from the cells and stored at -20°C prior to batch analysis at the end of the study. In all studies, plasma samples were analysed within 3-4 months. All samples were batch analysed at the end of the study to minimise inter-assay variability.

2.3.1 Analysis of plasma TAG

TAG was measured in duplicate by the clinical chemistry analyser- ILab 650 (Instrumentation Laboratories, Warrington, UK). The TAG kit (Instrumentation Laboratories, Warrington, UK) liberates glycerol and fatty acid from TAG molecules. Glycerol breaks down to H₂O₂ (Hydrogen peroxide) proportionally to the concentration of TAG in the sample. The concentration of quineimine generated in the reaction is also directly proportional to the concentration of TAG in the sample, and it was determined by measuring the absorbance at 510 nm. See (Appendix D). Quality controls (QCs) were measured at the beginning and the end of the analysis. QCs were checked to show they were within the correct range for TAG analysis. Intra-assay Coefficient of Variability (CVs) were between (1.4- 1.8) % and inter CVs were between (3.3- 3.5) %.
2.3.2 Analysis of plasma NEFA

NEFA was measured in duplicate by the clinical chemistry analyser- ILab 650 (Instrumentation Laboratories, Warrington, UK) using an enzymatic colorimetric method kit (Randox Laboratories, County Antrim, Ireland). In a 2 step enzymatically catalysed reaction, hydrogen peroxide was produced from NEFA, which then reacted with N-ethyl-N-(2hydroxy-3-sulphopropyl)m-toluidine (TOOS) and 4-aminoantipyrine (4-AA) to form a purple adduct.

The concentration of this purple adduct is directly proportional to the concentration of NEFA in the sample, which was determined by measuring the absorbance at 550nm. See (Appendix D). QCs were measured at the beginning and the end of the analysis. QCs were checked to show they were within the correct range for NEFA analysis. Intra-assay CVs were between (4.1- 4.3) % and inter-assay CVs were between (11.3- 13.2) %.

2.3.3 Analysis of plasma glucose

Glucose was measured in duplicate by the clinical chemistry analyser- ILab 650 (Instrumentation Laboratories, Warrington, UK). The concentration was determined by using an enzymatic colorimetric method kit (Instrumentation Laboratories Warrington, UK).

The concentration of the red dye is directly proportional to the amount of glucose in the sample and determined by measuring the absorbance of red quinoneimine at 510 mn. See (Appendix D). Two levels of quality control were monitored before and after the samples. Coefficients of variation (CV) were calculated for these QCs. Intra-assay CVs were between (4.6- 4.8) % and inter CVs were between (6.6- 14.9) %.

2.3.4 Analysis of plasma insulin

Plasma insulin concentrations were measured using an enzyme-linked immunosorbent (ELISA) Millipore assay kit (Millipore Corporation, Billerica, MA, USA). The plate was prepared according to the manufacturer’s instructions. The sample was sealed, agitated and
incubated for one hour at room temperature and subsequently a wash was preformed 3 times with a 300 µL wash buffer and shacked by an orbital microliter plate at (400 to 500 rpm). The plate was sealed, agitated and incubated again for 30 minutes. After adding the enzyme, the wash was preformed 5 times with a 300 µL wash buffer. 100 µL of substrate was added and shacked for 5-20 minutes. The colour change was measured by reading the absorbance at 450 nm.

The analysis was performed at room temperature, and all standard samples and QCs were run in duplicate. Plasma samples were defrosted and vortex at room temperature on the same day of the assay, for 20 minutes. The standard for assays was attached with the assay kit and the range from 0 to 200mU/L. The conversion factor for insulin is: 1uU/mL=6.00pmol/L (Vølund, 1993).

QCs were measured at the beginning and the end of the analysis. QCs were checked to show they were within the correct range for insulin analysis. Intra-assay CVs were between (5.2-7.5) % and inter-assay CVs were between (7.1-9.8) %.

2.3.5 Analysis of GLP-1

Total GLP-1 was measured at the Faculty of Medicine in Imperial College, London in collaboration with Dr Paul Beck, using an in-house radioimmunoassay (RIA) method (Kreymann et al., 1987). The antibody cross reacts with GLP-1 forms but not with glycine extended form (1-37) and (7-37) or any other GI peptide. This anti-body was raised in rabbits and against GLP-1 coupled to bovine serum albumin. The sensitivity of the assays was 2pmol/L for GLP-1. In all blood analysis, to eliminate inter-assay variation, each participant’s samples for HI and LI were analysed in the same run in duplicate. The intra-assay and inter-assay CVs were < 10% and < 15% respectively.
2.4 Statistical analyses

Data was collected and entered into Microsoft Excel. Statistical analysis of the data was carried out using SPSS version 22.0 (IBM SPSS Corp, Armonk, NY, USA) for Microsoft Windows and statistical significance assumed at P ≤ 0.05 unless otherwise stated. Parametric and non-parametric tests were used throughout this thesis as appropriate. Mean and standard error of mean (SEM) were used to present the data. All variables were checked for normality using the Kolmogorov-Smirnov test. Normal distribution was assumed if (P≥0.05). If the P value was not significant, data was assumed to be normally distributed.

The sample of lean participants was analysed first. Then the complete sample, including both lean and obese participants, was analysed. For each of these two samples:-

A paired t-test was performed when comparing HI versus LI for scalar outcomes such as RQ, EE during exercise, *Ad libitum* (pasta test-meal) and food and macronutrient intake (CHO, protein and fat) in the 24th, 48th hours post-exercise. Non-parametric testing was used (Wilcoxon matched pairs test) if the differences were not normally distributed. Statistical significance was assumed at P≤0.05.

An unpaired t-test was performed when comparing two groups of participants (males versus females) for outcomes such as RQ, EE during exercise, *Ad libitum* (pasta test-meal) and food and macronutrient intake (CHO, protein and fat) in the 24th, 48th hours post-exercise. Non-parametric testing was used (Mann-Whitney U test) if the outcomes were not normally distributed. Statistical significance was assumed at P≤0.05.

The effect of exercise (HI vs. LI), time (pre and post) and interaction between exercise and time on subjective feeling of hunger and fullness, from 5 minutes before breakfast until 180 minutes after breakfast (i.e. 2 hours post-exercise) were assessed by repeated measures analysis of the variance (rmANOVA). Also, rmANOVA was used to compare the exercise intensities
over time for the study day for each of three time points of EE, RQ and fat oxidation post-exercise. A pre-study screening measurement of EE, RQ and fat oxidation was included as a covariate but was found to be insignificant and therefore excluded.

To examine the effect of exercise intensity (HI vs. LI), along with time and (exercise*time interaction), on the change in VAS, blood glucose, TAG, NEFA, insulin and GLP-1, a 2 repeated measured ANOVA test was used. The change from baseline in VAS, blood TAG, NEFAa, glucose, insulin and GLP-1 were taken into account throughout the statistical analysis, with Time 60 scores subtracted from Time 90, 120, 150 and 180 scores. Results were presented graphically as mean ± SEM.

In order to investigate the effect of gender difference in the outcome, a 2-way mixed repeated measured ANOVA test was used, with exercise as the within-subjects factor and gender and obese as the between-subjects factor. More details on each statistical test are presented in the relevant chapters of this thesis.
Chapter three
3 Validation PRO-Diary against the traditional pen and paper (P&P) method in a laboratory setting using healthy young adults:

3.1 Introduction

Visual Analogue Scales (VAS) are a type of psychometric scale that have been used in clinical and research settings to measure and assess a range of subjective sensations, for example, pain, depression, quality of life and appetite (Stubbs et al., 2000). Typically, in appetite research, VAS takes the form of 100 mm horizontal lines, with extreme subjective phrases placed at both ends (Hill and Blundell, 1983). Participants are required to respond to a particular question by placing a mark on the scale, translating the subjective sensation at a specific moment in time. This allows the quantification of the subjective sensation (Gift, 1989) within an individual.

Traditional paper based questionnaires for use in appetite research were developed by Hill and Blundell in the 1980s (Hill and Blundell, 1983) and have been applied widely (Mattes et al., 2005). Traditionally, each VAS question is presented to participants separately using pen and paper (P&P), in order to eliminate the chance of the mark for the current question being linked with the previous questions (Stubbs et al., 2000). This traditional VAS is quick, convenient, simple and easy to use for the participant. However, it can be time consuming for researchers, due to the preparation and manual measurement and the transfer of the data into a spreadsheet or database after completion. This can also introduce an additional source of human error (Gibbons et al., 2011). Despite its simplicity, this traditional scale has a potential limitation in a free-living situation where the researcher has limited control over the participants, and when the VAS are completed in respect to food intake (Stratton et al., 1998). Recently, VAS on portable electronic devices have been developed, such as the hand-held electronic appetite rating system and the hand- watch electronic appetite rating system. These can be used easily and comfortably in a free-living situation. These new techniques are inexpensive and easy to
use (Whybrow et al., 2006). They use an audio alarm to remind the participant to complete the VAS question at the appropriate time. This feature helps to increase the compliance rate to 90% or more and results in less missing data than the P&P based system (Hufford and Shields, 2002, Gibbons et al., 2011) and has the advantage of transferring the data directly from the device to a spreadsheet for future analysis and data storage (Stubbs et al., 2001). In adult populations, many scientific laboratory studies have tested the agreement between traditional VAS and hand-held electronic appetite rating systems (EARS) (Delargy et al., 1996, Stubbs et al., 1997, Stubbs et al., 2000, Stubbs et al., 2001, Whybrow et al., 2006, Zabel et al., 2009, Gibbons et al., 2011).

Having reviewed the literature, for adult populations only one study has been published in abstract form (Hampton and Middleton, 2011) that has explored the agreement between the hand-watch electronic appetite rating system, PRO-Diary (CamNtech Ltd. and CamNtech Inc, Cambridge, UK) and the traditional paper method.

3.2 Aim

This study aims to validate PRO-Diary against the traditional P&P method in a laboratory setting using healthy young adults.

3.3 Analysis

The data were transferred either manually or directly to Microsoft Excel and then to SPSS Version 22 (IBM SPSS Corp, Armonk, NY, USA). The values are summarised as a Mean ± SEM. Within each method and each question for subjective appetite (hunger, prospective food consumption and fullness) (traditional VAS and PRO-Diary) the data were checked for normality in order to determine the appropriate statistical techniques to be used.
3.4 Participants

Thirty healthy volunteers (15 male and 15 female), aged 23 ±1 (18-34 y) years with a BMI of 22±1.5 kg/m² (21-24) were recruited to take part in the study. The participant exclusion criteria included past or present medical conditions such as heart disease, type 1 or 2 diabetes, eating disorders, cognitive eating restraints, past or current drug/alcohol abuse, smoking, and any medication that is known to affect bodyweight or appetite. The average score from the DEBQ for emotional, external and restrained were (1.7±0.7), (1.6±0.5) and (3±0.2) respectively. The study was approved by the University of Surrey Research Ethics Committee and written informed consent was obtained from all of the participants. Prior to inclusion, baseline screening data were taken from the participants; age, height, weight, BMI and body fat percentage (Tanita Corp, Japan).

3.5 P&P VAS and PRO-Diary

The traditional VAS method was presented to the healthy young individuals on separate pages for hunger, prospective food consumption and fullness. See chapter 2. The same VAS questions were uploaded onto the PRO-Diary devices and these appeared as separate VAS questions on the screen one at a time, in the same order of appearance as on the traditional VAS. The dimensions of the hand-watch PRO-Diary device were 34x51x8 mm. The participants were asked to hold the watch in front of them whilst answering the VAS questions in order to reduce the distortion of the screen. The Pro-Diary watch was not to be worn around the wrist, in order to avoid misjudgement of the appropriate length. The participants were asked to read each question carefully and touch the electronic slider located at the bottom of the PRO-Diary. The participants were instructed to move a vertical cursor along the horizontal VAS and then press the O button after placing the vertical cursor to confirm their response. The data were downloaded and transferred into Microsoft Excel. Each result was presented as a decimal
between 0.00 and 1.00. The data were converted from decimals to millimetres in order to match the results obtained from the traditional method.

3.6 Study design

The comparison between PRO-Diary against P&P method was conducted as a part of exercise study comparing high intensity exercise (HI) versus low intensity exercise (LI) See Figure 2.2. The study was an interventional cross-over study with participants undertaking clinical sessions. Each participant had to come for two visits with a 5 day washout in between. For the experimental study visits all of the subjects arrived for testing having eaten the same standardised evening meal (Bolognese Pasta Bake and Chocolate Sponges) and fasted for 12 hours. All of the participants were instructed to refrain from exercise and alcohol consumption for 24 hours prior to each visit. Appetite was rated on a visual analogue scale (VAS) (Haskell et al., 2007) during the fasting and every 30 minutes during the study. The participants completed the traditional VAS and PRO-Diary questionnaire in succession but independently, with each being removed immediately after they had completed it. A standard liquid meal was given immediately after the first VAS at time =0 (F). The correlation between the results obtained from the two methods will be examined retrospectively.

3.7 Statistical methodology

Hunger, prospective food consumption and fullness were measured using two methods: traditional P&P VAS and PRO-Diary watch. The agreement between these two methods was assessed using scatter plots, as outlined by Bland and Altman (Bland and Altman, 1986). A good agreement is indicated if all of the points adhere reasonably closely to the diagonal line of equality. A substantial disagreement is visually obvious. The further steps outlined by Bland and Altman were performed in order to produce numeric evaluations of agreement, notably the “limits of agreement”. With an intervention taking place 60 minutes post-prandial, the
relationship between the paper and Pro-diary scores may subsequently change. Furthermore, these changes may not be constant over time. This problem is alluded to by Bland and Altman (1986). Therefore only the measurements taken at 0, 30 and 60 minutes were used to assess the agreement, with the mean area under the curve (AUC) per minute being calculated for each participant over the 60 minutes. If a substantial disagreement was evident, because paper VAS has been validated and well established, this measurement alone would be determined to be appropriate for measuring the feeling of hunger and prospective food consumption. P < 0.05 was reported as being statistically significant.

3.8 Results

3.8.1 Hunger score

The Rating of hunger P&P and Pro-Diary pre, during and post-LI and HI exercise intensity is illustrated in Figure 3.1A during the 180 mins observation period for exercise study including the first 60 min of the validation between PRO-Diary against P&P method. These points (F-60) are displayed in Figure 3.1B. A detailed inspection of Figure 3.1B identified 4 notable misses (outliers), defined as a difference of 30 or more between the P&P and the Pro-Dairy. Table 3.1 illustrates the details of the notable misses between the methods.

Table 3.1 Differences of >30 between the P&P and the Pro-Diary for the hunger score.

<table>
<thead>
<tr>
<th>ID</th>
<th>*Sequence</th>
<th>Visit &amp; time point</th>
<th>P&amp;P</th>
<th>Pro-Diary</th>
<th>Difference (P&amp;P and Pro-Diary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>2</td>
<td>2nd week: 0 mins.</td>
<td>23.50</td>
<td>66.00</td>
<td>-42.50</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>2nd week: 0 mins.</td>
<td>68.00</td>
<td>11.00</td>
<td>57.00</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1st week: 30 mins.</td>
<td>46.00</td>
<td>82.00</td>
<td>-36.00</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>2nd week: 30 mins.</td>
<td>15.00</td>
<td>56.00</td>
<td>-41.00</td>
</tr>
</tbody>
</table>

*Sequence: 1= LI then HI, 2=HI then LI. Measuring P&P and Pro-Diary in (mm)

One notable miss was at 30 minutes at the first visit. Two notable misses were at the fasting point at the second visit, and the other notable miss was at 30 minutes at the second visit, a week later.
The mean AUC was calculated from fasting point to 60 min. The Pearson coefficient between the two methods is 0.922 (p<0.001), which is statistically significant and shows a positive correlation between P&P and Pro-Diary.

The mean difference relating to the mean AUC was -1.81 (95% confidence interval (CI) from -3.86 to 0.24), indicating no statistically significant difference between the P&P and Pro-Diary scores (paired t-test: p=0.083). Following the Bland-Altman procedure (Bland and Altman, 1986), the difference (P&P – Pro-Diary) was plotted against the average mean AUC with the limits of agreement as reference lines Figure 3.1C. This shows no association between the true mean AUC (as estimated by the average of the P&P and Pro-Diary mean AUC) and the measurement error (i.e. the difference). This is confirmed by a Pearson’s correlation test (r=-0.049; P=0.709).
Figure 3.1: Hunger (HUN) both pen and paper VAS (P&P) and pro-diary watch (Pro-Diary)

(3.1A) Rating of hunger (HUN) both pen and paper VAS (P&P) and pro-diary watch (pre, during and post-exercise. Low and High exercise intensity. Breakfast was given immediately after Fasting point F. The shading indicates the period of exercise intervention. Values are means ± SEM (n=30). (3.1B). Scatterplot of The limit of agreement between the P&P vs Pro-Diary including all observations at F, 30 minutes and 60 minutes in LI and HI. (3.1C) Bland Altman plot of difference against mean AUC by the two methods for HUN.
The limits of agreement are shown as thick dotted lines at -17.37 and 13.76. There are four points that lie outside the limits of agreement, and two (ID=8 Visit 1 and ID=22 Visit 2) of these involve the notable misses identified earlier. See Table 3.2, which shows the particular participants and their scores.

Table 3.2 Score of participants Identified by Bland-Altman procedure of outliers.

<table>
<thead>
<tr>
<th>ID</th>
<th>*Sequence</th>
<th>Visit &amp; time point</th>
<th>P&amp;P</th>
<th>Pro-Diary</th>
<th>Difference (P&amp;P and Pro-Diary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2</td>
<td>2nd_week</td>
<td>41.00</td>
<td>24.25</td>
<td>16.75</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>1st_week</td>
<td>82.63</td>
<td>67.75</td>
<td>14.88</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>2nd_week</td>
<td>30.75</td>
<td>51.88</td>
<td>-21.13</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>2nd_week</td>
<td>62.25</td>
<td>48.38</td>
<td>13.88</td>
</tr>
</tbody>
</table>

*Sequence: 1 = LI then HI, 2 = HI then LI. Measuring P&P and Pro-Diary in (mm)

These particular participants had no known demographic features to distinguish them from the other participants.

3.8.2 Prospective food consumption score

The Rating of prospective food consumption P&P and Pro-Diary pre, during and post-LI and HI exercise intensity is illustrated in Figure 3.2A during the 180 mins observation period for exercise study including the first 60 min of the validation between PRO-Diary against P&P method. These points (F-60) are displayed in Figure 3.2B. A detailed inspection of Figure 3.2B reveals three notable misses, defined as a difference of 30 or more between the P&P and the Pro-Dairy. Also, Figure 3.2A shows the rating of prospective food consumption P&P and Pro-Diary pre, during and post-LI and HI exercise intensity. Moreover, Table 3.3 showed the notable miss and the difference between the P&P and Pro-Diary for the Prospective food consumption score.
Table 3.3 Differences of >30 between the P&P and Pro-Diary.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sequence *</th>
<th>Time point</th>
<th>P&amp;P</th>
<th>Pro-Diary</th>
<th>Difference (P&amp;P and Pro-Diary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1</td>
<td>1st week:  0 mins.</td>
<td>50.00</td>
<td>81.00</td>
<td>-31.00</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>2nd week: 0 mins.</td>
<td>73.00</td>
<td>22.00</td>
<td>51.00</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>2nd week: 30 mins.</td>
<td>54.00</td>
<td>22.00</td>
<td>32.00</td>
</tr>
</tbody>
</table>

* Sequence: 1= LI then HI, 2=HI then LI. Measuring P&P and Pro-Diary in (mm)

One notable miss was at the fasting point at the first visit. One was at the fasting point at the second visit and the other was at 30 minutes at the second visit, a week later.

The mean AUC was calculated from the fasting point to 60 min. The Pearson coefficient between the two methods was 0.940 (p<0.001), confirming a positive correlation between the P&P and Pro-Diary.

The mean difference relating to the mean AUC was -1.06 (95% confidence interval (CI) from -2.83 to 0.72), indicating no statistically significant difference between the paper and watch scores (paired t-test: p=0.238). Following the Bland-Altman procedure (Bland and Altman, 1986), the difference (P&P – Pro-Diary) was plotted against the average mean AUC with the limits of agreement as reference lines Figure 3.2C. It shows no association between the true mean AUC (as estimated by the average of the P&P and Pro-Diary mean AUC) and the measurement error (i.e. the difference). This is confirmed by a Pearson’s correlation test (r=-0.140; p=0.228).
Figure 3.2: Prospective food consumption (EAT) for both pen and paper VAS (P&P) and pro-diary VAS watch (Pro-Diary)

(3.2A) Rating of Prospective food consumption (EAT) for both pen and paper VAS (P&P) and pro-diary VAS watch (Pro-Diary) pre, during and post-exercise. Low and High exercise intensity. Breakfast was given immediately after fasting point F. The shading indicates the period of exercise intervention. Values are means±SEM (n=30). (3.2B) Scatterplot of The limit of agreement between P&P vs Pro-Diary including all observations at F, 30 minutes and 60 minutes in LI and HI. (3.2C) The Bland Altman plot of difference against mean AUC by the two methods for EAT.
The limits of agreement are shown as thick dotted lines at -14.53 and 12.41. There are two points that lie outside the limits of agreement, and two (ID=8 Visit 2 and ID=18 Visit 2) of these involve the notable misses identified earlier. See Table 3.4, which shows the particular participants and their scores.

Table 3.4: Score of participants Identified by Bland-Altman procedure of outliers.

<table>
<thead>
<tr>
<th>ID</th>
<th>*Sequence</th>
<th>Visit &amp; time point</th>
<th>P&amp;P</th>
<th>Pro-Diary</th>
<th>Difference (P&amp;P and Pro-Diary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2</td>
<td>2nd_week</td>
<td>51.75</td>
<td>27.00</td>
<td>24.75</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>1st_week</td>
<td>83.50</td>
<td>64.63</td>
<td>18.88</td>
</tr>
</tbody>
</table>

*Sequence: 1 = LI then HI, 2 = HI then LI. These particular participants with their scores, which were identified as outliers, had no known demographic feature to distinguish them from the other participants. Measuring P&P and Pro-Diary in (mm)

### 3.8.3 Fullness score

The Rating of fullness P&P and Pro-Diary pre, during and post-LI and HI exercise intensity was illustrated in Figure 3.3A during the 180 mins observation period for exercise study including the first 60 min of the validation between two methods. These points (F-60) are displayed in Figure 3.3B. A detailed inspection of Figure 3.3B reveals 8 notable misses, defined as a difference of 30 or more between the P&P and Pro-Diary. Also, Figure 3.3A shows the Rating of fullness P&P and Pro-Diary pre, during and post-LI and HI exercise intensity. Moreover, Table 3.5 showed the notable misses and the difference between the P&P and Pro-Diary for Fullness score.
Table 3.5 Differences of >30 between the P&P and Pro-Diary.

<table>
<thead>
<tr>
<th>ID</th>
<th>*Sequence</th>
<th>Time point</th>
<th>P&amp;P</th>
<th>Pro-Diary</th>
<th>Difference (P&amp;P and Pro-Diary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>2nd week: 0 mins.</td>
<td>17.00</td>
<td>53.00</td>
<td>-36.00</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>2nd week: 0 mins.</td>
<td>28.00</td>
<td>74.00</td>
<td>-46.00</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1st week: 30 mins.</td>
<td>11.00</td>
<td>75.00</td>
<td>-64.00</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>2nd week: 30 mins.</td>
<td>81.00</td>
<td>20.00</td>
<td>61.00</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>1st week: 30 mins.</td>
<td>76.00</td>
<td>26.00</td>
<td>50.00</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>1st week: 30 mins.</td>
<td>32.00</td>
<td>65.00</td>
<td>-33.00</td>
</tr>
<tr>
<td>28</td>
<td>1</td>
<td>1st week: 60 mins.</td>
<td>18.00</td>
<td>50.00</td>
<td>-32.00</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1st week: 60 mins.</td>
<td>50.00</td>
<td>82.00</td>
<td>-32.00</td>
</tr>
</tbody>
</table>

*Sequence: 1= LI then HI, 2=HI then LI. One notable miss was at 30 minutes at the first visit. One was at the second visit, two were at 60 minutes at the first visit, and two were at 0 (F) minutes at the second visit.

The mean AUC was calculated from the fasting point to 60 min. The Pearson coefficient between the two methods is 0.802 (p<0.001), which is statistically significant and so the null hypothesis that there is no association between P&P and Pro-Diary is rejected.

The mean difference relating to the mean AUC is -1.7 (95% confidence interval (CI) from -4.13 to 0.74), indicating no statistically significant difference between the paper and watch scores (paired t-test: p=0.168). Following the Bland-Altman procedure (Bland and Altman, 1986), the difference (P&P – Pro-Diary) was plotted against the average mean AUC with the limits of agreement as reference lines (Figure 3.3C). It shows no association between the true mean AUC (as estimated by the average of the P&P and Pro-Diary mean AUC) and the measurement error (i.e. the difference). This is confirmed by a Pearson’s correlation test (r=-0.138; p=0.294).
Figure 3.3 Fullness (FULL) both pen and paper VAS (P&P) and pro-diary VAS watch (Pro-Diary) (3.3A) pre, during and post-exercise. Low and High exercise intensity. Breakfast was given immediately after fasting point F point. The shading indicates the period of exercise intervention. SEM (n=30), male (n=15) and female (n=15). (3.3B) Scatterplot of the limit of agreement between P&P vs Pro-Diary including all observations at F, 30 minutes and 60 minutes in LI and HI (3.3C) The Bland Altman plot of the difference against the mean AUC by the two methods for FULL.
The limits of agreement are shown as thick dotted lines at -20.16 and 16.72. There are three points that lie outside the limits of agreement, and three (ID=1 Visit 1 and ID=13 Visit 2 and ID=26 Visit 1) of these involve the notable misses identified earlier. See Table 3.6, which shows the particular participants with their scores.

Table 3.6: Score of participants Identified by Bland-Altman procedure of outliers.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sequence</th>
<th>Time point</th>
<th>P&amp;P</th>
<th>Pro-Diary</th>
<th>Difference (P&amp;P and Pro-Diary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1st_week</td>
<td>34.75</td>
<td>64.75</td>
<td>-30.00</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>2nd_week</td>
<td>64.50</td>
<td>33.50</td>
<td>31.00</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>1st_week</td>
<td>65.38</td>
<td>30.25</td>
<td>35.13</td>
</tr>
</tbody>
</table>

*Sequence: 1= LI then HI, 2=Hi then LI

These particular participants with their scores, which were identified as outliers, had no known demographic features to distinguish them from the other participants.

3.8.4 Discussion

The aim of this study was to validate the PRO-Diary as an electronic method of quantifying subjective appetite sensations. It was validated against the traditional P&P method in a laboratory setting, using healthy young adults. If proven to be equivalent to established methods, the PRO-Diary would have the advantage of being quicker and potentially more valuable, as it would remove the step of converting the VAS to a numerical value. Gibbons et al. (2011) reported no difference between P&P and Pro-Diary in a study that involved its sensitivity to the impact of a meal and the recovery of appetite during the post-prandial period. However, our study has avoided the meal factor when comparing the methods. The effect associated with the manipulation of a meal is recognised in the review by Stubbs et al. (2000).

Conversely of course, all portable electric devices require an adequate energy / battery source and may malfunction. Moreover, electronic devices may run out of energy and this
may lead to bias in the data due to missing data from people who have forgotten to charge their devices.

In all three components of the VAS, Pro-Diary displayed (see Figure 3.1A, 3.2A and 3.3A) a higher score (albeit not statistically significant for each component). This bias, although not statistically significant, is a concern. This phenomenon was not reported by Almiron-Roig et al. (2009). Their study used a different type of electronic device with a 70 mm screen, while this study used the Pro-Diary, which has a 51 mm VAS scale. Stubbs et al. (2001) and Whybrow et al. (2006) indicated the use of the extreme ends of EARS (an electronic device) scale were avoided. Inspection of 3.1B, 3.2B and 3.3B at the extreme ends shows a little evidence of any trend for the Pro-Diary scores to be farther away from the end point (0 mm or 100 mm) than P&P scores. So, the 51mm scale of Pro-Diary may not have the same problem as EARS.

The possible problem of bias by the scale being read upside down was addressed by specifically asking the subjects to hold the Pro-Diary rather than wearing it on their wrist when recording their appetite. Both studies used the same 100 mm VAS scale for the P&P.

In contrast, Rumbold et al. (2013) reported a lower mean for the Pro-Diary, albeit not statistically significant. This study was in 7-10 year olds children in a free-living environment, which was not similar to our age group or our environment, as ours was with young adults in a laboratory.

However, in line with our study, another adult study conducted by Whybrow et al. (2006) reported a higher mean score in an electronic device equivalent to the Pro-Diary (EARS) for hunger and prospective food consumption with the prospective food consumption being statistically significant. However, fullness was lower in the EARS.
In this study, the limit of agreement (Bland and Altman, 1986) is unacceptably high, demonstrating beyond sample bias effect the extended of disagreement between P&P and Pro-Diary. It can be seen from Figure 3.1B, the plot of the hunger score Pro-Diary versus P&P, that there are some extreme disagreements. For example: at time = F there was a score of 11 for the Pro-Diary corresponding with a score of 68 for the P&P. Also, at F, there was a score of 22 for prospective food consumption Pro-Diary and 73 for prospective food consumption P&P. Conversely, for hunger at F, there was also a score of 66 for the Pro-Diary and 23.5 for the P&P. These disagreements could be attributed to the participant using the Pro-Diary for the first time and reading the scale upside down. However, a similar extreme disagreement with a Pro-Diary score of 65 and corresponding P&P of 15 took place at 30 min. This was also seen for fullness, with a score of 20 for the Pro-Diary and 81 for the P&P along with 26 for the Pro-Diary and 76 for the P&P. Both at 30 min postprandial. Despite the participants learning how to use the Pro-Diary before the F point, substantial disagreement between the methods can still be seen. Although in general there is good agreement between the methods, in particular for the value of fullness, 9% of the points disagree by more than 30 mm and some worrying disagreements as have been identified.

Based on our findings, the Pro-Diary appears to show restricted agreement with the P&P, and so caution is advised.

The most important new finding to emerge from this study is that despite participant exclusion criteria and thorough participant training as described above, notable discrepancies between P&P and the Pro-Diary were identified all the way across the observation period. Our interventions were applied with at least a 5 day gap, which was less than Whybrow et al. (2006) who used a 7 day gap between the interventions, and Gibbons et al. (2011) who had 7-10 days in between. In contrast, others such as Almiron-
Roig et al. (2009), Hampton and Middleton (2011) had a gap of just 2 days. Stubbs et al. (2001) had a gap of at least 2 days gap as well. It seems that there is a variation between studies that needs to be considered for future tests.

In conclusion, this study adds to the body of evidence that suggests that the Pro-Diary is not a robust alternative to P&P; the Pro-Diary is seen to produce scoring disagreements with another type of electronic device across a range of experimental conditions, including young adults. We agree with the conclusion of Whybrow et al. (2006) that the two methods should not be used interchangeably and we would recommend the use of P&P, which is a validated technique.
Chapter four
4 Examining the acute effects of exercise intensity on subsequent appetite, food intake, resting energy expenditure and fat oxidation in lean participants.

4.1 Introduction

Obesity arises when there is a persistent energy imbalance with EI exceeding EE (Elks et al., 2010), underlying biological factors play a small role in the global obesity epidemic which has developed fairly rapidly in recent years (O'Rahilly and Farooqi, 2008). Exercise and PA have long been considered important factors in the control of body weight and prevention of obesity (Grundy et al., 1999), and in the context of current society, a ‘lack of time’ is commonly named as a barrier to carrying out exercise, which can contribute to levels of obesity (Trost et al., 2002).

The beneficial effects of exercise may not only be due to its ability to encourage a negative energy balance through EE, but may also be due to its effects on gastrointestinal hormones which modulate appetite (Martins et al., 2008a). Studies have suggested that the intensity of exercise is important, while a LI (50 W) session carried out for 30 min had no effect on either hunger or hunger appetite sensations and food intake (Erdmann et al., 2007), subjective ratings of hunger were reduced during and after HI sessions (Burns et al., 2007). Other studies have suggested that HI lead to a greater acute negative energy balance by exerting a suppression of EI (Thompson et al., 1988, Reger WE et al., 1986, Blundell and King, 1999). Their data suggests that investigations into HI exercise protocols may help to understand the influence of the exercise mode on appetite and EI responses, although both HI and LI need to be matched for total workload and energy cost (Deighton et al., 2013b).

4.2 Aim

To investigate the difference between the effect of HI versus LI on: short-term appetite, gut peptide secretion, EI, EE and substrate utilisation and oxidation in lean individuals.
4.3 Methods

Methods were fully described in chapter 2.

4.4 Results

4.4.1 Participant characteristics and measurements

A total of 56 participants were initially screened for the study. The average scores of emotional, external and restrained eating from the DEBQ were (1.7 ± 0.7), (1.6 ± 0.5) and (3.0 ± 0.2), respectively. Of these 56 participants, 14 participants did not meet the inclusion criteria. Two subjects were excluded from the study due to an irregular ECG trace during screening. Three participants failed to attend their screening visit and three withdrew from the study due to personal reasons; leaving 30 participants to complete both study days. However, blood sample data was available for only 23 participants (8 males and 15 females) due to human error. Figure 4.1 of the Participants flow diagram was adapted from (Moher D, 2001).
Figure 4.1 Lean participants flow diagram.
Baseline characteristics of the 30 participants who completed both visits are displayed in Table 4.1.

**Table 4.1** Baseline characteristics of lean participants who completed the exercise study intervention.

<table>
<thead>
<tr>
<th>N= 30</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.9 ± 0.3</td>
</tr>
<tr>
<td>REE (KJ/day)</td>
<td>5974 ± 132</td>
</tr>
<tr>
<td>Fasted RQ (at rest)</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>VO₂₉₀₉max (ml/min/kg)</td>
<td>36.3 ± 1.1</td>
</tr>
<tr>
<td>Workload at 50% VO₂₉₀₉max (W)</td>
<td>99.5 ± 3.5</td>
</tr>
<tr>
<td>Workload at 95% 50% VO₂₉₀₉max (W)</td>
<td>189.1± 6.7</td>
</tr>
<tr>
<td>Random glucose (mmol/L)</td>
<td>5.2 ± 0.4</td>
</tr>
</tbody>
</table>

Participants exercised less than three times a week (Wilmore JH, 2005). Results expressed as Mean ± SEM. BMI= body mass index; REE= calculated resting energy expenditure; RQ= respiratory question; VO₂₉₀₉max= maximal oxygen uptake.

No significant difference was observed in BMI between both legs of the study day (P= 0.23).

### 4.4.2 During exercise measurement

#### 4.4.2.1 Energy Expenditure (EE) and work load

According to the study hypothesis, paired t-test showed no significant difference between LI and HI in terms of the energy cost of the exercise session (P> 0.05). Therefore, the energy costs of both intensities were matched, leading to achieving the primary objective of the study; matching LI and HI energies. See Figure 4.2
Approximate energy cost of this protocol is 750KJ (Little et al., 2010). Values are presented as mean ± SEM. 720±14 KJ for Low and 755±21 KJ for High exercise, (n= 30; 15F, 15M). No significant difference between exercise protocols was found (P> 0.05)

4.4.2.2 Substrate Utilisation during exercise

The results showed a significant difference in the mean RER between LI and HI during the exercise intervention (P= 0.001). See Figure 4.3.
4.4.3 Post-exercise measurements

4.4.3.1 Subjective appetite ratings

Repeated measures ANOVA comparing the exercise intensities over time for the study day for each of the eight VAS questions showed the expected change in appetite response over time. However, there was no significant difference as a response to exercise intensity alone as an intervention in any of the components of appetite regulation except in prospective food consumption, which was found to be approaching significance (P= 0.051). HI was lower than LI immediately post-exercise and until the end of the study. However, (time*exercise) interaction between the two exercise intensities was observed for fullness (P= 0.04). Fullness peaked immediately post-exercise in HI, whilst at the same point, it reduced in LI. After this point, fullness levels were matched for both legs. See Figure 4.4 that showed the effects of Low versus High on Subjective Appetite Ratings.
The effects of Low versus High intensity exercise on subjective appetite ratings in lean participants. 500kcal breakfast was given immediately after Fasting (F). Shading indicates the period of the exercise intervention. *Ad libitum* pasta test meal was given at 220 minutes. Values are presented as mean ± SEM (n= 30; 15F, 15M). ANOVA showed that there was a significant effect of time on all appetite question’s (P < 0.001) and a significant effect of interaction (exercise*time) on fullness (P= 0.04). The effect of exercise for prospective food consumption was approaching significant (P= 0.051).
4.4.3.2 Plasma metabolites and glucagon-like peptide-1 (GLP-1)

4.4.3.2.1 Triacylglyceride (TAG)

No significant effect of exercise intensity was observed in TAG plasma levels, but, a significant effect on (exercise*time) (P= 0.037) and time (P< 0.001) were found. HI was higher and LI was lower until 60 min post-exercise where they matched. Figure 4.5 shows the mean of TAG plasma levels pre, during and post-exercise.

Figure 4.5 Plasma triacylglyceride (TAG) concentrations (mmol/L) over time pre, during and post exercise in lean participants.

A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are presented as mean± SEM (n= 23; 15F, 8M). ANOVA showed that there was no significant main effect of exercise intensity alone (P= 0.358).

4.4.3.2.2 Non-esterified fatty acid (NEFA)

There was a significant effect of exercise intensity on post-prandial plasma NEFA concentrations (P= 0.004) with a significant (time*exercise) interaction (P= 0.021) and the effect of time (P< 0.001). Mean NEFA started to increase immediately post-exercise for both legs. This increase in NEFA was significantly greater in the LI intervention from the end of exercise period and for the 90 mins post-exercise. Figure 4.6 shows the mean values of NEFA plasma level pre, during and post-exercise.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are presented as means ± SEM (n= 23; 15F, 8M). ANOVA showed that there was a significant effect of exercise alone (P= 0.004).

4.4.3.2.3 Glucagon-like peptide-1 (GLP-1)

No significant effect on exercise intensity (P= 0.392) or (exercise*time) interaction (P= 0.192) were observed in plasma GLP-1 levels, while a significant effect of time (P< 0.001) was found. The mean GLP-1 plasma level was elevated in LI and HI at the end point of exercise session compared with pre and post-exercise period. Figure 4.7 depicts GLP-1 levels pre, during and post-exercise.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise the intervention. Values are presented as mean ± SEM (n= 23; 15F, 8M). ANOVA showed that there was no significant main effect of exercise alone (P= 0.392).

**4.4.3.2.4 Glucose and Insulin**

No significant main effect of exercise or (time*exercise) interaction was observed in plasma glucose and insulin concentrations. As expected, the plasma glucose and insulin profiles mirrored one another. Both metabolites increased until 30 minutes post-breakfast and decreased until 60 minutes to the commencement of the exercise. At this point, glucose gradually increased in both exercise protocols until 90 minutes. Figure 4.8 depicts the mean of glucose and insulin plasma level pre, during and post-exercise.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of the exercise intervention. Values are presented as mean ± SEM (n= 23; 15F, 8M). ANOVA showed that there was a significant main effect of time alone (P< 0.001) for both glucose and insulin plasma levels.

Figure 4.8 Plasma glucose concentrations (mmol/L) (A) and insulin concentrations (pmol/L) (B) over time pre, during and post exercise in lean participants.
4.4.3.3 Energy expenditure (EE)

Comparing the exercise intensities over time for the study day for each of the three time points of EE post-exercise, revealed a significant effect of exercise intensity on EE (P=0.01) and time (P<0.001). However there was no significant effect of (time*exercise) interaction (P=0.135) between the two intensities. HI resulted in a higher amount of energy expenditure than LI at 5, 45 and 75 minutes post-exercise. Figure 4.9 illustrates EE post-exercise

Figure 4.9 Energy expenditure (EE) following either Low or High exercise measured at 15, 45 and 75 minutes post exercise by indirect calorimetry in lean participants.

Values are presented as mean ± SEM (n= 30; 15F, 15M). ANOVA showed that there was a significant effect of both exercise and time (P= 0.001) and (P< 0.001), respectively.

4.4.3.4 Substrate utilisation

Substrate utilisation was determined using the RQ value. There was a significant difference between the exercises intensities post-exercise in terms of the substrate utilised. This was evidenced by the significant effect of exercise (P= 0.02) and (exercise*time) interaction (P= 0.014). HI had a lower RQ value at the 5 and 45 minutes time points. However by 75
minutes, this difference in post-exercise substrate utilisation disappeared, and the substrate utilisation continued to be elevated to baseline pre-exercise levels. Figure 4.10 shows the mean RQ post-exercise.

**Figure 4.10** Mean Respiratory Quotient (RQ) post-exercise at 15, 45 and 75 minutes measured by indirect calorimetry in lean participants.

Values are presented as mean ± SEM (n= 30; 15F, 15M). ANOVA showed that there were significant effects of exercise (P= 0.02), (exercise*time) interaction (P= 0.014) and time (P= 0.001).

### 4.4.3.5 Fat oxidation

Repeated measures ANOVA was used to examine the effect of exercise intensity on fat oxidation post-exercise. There was a significant effect of exercise (P< 0.001), (exercise*time) interaction (P= 0.020) and time (P< 0.001) on the calculated fat oxidation rates post-exercise between LI and HI. The mean calculated amount of oxidised fat over this post-exercise period was higher in HI (1.31 ± 0.05) g/min compared with LI (1.17 ± 0.04) g/min. Value of AUC for LI was (81.75 ± 02.85) g and for HI was (91.26 ± 04.06) g. Figure 4.11 showed fat oxidation rate post-exercise.
Values are presented as mean ± SEM (n= 30; 15F, 15M). ANOVA showed significant effect of exercise (P< 0.001), (exercise*time) interaction (P= 0.020) and time (P< 0.001).

4.4.3.6 Ad Libitum pasta test meal intake and 48 hours post-exercise intake

After analysing the data using a paired t-test and Wilcoxon matched pairs test, it was found that there was no significant difference in the mean of ad libitum test meal or the 48 hour intake records, following exercise, between the two intensity periods.

Table 4.2 shows the Mean (± SEM) of ad libitum pasta intake post-exercise and energy intake with macronutrient intake in the 48 hour post-exercise. See Table 4.2.
Table 4.2 *Ad libitum* Pasta test meal post-exercise and the total two days of energy and macronutrient intake following Low or High exercise in lean participants.

<table>
<thead>
<tr>
<th></th>
<th>Mean Low</th>
<th>Mean High</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ad libitum</em> pasta meal (KJ)</td>
<td>4507 ± 255</td>
<td>4496 ± 240</td>
</tr>
<tr>
<td>0- 24 h intake post-exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (KJ)</td>
<td>8423 ± 652</td>
<td>8729 ± 741</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>82 ± 7</td>
<td>83 ± 7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>75 ± 6</td>
<td>78 ± 9</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>251 ± 23</td>
<td>274 ± 24</td>
</tr>
<tr>
<td>24-48 h intake post-exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (KJ)</td>
<td>9356 ± 771</td>
<td>8224 ± 567</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>84 ± 6</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>83 ± 7</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>261 ± 24</td>
<td>221 ± 14</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n= 30).
4.5 Discussion

The main aim of this study was to investigate whether the acute effect of exercise intensity could induce differences in appetite, an appetite related hormone (GLP-1), EI, EE and fat oxidation. This was conducted using a cross-over study design with sedentary, healthy lean volunteers (both male and female).

The novel finding of this study was that acute HI exercise induced a greater suppressive effect on one appetite component, increased EE and fat oxidation compared with LI, without changes in the post-prandial appetite hormone (GLP-1) or increases in post-exercise EI, despite there being no significant difference in the energy cost of the exercise session. In order to ensure that the energy cost was matched between both types of exercise, the protocol was calculated by MET’s (Ainsworth et al., 1993), which allowed this study to investigate the independent effect of exercise intensity. The study was further tailored as each exercise intensity protocol was adjusted for each individuals exercise capacity, which meant the HI exercise was attainable and achievable for all participants.

The study was conducted using thirty young Caucasian (white) volunteers who were in the healthy range of BMI (20-25 Kg/m²) and their average VO\textsubscript{2max} was (36.3 ±1.1 ml/kg/min) demonstrating that they were non-athletes according to Wilmore JH (2005). This suggests the results of this study could be applicable to a general healthy population.

In this study, the approximate energy cost of the protocol was designed to be 750 KJ (Little et al., 2010). The results of measuring the energy cost during the different exercise intensities showed no significant difference, suggesting both intensities were successfully matched, allowing any differences in the other factors measured to be attributed to the difference in intensity not energy cost. The results for the measurement of RER show there was a significant difference in the mean between LI and HI during the exercise intervention,
which suggests there were differences in the fuel utilisation of each intensity. It is well established that during exercise an RER value of 1.00 equates to 100 % CHO oxidation and of 0.7 to 100 % fat oxidation (McArdle et al., 2010). Therefore, as the mean of the LI exercise session was close to 1.00, this would indicated that the volunteers tended to use CHO as the main fuel source. The results for the HI sessions showed the mean was higher than 1.00, which indicated the body tended toward anaerobic metabolism to provide the body with the energy needed to complete the HI session (McArdle et al., 2010).

VAS questions regarding appetite were not measured during the pre-study day in any of appetite components for both intensities. Nevertheless, a standardized meal was given (12 hours) pre-study day along with a standardized breakfast preload at the beginning of the study day. A previous study suggested that hunger and satiety ratings do not change significantly over time and can lead to identical pre-prandial satiety and hunger scores (Erdmann et al., 2007).

Many studies have reported that the feeling of hunger or fullness is not significantly affected by exercise (Reger WE et al., 1986, Reger and Allison, 1987). Our finding supported these studies as despite post-exercise ratings of hunger, prospective food consumption and fullness showing a significant effect of time, there was no effect of exercise intensity either on the hunger and the fullness scores. In fact, our study was based on the work of other groups that compared HI vs LI in lean people (Imbeault et al., 1997, Pomerleau et al., 2004, Jürimäe et al., 2007, Erdmann et al., 2007, Deighton et al., 2013a), all of which matched the energy cost of exercise (Deighton et al., 2013b). Nevertheless, our result was in contrast with the study of King et al. (1994) that showed a suppressive effect of acute exercise on appetite and hunger score in comparison with the rest condition.
Within our study the prospective food consumption was found to be approaching significance (P= 0.051) as a result of the exercise intervention, with HI exercise having a lower score than LI post-exercise. The results also showed that the feeling of fullness immediately post-exercise was influenced over time by the type of exercise undertaken; there was a (time*exercise) interaction between the two exercise intensities observed for fullness, we observed fullness following HI was higher immediately post-exercise until 30 min post-exercise. This finding is similar to that of Ueda et al. (2009a) who reported a transient suppression in feelings of hunger and a reduced EI one hour post-LI and HI cycling, albeit their results were in comparison to the rest condition, whereas our study compared LI versus HI. There are a multitude of studies on the effect of exercise on hunger or appetite sensations and EI, but the result of these studies are fairly conflicted and appear to be dependent on the duration or intensity of exercise undertaken (Warren et al., 2009, King et al., 1997b, Pomerleau et al., 2004, King et al., 1994). Our study aimed to help to determine the influence of exercise intensity on hunger and appetite after correction for energy cost.

There was a significant difference in the TAG pattern over time post-exercise; HI had higher TAG and LI had lower TAG immediately post-exercise until 60 min post-exercise where the levels became the same, but there was no significant change in TAG concentration found between the exercise intensities, probably due to the short duration of the study as a relatively small lipid does provided.

In addition the results for the other plasma metabolites showed that the exercise intervention significantly influenced the mean NEFA concentration. A significant difference was observed in the NEFA levels between HI and LI post-exercise (P= 0.004); the mean concentration of NEFA in the 23 healthy subjects rose during exercise for both legs. However, the result for HI were significantly lower than LI, both during, and until 90
min post-exercise. To explain this result, the mechanism behind NEFA responses to HI vs LI needs to be considered. Studies have reported that there was an association between catecholamine concentrations and NEFA, with catecholamine release increasing with exercise intensity (Maughan, 2008). During exercise at 85 % VO$_2$max (HI), the total fat oxidation was reduced due to a suppression in the rate of appearance (Ra) of NEFA into the plasma, possibly caused by an increase in catecholamine concentration, which stimulates plasma glycogenolysis and glucose uptake (Maughan, 2008). This means that the significant catecholamine response to HI is in contrast to moderate or LI steady state aerobic exercise, all of which result in relatively smaller increases in adrenaline and noradrenaline (Zouhal et al., 2008). The impact of plasma catecholamine may last until the post-exercise period, which may explain our NEFA findings post-exercise (Trapp et al., 2007, Christmass et al., 1999).

Our results for NEFA could also be explained by considering the study by Erdmann et al. (2007) who compared 30 min of LI cycling (50 W) with HI cycling (100 W) and 45 min rest in lean individuals. Their results showed that adrenaline was elevated after 15 min of LI by 42 %, while in the HI group adrenaline rose by 178 % at 15 min during exercise. In addition, the exercise-induced rise in noradrenaline was significantly greater at both intensities compared to controls; following the LI it increased by 83 % over the baseline within 30 min, whereas for HI noradrenaline rose by 485 % over the baseline at 30 min. These findings could explain the reversal in the trend of NEFA concentration with catecholamine concentrations.

Experimental evidence reports that an increase in exercise intensity may promote fat loss (Tremblay et al., 1994). Studies have shown that adrenaline is responsible for exercise-induced lipolysis and this still has an effect after exercise, with an increase in lipid mobilisation, suggesting that during acute exercise lipolysis is mediated and stimulated by
adrenaline in addition to insulin (de Glisezinski et al., 2009). Studies have also shown that fat oxidation and NEFA concentrations are increased during and after a second bout of exercise following a short rest interval (Goto et al., 2007), and fat oxidation generally increases during exercise at 55-56% of VO$_{2\text{max}}$ and declines with a higher intensity but the reason behind this is still unclear (Romijn et al., 1993). All this suggests that the fat oxidation during exercise might be continued into the post-exercise period, which could also explain the lower level of NEFA post-HI than LI.

During exercise and rest CHO is the main fuel used by muscle (Basu et al., 1960), and NEFA are used as an energy source by other tissues during times of energy deprivation (Lafontan and Langin, 2009). For LI to moderate impact exercise (but not HI) the Ra NEFA is usually 2-3 times more than at rest, which is a result of the coupling between the release of NEFA to the blood and oxidation in the muscle (van Loon et al., 2001). This balance could possibly lead to higher a mean level of NEFA for LI post-exercise, and this could explain our result which showed that HI (95% VO$_{2\text{max}}$) reduced NEFA plasma concentration in the absence of any differences in insulin levels when compared to LI.

Studies suggest that when exercise intensity is increased to 75% W$_{\text{max}}$, both muscle glycogen and plasma glucose oxidation rates rise noticeably, and the fat oxidation rate decreases (Loon et al., 2001). This decrease in fat oxidation rate results in a significant decrease in both of the plasma NEFA oxidation rate, and the TAG fat sources (sum of intramuscular and lipoprotein-derived TAG) (Loon et al., 2001). The data suggests that the reduction in plasma FFA oxidation was not a consequence of a decrease in plasma NEFA availability; instead it was suggested that a mechanism involving the down-regulation of Carnitine palmitoyltransferase (CPT I), either by a decrease in free carnitine availability or by a decrease in pH, was the most likely cause of the decrease in fat oxidation during HI.
(Loon et al., 2001). This change might continue into the post-exercise period until the balance is restored.

Our difference in NEFA concentration between HI and LI could be interpreted by using other evidence that showed there was a decrease in blood flow in adipose tissues, with the inability to generate ATP from NEFA oxidation, in HI and this was responsible for a decrease in NEFA and reduction in the activity of CPT-1 observed (Melanson et al., 2009, Achten and Jeukendrup, 2004). This change could continue into the post-exercise period as shown in our study, where NEFA was lower in HI than LI.

There is plenty of evidence that adrenaline is a potent stimulus which increases lipolysis in men under physiological conditions (Macdonald et al., 1985). It is less clear whether noradrenaline in the circulation can stimulate lipolysis under physiological conditions, but it is one of the principal regulators of lipolysis when the catecholamine is released from sympathetic postganglionic neurones innervating adipose tissue (Macdonald et al., 1985).

Studies have shown that during LI (30 % and 50 % VO$_{2\text{max}}$), catecholamine increased lipid mobilisation and decreased insulin concentration in the plasma (Arner et al., 1990), however, our results did not agree with these findings as they showed that there was no difference in plasma glucose and insulin concentration observed after either exercise condition. Therefore, we cannot confirm this in our study. Our results are supported by the work of Ueda et al. (2009a) who compared 30 min of HI (70 % VO$_{2\text{max}}$) with LI (30 % VO$_{2\text{max}}$) and rest, and reported no significant effect of exercise on insulin plasma levels; and also by another study that found the post-prandial glucose and insulin responses between LI, HI and rest conditions were not statistically different (Erdmann et al., 2007).

Moreover, in a study that compared 6.5 km running below and above anaerobic threshold (AT), no changes in glucose concentrations were observed for any of the exercise sessions (Jürimäe et al., 2007). Similar results have also been found between the resting and post-
exercise insulin levels (Ferguson et al., 2004) and glucose concentrations (Jürimäe and Jürimäe, 2005), all of which support our results.

The effects of circulating catecholamines on CHO metabolism, lipid metabolism and the metabolic rate (thermogenesis) are not independent; there is evidence that they may cause indirect effects by affecting the release of other hormones e.g. PYY, and by influencing the blood supply to the metabolically active tissues, which could influence NEFA oxidation during and post-exercise (Macdonald et al., 1985).

GLP-1 is an incretin hormone, which means that it stimulates glucose dependent insulin secretion by the pancreas and inhibits glycogen release from the liver, and is an important factor in hunger and appetite, therefore the levels of this hormone were measured in response to different intensities of exercise (Little et al., 2006) in this study. Our experiments previously showed that there was no satiety effect linked with exercise intensity, or a direct suppression in hunger score. The results for GLP-1 in this study were in line with the earlier appetite results, and showed that there was no significant difference between exercise intensities. However, the concentration of GLP-1 was higher in HI immediately post-exercise, which was consistent with the findings from the lower score of prospective food consumption (VAS), in HI compared to LI. Nevertheless, our results also show there were no significant differences between LI and HI sessions in the score of hunger and that of GLP-1 levels, but GLP-1 appeared to be higher and the score prospective food consumption (VAS) was lower in HI post-exercise, even if it was just outside of significance (P = 0.051). At the end point of HI the GLP-1 plasma level was 49 ± 4.2 pmol/L, which was lower than levels achieved in other studies such as Flint et al. (1998), which had a peak value of about 60-90 pmol/L that showed the significant reduction in hunger. Overall, our results agree relatively well with that from Ueda et al. (2009a) who found no change in GLP-1 level, which suggests the levels of GLP-1 achieved in our study
may not be great enough to have a significant effect on hunger. However, It is worth mentioning that Ueda et al. (2009a) also found a large increase in PYY\textsubscript{3–36} plasma levels at HI despite a similar increase in GLP-1 levels and, a similar reduction in absolute EI and hunger feelings in both exercise conditions. It is well established that PP and PYY have an anorectic effect on food intake (Chaudhri et al., 2008), and it has been shown that generally gut peptides (for example PYY and GLP-1) work in a complementary manner to inhibit food intake (Neary et al., 2005), therefore other gut peptides such as PP and PYY should be measured to further investigate the effect of the complex relationship between HI and LI exercise and gut peptide release.

Further evidence for the influence of exercise on gut hormones comes from a study of one hour of cycling at 65 % of Maximum heart rate (HR\textsubscript{max}) in young lean males and females that found that exercise increased the release of satiety hormones such PYY and GLP-1 in lean individuals (Martins et al., 2007a). Additionally, an opposing pattern of time course curve was observed between these hormone levels and the feeling of hunger (Martins et al., 2007a). Hence, we set out to determine whether circulating GLP-1 levels are affected by exercise intensity (LI or HI), and thereby affect subsequent EI, and we found that, at least in this study, GLP-1 plasma levels post-exercise were similar between the two different intensities. Another possible cause of this absence of response is the shorter duration of exercise (30 min) in the present study and the study by Ueda et al. (2009a) compared exercise with a longer time (60 min) seen in other studies (Martins et al., 2007a) (Broom et al., 2009).

In this study, EE, RQ and calculated fat oxidation were measured immediately post-exercise and continued for 75 min (chapter 3). This study provides evidence that EE and fat oxidation are significantly higher after HI at 5 and 45 min post-exercise, in comparison with LI, and they are inversely associated with the RQ curve. Furthermore, this difference
was present until 75 min post-exercise when the final measurement was made. The mean RQ was 0.86 after HI, which implies the utilisation fat as a fuel for the recovery post-exercise, whereas the mean RQ was 0.96 after LI which suggests CHO was being utilised as the primary energy source. These findings are consistent with previous work which demonstrated that when EE during exercise was matched, increasing the exercise intensity reduced total fat oxidation during exercise and increased EE and fat oxidation during the 90 min period post-exercise, although underlying mechanisms that cause this are unclear (Warren et al., 2009, Romijn et al., 1993). Among the plausible explanations for these findings is that the observed higher rate of fat oxidation after HI versus LI was a reflection of both augmented EPOC and a specific alteration of the post-exercise RER kinetics, suggestive of higher relative fat oxidation (Warren et al., 2009).

Unlike glucose oxidation, TAG lipolysis/ NEFA uptake and oxidation are not as tightly regulated in relation to metabolic requirements (Issekutz Jr et al., 1967). FFA availability, which, in part, influences fat oxidation is affected by hormone-sensitive lipase (HSL) activity (both adipose and intramyocellular) (Issekutz Jr et al., 1967, Watt et al., 2004). Therefore the greater catecholamine response to HI exercise is a potential stimulant of post-exercise mitochondrial respiration; increasing adipose lipolysis via activation at HSL and oxidation (Mulla et al., 2000), and/or increasing oxidation of intramyocellular TAG-derived FA (Kiens and Richter, 1998), and it is also possibly responsible for FA release from visceral fat stores (Issekutz, 1978). This could explain the higher fat oxidation in HI than LI within our result, nevertheless lipolysis of peripheral adipose tissue and intramuscular TAG are regulated differently (Maughan, 2008).

We did not measure circulating catecholamines directly in this study, but the significant reduction in NEFA post-HI in the absence of any difference in insulin is likely to reflect this. It is also been suggested that the higher rates of adipose lipolysis and catecholamines
as a result of HI could possibly lead to enhanced fat oxidation after 8–24 s of HI cycling intervals interspersed with 12–36 s of recovery (Trapp et al., 2007). One other possible explanation for having higher fat oxidation post-HI was to offset the inhibition of fat oxidation during HI exercise at 85 % of VO$_{2\text{max}}$ (Warren et al., 2009). The increased EE and fat oxidation could also be the consequence of the increased need to resynthesize glycogen and to remove H$^+$ and lactate, as there is an elevated pH level after a HI bout (Nevill et al., 1996).

A HI study not dis-similar to our trial, consisting of numerous repeat sprints (e.g., ten 6-second bouts of maximal sprinting), indicated there was an inhibition of anaerobic glycogenolysis and ATP resynthesis was mostly derived from PCr degradation and intramuscular TAG stores (Gaitanos et al., 1990). Another study showed that increased venous glycerol concentration accompanied HI in both trained female cyclists and untrained women, (Trapp et al., 2007), and this reinforces the concept that acute HI gradually leads to a higher NEFA availability, which improved fat oxidation post-exercise (Trapp et al., 2007).

It has been reported that for a certain EE, HI could be more influential in inducing a negative energy balance and fat loss, than LI (Tremblay and Drapeau, 1999). Some authors have suggested that HI may be more beneficial for fat loss than continuous LI/moderate intensity exercise due to greater post-exercise decrease in appetite and increase in EE (Boutcher, 2010, Tremblay et al., 1994). However, our results go further by demonstrating this significant effect on EE and fat oxidation without an increase in subsequent appetite.

Our findings highlight the lack of difference in ad libitum food intake and total EI between HI and LI when measured for 48 hours post-exercise. This finding is supported by the results of Ueda et al. (2009a) who showed that a significant decline in EI was detected in moderate and HI exercise sessions, when compared with the resting condition, without any
significant difference between the exercise intensities, which was similar to the results of Deighton et al. (2013b), who also matched the energy cost of the exercise.

A review by Malkova et al. (2008) proved that post-exercise ratings of hunger rise over an extended period of time, indicating that exercise-induced appetite suppression is transient and exercise may prompt a compensatory increase in the drive to eat several hours post-exercise. Additionally, it has been shown that an increase in blood glucose circulation reduced the short-term EI, in addition to an increase in the ability of skeletal muscle to utilise lipids, which tends to decrease EI (Scheurink et al., 1999, Tremblay et al., 1994). Also, the increase in GLP-1 levels were significantly and oppositely related to a decrease in EI post each exercise intervention (Ueda et al., 2009a). This is consistent with our results as there was no difference in hunger score, TAG, glucose, inulin and GLP-1 level between both intensities, which suggests no compensatory response was observed in EI. Moreover, EI might need a longer exercise session (more than 20 min) to be increased with other metabolites, for example: after a longer duration (120 min) of exercise at (50 W), EI was significantly greater after exercise (Erdmann et al., 2007).

The majority of the studies in this area have compared the exercise invention to the rest control, for example: it was reported that exercise increased subsequent EI (Pomerleau et al., 2004), or decreased EI post-exercise (Ueda et al., 2009a), or had no effect on EI (Imbeault et al., 1997). However some studies have compared the intensity of exercise, and the results showed that EI was lower after HI compared with LI in a lean group (Kissileff et al., 1990). Hence, our work matched the energy cost of both intensities to allow comparison of its effect on EI.

In the present study, the bout of exercise only occurred once with a five day washout, which meant that the participants did not adapt to this intervention. A longer period of exercise training would be needed to investigate if there are significant differences between exercise
intensities. This is supported by the finding that even after an increase in EE for about 14 days there was still no increase in EI (Whybrow et al., 2008).

Overall, the results in this chapter found that an acute bout of HI significantly decreased NEFA plasma levels during and post-exercise, with a near significant reduction in prospective food consumption, and an increase in EE and fat oxidation post-exercise, but, there was no effect on hunger, TAG, glucose, insulin and GLP-1 levels, together with EI directly post-exercise.

Further studies are needed to elucidate the optimal intensity and duration of exercise to suppress appetite and reduce EI, with particular focus on the mechanism that improves appetite control.

There are some limitations of this study that should be considered, for instance: the possibility to control some external factors (cognitive or/and environmental) is limited, but they could have affected any of the study outcomes. For example the myth that food is a reward for exercise can influence the amount of food eaten post-exercise due to cognitive factors rather than hunger (King, 1999). Another factor to consider was that the test meal for the study was pasta ad libitum post-HI and LI, so the subjects already knew and could possibly estimate the amount of food intake from the first study.

The mechanism and the physiological differences associated with changes in the GLP-1 and other gut hormones in response to changes in exercise intensity and energy balance regulation needs to be investigated, as the mechanisms that illustrate time-course changes in plasma PYY and GLP-1 during exercise is unclear (Adrian et al., 1985). Additional mechanisms might be involved in order to control hormone release during exercise. The differences between countries and ethnicities in terms of prevalence and definition of obesity, should be taken in account with the pathophysiological involvement of BMI with obese-related diseases (Yamamoto et al., 2002).
Chapter five
5 The independent effect of exercise intensity on appetite, energy intake and energy expenditure: is there a gender difference?

5.1 Introduction

Gender is one of the key factors that could influence the motivation to eat and the amount of food intake in response to acute exercise (Jan Bilski, 2009). The results from current studies remain unclear as to whether there is a difference in the acute effect of exercise on EI between men and women (Hagobian et al., 2012). Some researchers have already demonstrated that intense exercise decreases EI acutely, but controversy remains (King et al., 1996). Most of the previous intervention studies only recruited men, even though the food intake pattern differs for women (King et al., 1996). To date, most studies that have investigated the effect of exercise on appetite, EI and EE have only been carried out in one gender, or had a small number of participants, which has not allowed a comparison between genders. Therefore, this study aimed to experimentally investigate if gender could be a factor that would influence the effect of acute HI verses LI exercise on appetite, GLP-1, EI, EE and fat oxidation post-exercise.

5.2 Aim

To investigate whether there is a difference between genders in response to the acute HI versus LI.

5.3 Methods

Methods were fully described in chapter 2.

5.4 Statistical analyses

This is a post-hoc comparison of the data from an earlier study (chapter 4). An unpaired t-test was performed to compare two groups of participants (males versus females) for a
number of outcomes such as RER, EE during exercise, *Ad libitum* (pasta test-meal) and food and macronutrient intake (CHO, protein and fat) in the 24 and 48 hour post-exercise. Non-parametric testing was used (Mann-Whitney U test) if the outcomes were not normally distributed. Statistical significance was assumed at \( P < 0.05 \).

In order to investigate the effect of gender on the time-course outcomes, a 2-way mixed repeated measured ANOVA was used, with exercise as the within-subjects factor and gender as the between-subjects factor.
5.5 Results

5.5.1 Participant characteristics and measurements

Baseline characteristics of the 30 participants who completed both visits are displayed in Table 5.1.

**Table 5.1** Baseline characteristics of lean participants (males and females) who completed the exercise study intervention.

<table>
<thead>
<tr>
<th>N= 30</th>
<th>n= 15 males</th>
<th>n= 15 females</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>24 ± 1</td>
<td>22 ± 1</td>
<td>0.47</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.9 ± 1.8</td>
<td>164.6 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.2. ± 1.6</td>
<td>57.8 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23 ± 0.5</td>
<td>21 ± 0.4</td>
<td>0.61</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>16.5 ± 1.6</td>
<td>22.27 ± 1.4</td>
<td>0.01</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>83.53 ±1.6</td>
<td>77.73 ± 1.5</td>
<td>0.01</td>
</tr>
<tr>
<td>REE (KJ/day)</td>
<td>6339 ±178</td>
<td>5608 ± 148</td>
<td>0.004</td>
</tr>
<tr>
<td>REE (KJ/day) per FFM</td>
<td>105 ± 4</td>
<td>126 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RQ at rest</td>
<td>0.87 ±0.01</td>
<td>0.89 ± 0.02</td>
<td>0.55</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml/min/kg)</td>
<td>37.5 ± 1.6</td>
<td>35.13 ± 1.5</td>
<td>0.28</td>
</tr>
<tr>
<td>Workload 50% VO$_{2\text{max}}$ (W)</td>
<td>113.6 ± 3.7</td>
<td>85.5 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Workload 95% VO$_{2\text{max}}$ (W)</td>
<td>215.8 ± 6.9</td>
<td>162.38 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Random glucose (mmol/L)</td>
<td>5.1 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Participants were exercised less than three times a week (Wilmore JH, 2005). Results expressed as Mean ± SEM. BMI= body mass index; REE= calculated resting energy expenditure; RQ= respiratory question; VO$_{2\text{max}}$= maximal oxygen uptake; FFM= fat free mass.
5.5.2 During exercise measurement

Men and women did not shown any gender differences in EE or RER during exercise (data not shown).

5.5.3 Post-exercise measurements

5.5.3.1 Subjective appetite ratings

A repeated measures ANOVA comparing gender over time for the study visits for each of the VAS questions showed that there was no gender difference in any appetite component, while there was a significant (time*gender) interaction (P= 0.018) in the hunger score. Mean hunger score in females for LI and HI were lower at the end point of the exercise session until 30 min post-exercise and until the end of the measurement period. Although, there was no difference in any of appetite component with respect to exercise intensity between the two genders. However, a trend toward (exercise*gender) interaction (P= 0.074), was shown in hunger. See Figure 5.1.

Figure 5.1 Rating of hunger pre, during and post-exercise for each gender.

A 500 kcal breakfast was given immediately after Fasting (F). Shading indicates the period of exercise intervention. Values are presented as mean ± SEM (n= 30; 15F, 15M). ANOVA showed that there was a significant (time*gender) interaction (P= 0.018). There were no significant differences in (exercise*gender) interaction (P= 0.074) or gender alone (P= 0.325).
Moreover, there was a significant effect of (exercise*gender) interaction (P= 0.017) and (time*gender) interaction (P= 0.004) in the prospective food consumption score. But, no effect of gender alone (P= 0.136) was observed. For LI, male scores were elevated from the end point of exercise until post-exercise. However at HI, the score was lower at the end of the exercise session and was steady for 120 minutes, when it increased for 60 minutes. For females, LI and HI scores were lower at the end point of exercise session and increased again thereafter. See Figure 5.2.

**Figure 5.2** Rating of prospective food consumption pre, during and post-exercise for each gender.

![Graph showing prospective food consumption pre, during and post-exercise for each gender.](image)

A 500 kcal breakfast was given immediately after Fasting (F). Shading indicates the period of the exercise intervention. Values are presented as mean ± SEM (n= 30; 15F, 15M). ANOVA showed that there was a significant (exercise*gender) interaction (P= 0.017) and (time*gender) interaction (P= 0.004). There was no significant gender difference between male and female participants (P= 0.136).

### 5.5.3.2 Plasma metabolites and GLP-1

There was no gender difference in TAG and GLP-1. In contrast, a significant difference in (time*gender) interaction (P= 0.011) for NEFA concentrations between male and female groups was found. Overall, HI led to lower NEFA levels for both genders, although males exhibited lower NEFA levels than female. See Figure 5.3.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are presented as mean ± SEM (n= 23; 15F, 8M). ANOVA showed that there was a significant (time*gender) interaction (P= 0.011). No significant difference was found in (exercise*gender) interaction (P= 0.512) and for gender alone (P= 0.655).

There was no gender difference in the glycaemic response (data not shown). However, there was a significant (time*gender) interaction (P= 0.035) for the plasma insulin response between genders. See Figure 5.4.

**Figure 5.3** Plasma non-esterified acid (NEFA) concentrations (mmol/L) over time pre, during and post-exercise for each gender.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of the exercise intervention. Values are presented as mean ± SEM (n= 23; 15F, 8M). ANOVA showed that there was a significant (time*gender) interaction (P=0.035). No significant difference was found in (exercise*gender) interaction (P= 0.343) or for gender alone (P= 0.780).

5.5.3.3 EE and substrate utilisation

There was no gender difference in RQ post-exercise (data not shown). However, there was a significant difference between male and female mean estimates of EE post-exercise (P< 0.001). Males had a greater mean EE (5.32 ± 0.123) KJ than female (3.89 ± 0.123) KJ. Also, there was a significant (time*gender) interaction (P= 0.048). Figure 5.5 shows the mean of energy expenditure (EE) post-exercise.

Figure 5.4 Plasma insulin concentrations (pmol/L) over time pre, during and post-exercise for each gender.
Values are presented as mean ± SEM (n= 30; 15F, 15M). There were a significant effect of gender (P< 0.001) and (time*gender) interaction (P= 0.48). No significant effect of (exercise*gender) interaction was found (P= 0.078).

The energy expenditure values were corrected for body composition (measured through impedance) then expressed per unit fat free mass (FFM). In this way, repeated measures ANOVA showed an effect of exercise (P= 0.002) and time (P< 0.001), however, no significant (exercise*time) interaction (P= 0.149), (exercise*gender) interaction (P= 0.168), (time*gender) interaction (P= 0.307) or gender difference (P= 0.953) were found. Figure 5.6 illustrates EE per kg of FFM for male and female participants.
Values are presented as mean ± SEM (n= 30; 15F, 15M). There was no difference in (exercise*time) interaction (P= 0.149), (exercise*gender) interaction (P= 0.168), (time*gender) interaction (P= 0.307) and gender difference (P= 0.953). Significant effects of exercise intensity (P= 0.002) and time (P< 0.001) were found. FFM= fat free mass

5.5.3.4 Fat Oxidation

There was a significant difference in mean fat oxidation between genders (P< 0.001). Males had a greater calculated fat oxidation (1.44 ± 0.036) g/min than females (1.02 ± 0.036) g/min. Statistically, there were significant effects in (exercise*gender) interaction (P= 0.029) and (time*gender) interaction (P= 0.020). The difference between LI and HI for male was of a greater magnitude than the difference between LI and HI for female. Value of AUC for males at HI was (108.03 ± 04.24) g and at LI was (91.71 ± 02.10) g, and for females at HI was (74.50 ± 03.16) g and at LI was (69.78 ± 03.46) g. Figure 5.7 shows the mean of fat oxidation post-exercise for male and female participants.
Values are presented as mean ± SEM (n= 30; 15F, 15M). There were significant effects of gender (P< 0.001), (exercise*gender) interaction (P= 0.029) and (time*gender) interaction (P= 0.020).

As with EE, when corrected for body composition and values were expressed per unit of FFM, repeated measures ANOVA revealed that there was an effect of exercise (P= 0.001), time (P< 0.001) and (exercise*time) interaction (P= 0.028). However, there was no longer an (exercise*gender) interaction (P= 0.092), (time*gender) interaction (P= 0.252) or gender effect (P= 0.739). Value of AUC for males at HI was (1.79 ± 0.09) g/kg and at LI was (1.52 ± 0.06) g/kg, and for females at HI was (1.68 ± 0.07) g/kg and at LI was (1.57 ± 0.07) g/kg See Figure 5.8.

**Figure 5.7** Calculated fat oxidation at 5, 45 and 75 minutes following Low and High exercises for each gender.
**Figure 5.8** Fat oxidation in (gram /minute/ FFM) at 5, 45 and 75 minutes following Low and High intensity exercises for each gender.

Values are presented as mean ± SEM (n= 30; 15F, 15M). There was a significant main effect of exercise (P= 0.001), time (P< 0.001) and (exercise*time) interaction (P= 0.028). However (exercise*gender) interaction (P= 0.092), (time*gender) interaction (P= 0.252) and gender difference (P= 0.739) were no longer significant.

FFM= fat free mass

### 5.5.3.5 Food intake post-exercise

The *ad libitum* pasta meal post-HI and, 0-24h EI post-HI were significantly greater for males than females. However due to the discordant body sizes between the groups and the differences in basal energy requirements, these values were then adjusted and expressed relative to REE. Hence, the 0-24h post-HI adjusted and expressed relative to REE was significantly greater for males than females. See Table 5.2 and Table 5.3.
Table 5.2 Significant mean difference of *ad libitum* pasta intake post-exercise and, energy intake with macronutrient intake in the first 24 second of the 24 hour post-exercise at Low and High for each gender.

<table>
<thead>
<tr>
<th></th>
<th>Male (n=15)</th>
<th>Female (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ad libitum</em> pasta meal (KJ)</td>
<td>4786.2±220.9</td>
<td>4228.2±457</td>
<td>0.285</td>
</tr>
<tr>
<td>0- 24h EI (KJ)</td>
<td>9926±1068</td>
<td>6919±547</td>
<td>0.021</td>
</tr>
<tr>
<td>24- 48h EI (KJ)</td>
<td>10630±1181</td>
<td>8080±913</td>
<td>0.099</td>
</tr>
<tr>
<td>0- 24h fat (g)</td>
<td>86.7±9.6</td>
<td>62.8±6.1</td>
<td>0.045</td>
</tr>
<tr>
<td>0- 24h CHO (g)</td>
<td>319.8±35.7</td>
<td>182±15.9</td>
<td>0.002</td>
</tr>
<tr>
<td>24-48 CHO (g)</td>
<td>313.4±36.2</td>
<td>209.2±24.7</td>
<td>0.025</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ad libitum</em> pasta meal (KJ)</td>
<td>5058.6±297.4</td>
<td>3932.5±324.4</td>
<td>0.016</td>
</tr>
<tr>
<td>0- 24h EI (KJ)</td>
<td>11087±1044</td>
<td>6372±623</td>
<td>0.001</td>
</tr>
<tr>
<td>24- 48h EI (KJ)</td>
<td>9231±921</td>
<td>7217±583.4</td>
<td>0.077</td>
</tr>
<tr>
<td>0- 24h fat (g)</td>
<td>98.3±12.3</td>
<td>58.1±10.6</td>
<td>0.019</td>
</tr>
<tr>
<td>0- 24h CHO (g)</td>
<td>349.5±34.9</td>
<td>198.1±16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-48 CHO (g)</td>
<td>234.7±23.9</td>
<td>207.1±15.4</td>
<td>0.340</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n= 30; 15F, 15M).
Table 5.3 The adjusted and expressed relative to resting energy expenditure (REE) in Low and High intensity exercise in each gender.

<table>
<thead>
<tr>
<th></th>
<th>Male (n=15)</th>
<th>Female (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0- 24h EI/REE</td>
<td>1.3± 0.11</td>
<td>1.6± 0.18</td>
<td>0.110</td>
</tr>
<tr>
<td>24-48h EI/REE</td>
<td>1.5± 1.7</td>
<td>1.7± 0.18</td>
<td>0.386</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0- 24h EI/REE</td>
<td>1.2± 0.14</td>
<td>1.8± 0.15</td>
<td>0.004</td>
</tr>
<tr>
<td>24-48h EI/REE</td>
<td>1.3± 0.11</td>
<td>1.5± 0.15</td>
<td>0.401</td>
</tr>
</tbody>
</table>

Values are presented as means ± SEM (n=30; 15F, 15M). EI= energy intake.
5.6 Discussion

Studies have shown that gender can play a role as a factor in modulating food intake in response to acute exercise, suggesting there is an interaction between gender and body weight affecting post-exercise appetite (Westerterp-Plantenga et al., 1997, Pomerleau et al., 2004). The present study aimed to compare genders in a cross-over study exploring the effect of HI and energy matched LI on appetite and GLP-1 (an appetite hormone), EE and EI post-exercise in both healthy men and women.

The EE protocol was calculated by MET’s (Ainsworth et al., 1993), and it was measured by adjusting the VO$_{2\text{max}}$ for each individual in order to ensure that the energy cost was matched between both intensities.

The comparison included thirty Caucasian (white) young volunteers (15 men and 15 women), all of which were in the range of healthy range of BMI (20-25 g/m$^2$). Moreover, the average VO$_{2\text{max}}$ for men was 37.5 ± 1.6 ml/kg/min, and for women was 35.13 ± 1.5 ml/kg/min, which fits the criteria of non-athletes, with no significant difference between genders (Wilmore JH, 2005). So, the results of this study could potentially be applicable and translatable in the healthy population.

The same inclusion criteria was used to recruit male and female volunteers which meant they had the same age range, but as expected, the groups were different in terms of FFM, REE and workload, as generally women have a greater percentage of fat mass than men (Blaak, 2001). Therefore in our study men had a higher FFM, REE and workload than women. In contrast, women had a higher REE per FFM unit, which may be a confounding factor in the interpretation of the results.
In this study, the approximate energy cost of the protocol was designed to be 750 KJ (Little et al., 2010). By measuring the energy cost during exercise, the result showed there was no significant difference between LI and HI exercise sessions for both men and women.

It has previously been demonstrated that, in healthy lean volunteers (both males and females), acute exercise of 60 min cycling at 65% HRmax (~1300-1450 KJ), temporarily decreased hunger sensations when compared with the rest condition, without mentioning the difference between the genders (Martins et al., 2007a). Moreover, hunger has been shown to be reduced immediately post 35 min of HI exercise at 80% VO2max in both men (n = 9) and women (n = 10) compared with rest (Laan et al., 2010). In contrast, another study concluded that LI (50 W) and HI (100 W) exercise for 30 min in six lean healthy men and eight women, had no effect on hunger/satiety sensations when compared to rest (Erdmann et al., 2007). The data suggest that the effect of exercise on appetite is inconsistent and with no general agreement; also there has been no post-hoc analysis in respect to gender differences in these small sample size studies. Therefore in this study, the primary goal was to investigate whether appetite response post-exercise differs between genders.

Our results for the 15 men and 15 women in this study showed there was no significant difference between LI and HI intensity between genders for VAS questions post-exercise. The results also showed that there was a significant difference found in the (time*gender) interaction for hunger and prospective food consumption post-exercise, with a significant difference in (exercise*gender) for prospective food consumption. These results mean that in this study each gender my have responded differently to the exercise regardless of the intensity. Male scores were lower during HI than during LI, and were elevated post-exercise for hunger and prospective food consumption. At the same time, mean hunger and prospective food consumption scores in females in both legs were lower during exercise
and until 30 min compared to men post-exercise, This is in line with the results of other studies that suggests that women do not exhibit suppression of hunger immediately post-exercise (Jan Bilski, 2009).

Our results are comparable to a study conducted by Hagobian et al. (2012), which compared the effect of acute exercise on appetite and EI verses the rest condition in 11 lean healthy men compared to 10 lean women. The exercise treatment was carried out at 70 % VO$_{2\text{max}}$ until 30 % of total daily EE (men 4079.4 ± 815.88 KJ in 82 ± 13 min; women 2983.192 ± 359.824 KJ in 84 ± 17 min) was achieved. The results showed that there was no significant difference between the genders or conditions used in any appetite rating. This is also illustrated by Broom et al. (2007) who found that despite hunger being significantly lower 1 h post-exercise compared to rest, there were no gender differences found between nine well-trained women and men undertaking 1 h running moderate intensity exercise (75 %VO$_{2\text{max}}$).

A recent study has shown that exercise between 50 % - 75 % VO$_{2\text{max}}$ can have the same influence on GLP-1 concentrations during and post-exercise (Ueda et al., 2013). Our results also revealed no gender difference in mean GLP-1, insulin and glucose level for either intensities post-exercise, however there was a significant difference in (time*gender) interaction in insulin concentration 90 min post-exercise. This suggests that the pattern of insulin response appeared to be different between men and women post-exercise. This result was in line with previous studies that found no gender difference in insulin between exercise and rest (Hagobian et al., 2012, Burns et al., 2007).

In our study, women had a higher insulin concentration until 30 min post-exercise compared to men, which has also been detected in another study that demonstrated 90 min of acute exercise at moderate intensity led to a lower peripheral insulin sensitivity 3–4 h
post-exercise in ten lean healthy men when compared to ten lean healthy women; however, no gender difference in suppression of hepatic glucose production was observed (Perreault et al., 2004a). Studies have shown that during 30 min exercise at 60 % VO2max, women have a higher insulin sensitivity compared to men (Boisseau et al., 2000), but it is unknown whether this difference could help to investigate the gender differences in insulin action post-exercise (Perreault et al., 2004a).

Our result showed that, despite no gender differences being found in NEFA and TAG plasma levels, there was a significant difference in the pattern of NEFA response between genders post-exercise, depicted by the (time*gender) interaction observed. This result could be in response to a difference in NEFA storage in the body depots between men and women. The higher NEFA plasma concentration in females may simply be the result of increased adipose tissue storage (Koutsari et al., 2008). In addition, the difference in the NEFA pattern between genders could be as a result of smaller increase in adrenaline in women compared to men as shown during running at 65 % VO2max for ~ 90-101 min (Tarnopolsky et al., 1990), which may lead to the difference in the NEFA mobilisation observed between genders. As insulin plays an important role in lipid and glucose metabolism at the post-exercise stage (Perreault et al., 2004b). The gender undertaken demonstrated in this study might be important. However, adrenaline was not measured in our study therefore these results cannot be confirmed.

Research by Henderson et al. (2007) also points towards noradrenaline and growth hormone playing a key role in the elevated lipolysis, NEFA mobilisation and NEFA oxidation observed in post-exercise recovery in men. Although the lipid mobilisation and utilisation were increased to a greater extent in men than in women, this does not mean that men will have a higher plasma NEFA than women (Henderson et al., 2007). However,
these hormones were also not measured in our study, therefore the differences can only be speculative.

In the present study we also found that the mean EE and fat oxidation were significantly higher in men than women post-exercise, with a significant difference in the gender response post-exercise. However, these differences became insignificant when the results were corrected and expressed per FFM. These findings supports the work by Henderson et al. (2007) who investigated 10 lean men and 10 women during three different iso-energetic trials: exercise for 90 min at 45 % VO$_{2\text{max}}$, 60 min at 65 % VO$_{2\text{max}}$ and the resting condition. Their results showed that over the 3 h of post-exercise recovery, the rate of total EE and fat oxidation were increased to a greater extent in men than women as a result of a higher lipolysis rate (Henderson et al., 2007). Fat oxidation remained increased until approximately 21 h post-exercise intervention, but only in men and not in women (Henderson et al., 2007). Similar to our study it appears fat oxidation was influenced by HI in men, but our measurement was only for one and a half hours post-exercise, therefore the full extent of the difference post-exercise may not have been seen. In addition, post-exercise metabolism was more dependent upon the EE of the exercise than on the intensity of the prior exercise session (Henderson et al., 2007). However, in our results when the energy cost is matched, HI still has a higher impact on post-exercise fat oxidation and utilisation.

Generally, the increase of growth hormone levels post-exercise may contribute to increased EE and fat oxidation (Boutcher, 2010). After exercise an increased EE and fat oxidation could be due to the energy substrate selection in non-muscle tissues being the site of raised FA utilisation in men, together with a coordinated improved mobilisation of adipose tissue to meet demand (Henderson et al., 2007). This could explain the higher EE and fat oxidation in men in our study post-exercise. In fact, Perreault et al. (2004a) has suggested
that it is unknown whether gender-specific patterns of exercise fuel utilisation affects the metabolism post-exercise. Overall this suggests more investigation is required to explore the mechanism behind the different pattern of EE and fat oxidation between genders with a longer duration of post-exercise measurements taken. It could also help to illustrate our result as men have a higher response to HI and higher EE and fat oxidation post-exercise.

Most of the research into the effect of exercise on EI have been carried out in men and has found a greater acute negative energy balance post-HI (Reger WE et al., 1986, Imbeault et al., 1997). Also, some research has noted an absence of compensation for the exercise energy cost at the meal post-HI in women (George and Morganstein, 2003, King et al., 1996). The absence of the compensation in EI post-HI is in line with our study as for both men and women EI was similar post both intensities.

One of the key findings to emerge from our study is that exercise intensity per se does not explain the effect on ad libitum and food intake, and that men have higher amount of EI post-exercise than women for both intensities. Moreover, our finding showed that after adjusting and expressing relative EI to REE due to discordant body sizes, it was shown that after 24 hours, EI was still higher in men than women following both intensities. Our result were in line with a recent study by Hagobian et al. (2012) which assessed EI matching both genders at the same EE, and showed that there was no gender difference in the effect of acute exercise on EI, albeit that men had a higher EI than women. This study also found that acute exercise suppressed relative EI in both genders (Hagobian et al., 2012). Our results are consistent with findings of past studies, suggesting that both genders do not tend to compensate for the induced EE after an acute bout of exercise (Erdmann et al., 2007, Burns et al., 2007).
Overall our results show there was no gender difference found in hunger or appetite sensation, GLP-1, glucose and TAG plasma levels, however there was a different response for hunger, prospective food consumption, NEFA, and insulin between the genders post-exercise. Although, no gender difference was found in EI, however, men had a higher response to the exercise in terms of EE and fat oxidation with an expected higher amount of EI post-exercise. Based on these results a number of possible future investigations using a similar exercise protocol can be investigated, for example, it would be valuable to study the gender difference in the effect of exercise intensity for a chronic period, e.g. over a period of training.
Chapter six
6 Short-term appetite control in response to an acute exercise intervention in overweight volunteers

6.1 Introduction

Obesity is recognised as a global epidemic and the treatment of obesity-related diseases is rapidly becoming the greatest economic burden faced by national health care services across the world (Withrow and Alter, 2011). A long term imbalance between a high calorie EI and a lower EE is often associated with an increase in body weight and the development of obesity (WHO, 2006), and it is widely acknowledged that PA plays an important role in preventing weight gain (Haapanen et al., 1997). Obese people show a measureable functional limitation in their motor activity, which generally reduces their quality of life and causes a lack of mobility, which has an impact on their daily activities and can contribute to a general sense of fatigue (Larsson et al., 2002). Hence, continuous and extended HI may not be maintainable in a sedentary overweight population. However, a mixed exercise protocol that includes short bouts of HI spread out through periods of LI may be more suitable. Recently, the popularity of this type of exercise as a treatment has increased, given the notable progress in several comorbidities of obesity (Kessler et al., 2012).

The ability of exercise to create a negative energy balance relies directly on its impact on EE, and also indirectly on its potential to modulate EI (King et al., 1997b). The effect of acute exercise on appetite sensation and subsequent food intake remains controversial (Blundell and King, 1999). The majority of studies suggest that acute exercise does not stimulate hunger or food intake (King et al., 2013). In addition, studies found that exercise improved appetite control, and people who exercise regularly can detect and manipulate the differences in the energy content between meals in comparison with sedentary people (Van Walleghehen et al., 2007). Short term (1-2 days) energy balance can be greatly
influenced by acute bouts of exercise (Blundell et al., 2003), and this alteration has previously been shown to influence EI and sensations of appetite, such as hunger and satiety (King et al., 2010a, Pomerleau et al., 2004). A large number of studies have been conducted in a healthy population and have investigated the link between exercise and bodyweight. However, the effect of exercise intensity on appetite in overweight individuals needs further study (Martins et al., 2014), and there is a need to investigate if different populations respond differently with regard to post-exercise appetite regulation.

It was shown (chapter 4) that in lean individuals HI only increased EE, NEFA utilisation and fat oxidation without any influence on appetite, TAG, glucose insulin, GLP-1 and EI post-exercise. Therefore it was hypothesised that HI would have a beneficial effect on increasing EE and the amount of fat utilised and oxidised post-exercise in overweight participants, and therefore to test this hypothesis the original protocol was repeated.

6.2 Aim

To examine the acute effect of exercise intensity on appetite and EI versus EE, substrate utilisation and oxidation in overweight individuals.

6.3 Methods

Methods were fully described in chapter 2.

6.4 Results

6.4.1 Participant characteristics and main measurements

A total of 30 participants were initially screened for the study. The average score of emotional, external and restrained eating from DEBQ were (2.7 ± 0.2), (3.3 ± 0.1) and (2.5 ± 0.2), respectively. Of these 30 participants, 9 (3 men+ 6 women) participants did not meet the inclusion criteria. One female subject was excluded from the study due to an irregular
ECG trace. 2 (one male and one female) participants failed to attend their screening day. Moreover, 7 (5 males and 2 females) withdraw from the study due to personal reasons, leaving 10 participants (6 men and 4 women) who completed both study days while only one male participant could not complete both days due to a sudden hypoglycaemic response to exercise. Figure 6.1 depicts the participants flow diagram adapted from (Moher D, 2001).

Figure 6.1 Overweight participants’ flow diagram.
The baseline characteristics of 10 participants who completed both visits are displayed in Table 6.1.

Table 6.1 Baseline characteristics of overweight participants who completed the exercise study intervention.

<table>
<thead>
<tr>
<th>N= 10</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176 ± 4</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>89.6 ± 4.8</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.6 ± 0.7</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>28.9 ± 3.2</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>71.1 ± 3.2</td>
</tr>
<tr>
<td>REE (KJ/day)</td>
<td>6983 ± 400</td>
</tr>
<tr>
<td>Fasted RQ (at rest)</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td>VO2max(ml/min/kg)</td>
<td>28.5 ± 1.7</td>
</tr>
<tr>
<td>Workload (50%) (W)</td>
<td>100 ± 10.6</td>
</tr>
<tr>
<td>Workload (95%) (W)</td>
<td>190 ± 20.1</td>
</tr>
<tr>
<td>Random blood Glucose (mmol/L)</td>
<td>5.5 ± 0.2</td>
</tr>
</tbody>
</table>

Participant were exercised less than three times a week (Wilmore JH, 2005). Results are expressed as Mean ± SEM. BMI= body mass index; REE= calculated resting energy expenditure; RQ= respiratory question; VO2max= maximal oxygen uptake; FFM= fat free mass. No significant difference was observed in BMI in Low and High (P= 84.2).

6.4.2 During exercise Measurements

6.4.2.1 Energy expenditure and work load

According to the study hypothesis, paired t-test showed no significant difference between LI and HI in terms of energy cost during exercise (P= 0.378). The energy costs of both intensities were matched, and therefore the primary objective of the study in terms of energy was achieved. Figure 6.2 shows mean EE during Low and High exercises.
Figure 6.2 Measured energy expenditure (EE) during 30 minutes of Low (50% VO₂max) and 20 minutes of High (95% VO₂max) exercise on each study day in overweight participants.

Values are expressed as mean ± SEM (n= 10; 6M, 4F). Low (580± 35) versus High (567±29) exercise. No significant difference between exercise protocols (P= 0.378) was found.

6.4.2.2 Substrate utilisation

The results showed that there was a significant difference in the mean RER between LI and HI during the exercise intervention (P= 0.002). Figure 6.3 depicts the mean RER during Exercise.
Values are expressed as mean ± SEM (n= 10; 6M, 4F). Low (0.977 ± 0.007) versus High (1.057 ± 0.020) exercise. A significant difference between interventions (P= 0.002) was observed.

6.4.3 Post-exercise measurement

6.4.3.1 Subjective appetite ratings

Repeated measures ANOVA comparing the exercise intensities over time for the study day for each of the eight VAS questions showed the expected change in appetite response over time. There was, however, no significant difference in response to exercise intensity alone and in terms of intervention and (time*exercise) interaction in any of the components of appetite regulation (P> 0.05) between the two exercise intensities. Figure 6.4 shows the effect of Low versus High exercise on first Subjective Appetite Ratings.
A 500 kcal breakfast was given immediately after fasting (F). Shading indicates the period of exercise intervention. *Ad libitum* pasta test meal was given at 220 minutes. Values are expressed as mean ± SEM (n=10; 6M, 4F). ANOVA showed that there was a significant effect of time for all VAS questions (P<0.001). No significant effect of exercise or (exercise*time) (P>0.05) was observed for any appetite rating.
6.4.3.2 Plasma metabolites and GLP-1

6.4.3.2.1 Triacylglyceride (TAG)

No significant effect on exercise intensity or (exercise*time) interaction observed in TAG plasma levels (P= 0.180) and (P= 0.344), respectively, was observed. A significant effect on time (P< 0.001) was, however, found during the study. Mean TAG plasma level was elevated in LI and HI at the end point of the exercise session compare with pre and post-exercise as showed in Figure 6.5

**Figure 6.5** Plasma TAG concentrations (mmol/L) over time pre, during and post exercise in overweight participants.

A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are presented as mean ± SEM (n= 10; 6M, 4F). ANOVA showed that there was no significant effect of exercise alone (P= 0.344), but a significant effect of time (P< 0.0001) was found.
6.4.3.2.2 Non-esterified fatty acid (NEFA)

There was no significant effect of exercise alone on post-prandial NEFA concentration (P=0.327), while significance of (time*exercise) interaction (P=0.034) and effect of time (P=0.001) were found. Mean NEFA started to increase from the end point of the exercise session for both legs. It was lower in HI up to 180 minutes. Figure 6.6 shows the mean of NEFA plasma level pre, during and post-exercise.

Figure 6.6 Plasma non-esterified acid (NEFA) concentrations (mmol/L) over time pre, during exercise and post exercise in overweight participants.

A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are expressed as mean ± SEM (n= 10; 6M, 4F). ANOVA showed that there was significant effects of (time*exercise) interaction (P= 0.034) and time (P= 0.001).

6.4.3.2.3 Glucagon-like peptide-1 (GLP-1)

No significant effect of exercise intensity was found in plasma GLP-1 levels (P= 0.398) was observed. In contrast, significant effects on (exercise*time) interaction (P= 0.029) and time (P= 0.004) were found. Mean plasma GLP-1 level was elevated in LI and HI at the end point of the exercise session compared with pre and post-exercise. Overall, LI was greater than HI but did not reach the significant level. See Figure 6.7.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are presented as mean ± SEM (n= 10; 6M, 4F). ANOVA showed significant effects of (time*exercise) interaction (P= 0.029) and time (P= 0.004).

6.4.3.2.4 Glucose and Insulin

No significant effect of exercise or (time*exercise) interaction was observed in plasma glucose and insulin concentration. As expected, the plasma glucose and insulin profiles mirrored one another. Both metabolites increased for 30 minutes post-breakfast and decreased for 30 minutes, where in glucose increased in both exercise protocols for the remaining 60 minutes. A significant effect of time (P< 0.001) was, however, observed on plasma glucose level and insulin concentration. Figure 6.8 depicts the mean of glucose and insulin plasma concentrations pre, during and post-exercise.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are expressed as mean ± SEM (n= 10; 6M, 4F). ANOVA showed that there was a significant effect of time alone (P< 0.001) for both glucose and insulin concentrations.
6.4.3.2.5 Energy expenditure (EE)

Comparing the exercise intensities over time for each study day for each of the three time points of EE post-exercise, the results indicated no significant effect of exercise alone on EE (P= 0.696) and (exercise*time ) interaction (P= 0.587). However there was a significant effect of time (P< 0.001) between the two intensities. EE was declined over 75 min post-exercise for both legs, as depicted in Figure 6.9.

Figure 6.9 Energy expenditure (EE) following either Low or High exercise measured at 15, 45 and 75 minutes post-exercise by indirect calorimetry in overweight participants.

Values are presented as mean ± SEM (n= 10; 6M, 4F). ANOVA showed that there was a significant effect of time (P< 0.001). Mean Low results were (6.227 ± 0.279), (5.690 ± 0.257) and (5.517 ± 0.289) KJ/min. Mean High results were (6.208 ± 0.374), (5.805 ± 0.277) and (5.596 ± 0.291) KJ/min

6.4.3.2.6 Substrate Utilisation

Substrate utilisation was determined by RQ value. Despite no significant effect of exercise (P= 0.628) between both intensities post-exercise, there was a significant change in (exercise*time) (P= 0.026) and time (P< 0.0001). HI had a lower RQ value compared to LI, which had a higher RQ value. However, 45 minutes post-exercise substrate utilisation was nearly similar for both intensities. 75 minutes post-exercise, LI had a lower RQ value
compared to HI. Mean values for LI at 5, 45 and 75 minutes were (0.901 ± 0.012), (0.873 ± 0.023) and (0.820 ± 0.012). Mean values for HI at 5, 45 and 75 minutes were (0.838 ± 0.014), (0.881 ± 0.021) and (0.858 ± 0.013). Figure 6.10 illustrates the mean RQ Post-Exercise.

Figure 6.10 Mean respiratory quotient (RQ) post-exercise at 15, 45 and 75 minutes in overweight participants.

Values are expressed as mean ± SEM (n= 10; 6M, 4F). ANOVA showed that there was no significant main effect of exercise alone (P= 0.628).

6.4.3.2.7 Fat oxidation

Repeated measures ANOVA showed that there was no exercise or (exercise*time) interaction effect on fat oxidation post-exercise (P= 0.063) and (P= 0.920). However, there was a significant effect on time alone post-exercise (P< 0.001). Mean LI at 5, 45 and 75 minutes were (1.68 ± 0.07), (1.55 ± 0.07) and (1.53 ± 0.07) g/min. Mean HI at 5, 45 and 75 minutes were (1.70 ± 0.10), (1.57 ± 0.063) and (1.53 ± 0.082) g/min. Overall, fat oxidation declined until 75 min post-exercise for both legs. Value of AUC for LI was
(111.32 ± 05.38) g and for HI was (112.86 ± 05.50) g. Figure 6.11 depicts fat oxidation post-exercise.

Figure 6.11 Mean fat oxidation at 15, 45 and 75 minutes post-exercise in overweight participants.

![Fat Oxidation Graph](image)

Values are presented as mean ± SEM (n= 10; 6M, 4F). ANOVA showed that there was a significant effect of time (P< 0.001), however, no exercise (P= 0.063) and (exercise*time) interaction (P= 0.920) effect were found.

### 6.4.3.2.8 Ad Libitum pasta test meal intake and 48 hours intake.

After analysing the data by a paired t-test and Wilcoxon matched pairs test, it was found that there was no significant difference in the mean of *ad libitum* test meal or the 48 hour intake records following exercise, between the two intensity periods. See Table 6.2.
Table 6.2 *ad libitum* pasta intake post-exercise and energy intake with macronutrient intake in a 48 post-exercise.

<table>
<thead>
<tr>
<th></th>
<th>Mean Low</th>
<th>Mean High</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ad libitum</em> pasta intake (KJ)</td>
<td>5494±421</td>
<td>5241±363</td>
</tr>
<tr>
<td><strong>0-24 h intake post-exercise</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (KJ)</td>
<td>11312 ± 2070</td>
<td>7849 ± 1782</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>108 ± 18</td>
<td>85 ± 13</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>112 ± 25</td>
<td>91 ± 19</td>
</tr>
<tr>
<td>Carbohydrate CHO (g)</td>
<td>290 ± 55</td>
<td>288 ± 52</td>
</tr>
<tr>
<td><strong>24-48h intake post-exercise</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (KJ)</td>
<td>7899 ± 930</td>
<td>7995 ± 1444</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>84 ± 11</td>
<td>95 ± 15</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>79 ± 13</td>
<td>89 ± 19</td>
</tr>
<tr>
<td>Carbohydrate CHO (g)</td>
<td>221 ± 25</td>
<td>257 ± 43</td>
</tr>
</tbody>
</table>

*Ad libitum* Pasta test meal post-exercise and the total two days of energy and macronutrient intake following Low or High. Values are expressed as mean ± SEM (n= 10; 6M, 4F).
6.5 Discussion

Investigations of the current literature have produced no relevant papers that studied/compared HI vs LI of the same energy cost for overweight people. Hence at the acute level, it remains uncertain if and to what extent, exercise intensity impacts on appetite, GLP-1 and EI, especially for the overweight population. This chapter continues the investigation of the previous chapters by trying to understand the effect of exercise intensity on appetite, EI, EE and fat oxidation specifically in overweight people.

The total number of overweight people who could complete the intervention until the end were 10 participants. A high dropout rate was observed in this overweight study, which was one of the barriers to conducting the study in addition to the difficulty of initially recruiting overweight volunteers.

Of the ten Caucasian (white) young volunteers, 8 were in the overweight range (BMI >25 kg/m²), with 2 volunteers classified as obese (BMI >30 kg/m²). Their average VO_{2max} was (28.5 ± 1.1 ml/kg/min) which confirmed they were non-athletes, and this value was below the average VO_{2max} according to the Wilmore JH (2005) criterion. So, the results of this study could potentially be applicable to the healthy overweight population.

The present study investigated the transient effect of acute HI versus LI on subsequent EI, appetite, an appetite related hormone (GLP-1), EE and fat oxidation in sedentary, overweight people. In order to ensure that the energy cost was matched between both intensities, the protocol was calculated by MET’s and it was measured by adjusting for VO_{2max} in each individual (Ainsworth et al., 1993).

The results for the energy cost measurements showed no significant difference between LI and HI during each exercise session, which shows the energy costs of both intensities were matched, which was the primary objective of the study. In addition, there was a significant
difference in the mean RER between LI and HI during the exercise intervention, which suggests there was a difference in the fuel utilisation between the exercise intensities. It has been established that an RER value of 1.00 is equal to 100 % CHO oxidation and of 0.7, 100 % fat oxidation (McArdle et al., 2010), therefore, as the mean of LI (0.99 ± 0.007) was close to 1.00, this indicated that the subjects tended to use CHO as a main fuel source. The mean of the RER in HI (1.08 ± 0.012) was higher than 1.00 which signified the subjects used the anaerobic pathway to provide their bodies with the energy needed to complete HI session (McArdle et al., 2010).

Several studies have suggested that HI exercise can transiently suppress appetite when compared to rest (Thompson et al., 1988, Kawano et al., 2013), and that LI or moderate exercise has an influence by increasing appetite when compared to rest, especially for obese individuals (Tsofliou et al., 2003). These results were contradicted by the experiments of Unick et al. (2010), Cornier et al. (2012) and Ueda et al. (2009b) who suggested that LI/moderate exercise had no effect on appetite in obese/overweight individuals. None of these studies compared different intensities of exercise, which could provide insight into the best type of exercise to help overweight subjects lose weight.

Our result of VAS questions post-exercise showed that there was no effect of exercise alone or an (exercise*time) interaction for hunger, prospective food consumption and fullness scores, but there was a significant change in time alone. Overall these results are similar to those obtained recently by Martins et al. (2014) and Sim et al. (2013) who compared the effect HI vs moderate intensity on appetite in obese/overweight people. In contrast, Kissileff et al. (1990) demonstrated that the rating of hunger was increased after LI in comparison with HI and rest in obese individuals. Overall the experimental data are rather unclear and there is no general agreement between authors about the effect of exercise intensity on appetite. It could be argued the effect of HI may still be beneficial for
overweight people as it did not increase hunger or appetite post-exercise in a compensatory manner.

The appetite related hormone GLP-1 is released in response to food intake, generally following fat and CHO (Nauck et al., 2004). The rapid increase in GLP-1 concentrations found in adults after having a meal signifies that GLP-1 might play a physiological role in meal termination (Nauck et al., 2004). As described previously, our study has shown that HI had no direct suppression of hunger or stimulation of satiety in comparison with LI in overweight individuals. The results of GLP-1 concentration post-exercise similarly showed no significant difference between both intensities. However, it appears that the GLP-1 level was higher in LI until 30 min post-exercise (when compared to HI), but it did not reach a significant level. HI did not influence appetite or GLP-1 in these overweight individuals post-exercise.

Despite there being very few studies that have compared LI vs HI in obese/overweight participants, in one of the few studies performed in obese individuals, Ueda et al. (2009) showed that 60 minutes of LI cycling (50 % VO$_{2\text{max}}$) led to a significant suppression in insulin levels, a significant increase in PYY and GLP-1 concentrations, but no changes in subjective appetite feelings in obese men (Ueda et al., 2009b). Similar results were obtained in a recent study by Martins et al. (2014), which showed that HI and LI suppressed insulin level during exercise, increased the post-prandial release of GLP-1 during and post-exercise, yet had no impact on hunger or fullness, despite reducing the desire to eat in overweight/obese participants. Their results were in contrast with our results, however it should be noted that in these previous studies, exercise was in comparison to rest condition and the duration of Ueda et al. (2009b) was double the length of our intervention, which may have an impact on the hormone levels. The results of the study also showed that in overweight/obese individuals, acute iso-energetic bouts of HI or moderate lead to a similar
appetite response (Martins et al., 2014), but the mechanism by which acute exercise increases the plasma level of gut hormones such as GLP-1 remains unknown (Martins et al., 2007b). The absence of a significant difference in plasma GLP-1 levels in our study between both intensities post-exercise does not mean that the exercise intensity has not stimulated GLP-1 release directly, or indirectly via stimulation in sympathetic nervous system with rising catecholamine levels (Hersey III et al., 1994), but that the release may need a longer exercise duration to demonstrate a significant effect for both exercise types.

The idea to look at the effect of HI vs LI on appetite and GLP-1 is also linked to studies that found there was an effect of exercise on plasma glucose and insulin levels, with an improvement in glycaemic regulation in overweight adults (Houmard et al., 2004, Roberts et al., 2013), which was associated with improved muscle and/or replacement of muscle glycogen stores (Holloszy, 2005). Nevertheless, our study showed no difference in plasma glucose and insulin concentration after the exercise condition when comparing the two exercise types in the overweight volunteers. Our findings are different to a study by Little et al. (2014) who used a similar HI protocol, they found that one session of HI involving 10 * 1 min intervals, producing ~90 % HR_max, improved post-prandial glucose control in overweight/obese adults for up to ~24 hours following exercise (Little et al., 2014). Our results are also different to a study which showed that the effect of exercise 70 min of (50 % VO_{2max}) or 55min (65 %VO_{2max}) can be attributed, at least in part, to an acute increase in skeletal muscle insulin sensitivity that persist for ~24- 48 hours following each bout of exercise in obese people (Newsom et al., 2013). One of the key differences with these studies and ours is they found the effect within 24 h while in our study the measurements were every half an hour for only one and half hours, which may be too short a time period to detect a change.
Our findings showed both glucose and insulin plasma levels were elevated until 30 min post-exercise in both intensities, and returned back to the baseline level at 60 min post-exercise. Each exercise session might need a longer duration, or to apply the exercise intervention regularly i.e. in training interventions show a significant difference in glucose.

A recent study by Sim et al. (2013) showed that in 17 overweight males, their glucose level was higher after 30 min of very HI (170 % VO₂max), HI (100 %VO₂max) in comparison with and moderate (60 % VO₂max) and rest condition, but as with our results, there were still no differences observed between the intensities. Hence, further research is needed to investigate if the acute effect can be translated into training, or if repeated interventions over time in a bigger group of overweight and obese people produces a clear result.

In our study post-exercise measurements were only conducted for one and a half hours after 20 or 30 min of exercise, meaning the exercise duration and time of measurement were limited, especially as it was concluded by Kang et al. (1996) that an improved insulin sensitivity occurred after 7 days of exercise at 70 % VO₂max for 50 min in obese participants and might be linked to greater muscle glycogen utilization during exercise (Kang et al., 1996).

Future investigation is needed to determine the exact mechanisms that contribute to an enhanced post-prandial glycaemic regulation after HI, and to effectively determine how this type of exercise compares to the typical LI for adherence and improving wellbeing outcomes over time, which could related to insulin, GLP-1 and appetite regulation (Little et al., 2014).

Previous research has shown that 90 min of exercise at ~50 % VO₂max reduced both fasting and post-prandial TAG concentrations by 25 % in both the lean and centrally obese volunteers in compered to rest (Gill et al., 2004). However, our study of ten overweight
participants indicated that there was no significant difference in TAG level found between the exercise intensities post-exercise, which agreed with Ueda et al. (2009b) who also showed no significant difference for TAG in comparison with the rest condition, but this may be due to small number of participants and acute exercise intervention. The results may differ if a larger study was undertaken over a larger time period.

The results of our investigation show that, despite the significant change in the pattern of NEFA concentration over time post-exercise, exercise intensity did not significantly influence the mean NEFA concentration. This result could be explained by a previous study that showed that during HI exercise, the delivery of NEFA from adipose tissue to the muscle and the use of FA by the muscle are reduced (Jeukendrup, 2002). This reduction in NEFA concentration in the blood might continue to the post-exercise period. The results are consistent with past studies which confirmed that, despite no data indicating a particular exercise type that would improve NEFA oxidation in overweight participants, LI (50 % VO$_{2\text{max}}$) provided the highest acute fat oxidation compared with 30 % VO$_{2\text{max}}$ and 70 % VO$_{2\text{max}}$ in overweight men and women, and this it might affect the fat oxidation in relation to our result post-exercise (Pillard et al., 2007). Another study that showed no difference in NEFA plasma level between intensities also showed an increase in use of the non-plasma FA as a fuel during LI exercise in overweight /obese men, which could affect the NEFA plasma level post-exercise (Mittendorfer et al., 2004).

The present results suggests there is a need to take into consideration the EE and also the calculated fat oxidation findings post-exercise. In our study among overweight subjects during 75 min post-exercise, there was no significant difference in EE rate and calculated fat oxidation between HI and LI. Similarly, Saris and Schrauwen (2004) found that isocaloric HI cycling (three times 30 min of interval protocol, 2.5 min 80/50 % W$_{\text{max}}$) or LI cycling (three times 60 min continuously at 38 % W$_{\text{max}}$) did not differentially affect the
24 h post-exercise EE and substrate oxidation in 8 obese men. It has been suggested that overweight people have a lower capacity to oxidize FAs due to a limited muscular oxidative capacity and/or lipid mobilization (Pillard et al., 2007). The decrease in this last process among overweight people might be related to having a greater sedentary lifestyle compared with lean people. This suggestion is based on results from a previous study indicating that in overweight men, a four month aerobic training program, consisting of 5 d/wk of aerobic exercise, can enhance exercise-induced lipolysis and lipid utilisation (De Glisezinski et al., 2003). Moreover, it has been found that in 24 obese men, exercise training was effective in increasing fat oxidation when the exercise was performed at LI (40 % VO$_{2\text{max}}$) compared to rest, and also that fat oxidation was not significantly increased during HI (70 % VO$_{2\text{max}}$) exercise training (Van Aggel-Leijssen et al., 2002). This finding was in agreement with a comparable study of 21 lower and upper-body obese women, exercising at 40 % VO$_{2\text{max}}$ three times per week for 12 weeks, that found the exercise had no effect on fat metabolism in lower body obese women, but an increase in the contribution of fat oxidation to total TEE was observed in upper-body obese women, suggesting there could be an influence in the post-exercise condition, as was observed in our results (Aggel-Leijssen et al., 2001). Despite these results, there is still no understanding of the exact mechanism and the most effective intensity, however, the goal of this study was to find out how to improve the EE and fat oxidation post-exercise either by LI or HI. Achten and Jeukendrup (2004) suggested that the maximal fat oxidation rate occurs during LI (47 % - 52 % VO$_{2\text{max}}$) in a large sample of the general population including obese individuals. However, any analysis needs to take in account the impact of the exercise intensity on post-exercise fat oxidation to truly understand the processes occurring.
In 2000, Kim et al. (2000) published a paper in which they described defects at several levels in the catabolic process of lipid metabolism in obese skeletal muscle mitochondria, such as a defect in CPT-1 and β-hydroxy-acyl-CoA-dehydrogenase (β-HAD) activity, with an elevation in PFK activity. These findings also provide a possible reason for the reduced dependence on lipid oxidation shown in obese people (Kim et al., 2000). As there was no difference between exercise intensities in our study in terms of EE and fat oxidation post-exercise, this could allow overweight individuals to choose the convenient intensity to allow exercise to be carried out for a long duration or at more frequent intervals. These results could lead to new ideas about the effects of inter-changing between HI and LI in a long exercise duration, and to what extend that could help to improve fat oxidation. However, Pillard et al. (2010) found that in 10 young overweight men, HI cycling (~30 minutes at 70% VO2max) produced a greater EE and fat oxidation within 30 min post-exercise, compared to LI cycling (~60 minutes 35% VO2max), and HI produced a higher fat oxidation at 6 hours after ingestion of a large meal 30 min post-exercise than LI, which suggest there are differences between the intensities. In fact, the sample size of each overweight or obese study need to be increased in the future to allow the true differences between the intensities to be determined. Overall, these results suggest exercise can be beneficial in improving fat oxidation capacity and can simplify weight maintenance, which is likely to help prevent obesity. However, it is still unknown what the best exercise regime for the obese people is, especially considering other factors such as safety and compliance with HI exercise (Saris and Schrauwen, 2004).

In the present investigation there was no significant difference observed in ad libitum food intake and total EI between HI and LI at either 24 hours or 48 hours post-exercise, however, after 24 h EI post-HI was 3462 KJ lower than post-LI. The small (but insignificant) reduction in EI 24 h after HI suggests HI might be beneficial if this protocol is applied.
frequently for a chronic period and in a larger group with increased study power. The result is similar to the results from earlier literature that showed that obese individuals were not only less responsive, but possibly totally unresponsive, to the effects of exercise on EI, as no compensatory rise in EI was noticed despite increased EE through daily walking (Woo et al., 1982, Stensel, 2011).

Research to date has tended to focus on the effects of exercise intensity on EI in a small number of overweight participants such as: Alkahtani et al. (2014), Martins et al. (2014) and Ueda et al. (2009b). Some authors found that LI does not affect appetite regulation and EI following exercise (George and Morganstein, 2003, Tsofliou et al., 2003), which fits with our finding that HI also did not affect appetite regulation and EI following exercise in comparison with LI. It was also was reported that in overweight and obese women an acute bout of walking (70-75 % VO$_{2\text{max}}$) did not influence the feelings of hunger or post-exercise EI compared with rest (Unick et al., 2010). Our findings were compared with LI, which may have an effect on the results, and are different to the findings by Ueda et al. (2009b) who showed a reduction in post-exercise EI after 1 h of cycling at LI 50 % VO$_{2\text{max}}$. This difference may be due to of the longer (double) duration of the exercise in this study in comparison with our study.

Generally further study is needed to elucidate the effect of HI on EI as the current studies have mixed results. A recent study showed suppression of EI after an acute bout of exercise at very HI intermittent intensity (≥100 % VO$_{2\text{max}}$) in overweight men (Sim et al., 2013). However, only men were recruited, the exercise was performed in fasted states, and moreover the HI employed in that study was at levels that would be difficult to sustain in the obese population (compared to ours), which could explain the different outcomes observed. On the other hand a study by Martins et al. (2014) suggested that exercise intensity by itself does not appear, at least in overweight/obese individuals, to have an
impact on appetite. The study showed that there were no significant differences in absolute macronutrient or EI differences among all the exercise conditions investigated, with no significant effect of exercise intensity found on subjective feeling of appetite, or appetite hormones which also continued post-exercise (Martins et al., 2014). A study by Thivel et al. (2012) investigated if a single bout of HI (75 % VO2max) (1400 KJ) suppressed spontaneous EI in the subsequent 24 h compared with a single iso-energetic bout of LI (40 % VO2max) and rest, in 15 obese adolescents, their results suggested that acute HI did not increase appetite and EI in obese people. (Thivel et al., 2012). These results were in support of the implementation of HI, as it was observed that despite the fact that the HI (maximal 4-s sprints repeated every minute with LI between sprints) involved a larger amount of work, it also suppressed post-exercise EI in overweight boys (8-12 years old) compared with a continuous bout of moderate-intensity exercise alone (Crisp et al., 2012). However, the reaction to exercise could be different between age groups (Skinner, 2005).

In conclusion, our findings showed that acute HI did not influenced the appetite hormone (GLP-1), glucose, insulin, NEFA, or TAG level post-exercise in the overweight participants. Also, no change was seen in the appetite components (hunger, prospective food consumption and fullness), EE and fat oxidation post-exercise in overweight individuals. Despite there being no significant difference in the EI between both exercise types, however, participants tended to eat less in the 24 h post-HI. This finding could provide an advantage as it could give an option to this population to choose between the intensities.

Our results are consistent with findings that HI intermittent exercise, in which HI session is interspersed with LI bouts, has been observed to save time and makes a maintainable exercise protocol (Boutcher, 2010). This type of exercise is sustainable for untrained obese individuals, allowing for a better outcomes on body weight, body composition,
cardiovascular fitness and insulin resistance (Boutcher, 2010). This also agrees with the data from Imbeault et al. (1997) who suggested that in some cases HI cannot be prescribed alone, especially for some clinical conditions such as obese people who are not used to exercise, or who are at risk of tolerance to exercise. In these cases the most practical approach is still LI with a progressive increase in frequency and duration, however, increasing intensity toward HI might be relevant if this is compatible with the fitness and wellbeing of these individuals (Imbeault et al., 1997). In fact, a longer duration of the exercise intervention and more frequent sessions may favour an increase in appetite response and fat oxidation for overweight people rather than a reduction.

One main criticism of our study was the small sample size (only 10 participants), making it impossible to divide it into a smaller group and to compare the results in terms of gender. However, these results show promise and suggest the protocol could be applied to larger sample size as an exercise training method in order to produce a more accurate outcome.
Chapter seven
7 Effect of BMI on energetic and physiological responses to exercise intensity.

7.1 Introduction

Exercise has been widely acclaimed as a successful method for weight loss (Boileau et al., 1971). Several exercise guidelines prescribed LI/ moderate intensity in order to prevent and treat obesity, however, exercise prescription should be considered not only through the effects that occur during exercise but also the post-exercise effects (Tabata et al., 2014). HI training is proposed as a more effective training method for weight management, rather than weight loss, due to its capacity to increase fat oxidation and decrease waist circumference (Whyte et al., 2010, Boutcher, 2010).

It was suggested that HI training would be beneficial for obese people as it can improve several metabolic markers, including fat oxidation and abdominal fat loss (Gibala and Little, 2010). Moreover, at the individual level, there is little evidence available to support the notion that obese subjects are significantly less active than lean subjects, therefore, inactivity is a poor predictor of obesity, until obesity reaches a severity threshold where performing any type of movement becomes highly limited (Prentice et al., 1996).

Post-exercise appetite suppression is not commonly observed in overweight and obese people (Unick et al., 2010). This might be as a result of a differing response toward exercise in this population, or it might be because it is rare to undertake studies that investigate acute effects of exercise at >60 % VO$_{2max}$ on appetite in overweight and obese participants (George and Morganstein, 2003). Generally, despite the optimized fat oxidation during LI or moderate exercise in lean and overweight individuals, HI could be more beneficial as it prompts a higher fat oxidation post-exercise (Pillard et al., 2010). Furthermore, it can be seen from the limited number of studies in the literature for this population, that the understanding around this issue is complex.
Previous studies have primarily concentrated on the effects of exercise on appetite and food intake in lean participants, however, the exact effect of exercise on appetite and EI will differ in strength from one group of participants to the others depending on their individual physiological characteristics and the intensity and the duration of exercise. Hence, it is hard to predict the responses to the exercise as it is highly variable (Blundell et al., 2015). Investigations of the current literature have produced no relevant papers that studied/compared HI verses LI of the same energy cost for both lean and obese participants.

7.2 Aim
To investigate influence of BMI on the response to the acute exercise intensity.

7.3 Methods
Methods were fully described in chapter 2.

7.4 Statistical analyses
This is a Post-hoc comparison of the data from two earlier studies (chapters 4 and 6). An unpaired t-test was performed to compare two groups of participants (lean versus overweight) for outcomes such as RER, EE during exercise, ad libitum (pasta test-meal) and food and macronutrient intake (CHO, protein and fat) in the 24, 48 hour post-exercise. Non-parametric testing was used (Mann-Whitney U test) to normalise the results when required. Statistical significance was assumed at (P< 0.05). In order to investigate if there was any gender different in the outcome, 2-way mixed repeated measures ANOVA was used, with exercise as the within-subjects factor and overweight as the between-subjects factor.
7.5 Results:

7.5.1 Participants

The baseline characteristics of 40 lean and in overweight participants who completed both visits are displayed in See

Table 7.1

**Table 7.1** Baseline characteristics of lean and overweight participants who completed the exercise study intervention.

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Overweight</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 30)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>23 ± 1</td>
<td>25 ± 2</td>
<td>0.40</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.22 ± 1.98</td>
<td>176.5 ± 4.0</td>
<td>0.30</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.51 ± 1.94</td>
<td>89.6 ± 4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.9 ± 0.3</td>
<td>28.6 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>19.4 ± 0.3</td>
<td>28.9 ± 3.2</td>
<td>0.02</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>80.6 ± 1.2</td>
<td>71.1 ± 3.2</td>
<td>0.02</td>
</tr>
<tr>
<td>REE (KJ/day)</td>
<td>5974 ± 132</td>
<td>6983 ± 400</td>
<td>0.003</td>
</tr>
<tr>
<td>REE (KJ/day) per FFM</td>
<td>115 ± 3</td>
<td>111 ± 3</td>
<td>0.316</td>
</tr>
<tr>
<td>Fasted RQ (at rest)</td>
<td>0.88 ± 0.01</td>
<td>0.84 ± 0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>VO₂max (ml/min/kg)</td>
<td>36.33 ± 1.11</td>
<td>28.50 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Workload 50% VO₂max (W)</td>
<td>99.52 ± 3.53</td>
<td>100 ± 10.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Workload 95% VO₂max (W)</td>
<td>189.09 ± 6.70</td>
<td>190 ± 20.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Random glucose (mmol/L)</td>
<td>5.2 ± 0.4</td>
<td>5.5 ± 0.2</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Participants exercised less than three times a week (Wilmore JH, 2005). Results expressed as Mean ± SEM. BMI= body mass index; REE= calculated resting energy expenditure; RQ= respiratory question; VO₂max= maximal oxygen uptake; FFM= fat free mass.
7.5.2 During exercise measurements:

7.5.2.1 EE and substrate utilisations:

Lean and overweight have not shown any overweight or BMI differences in RER during exercise. However, unpaired t-test showed a significant difference in EE during exercise between lean and overweight (P< 0.001) for both legs. Overweight have lower EE than lean. Figure 7.1 shows the mean of EE during exercise for lean and overweight participants.

Figure 7.1 Energy expenditure (EE) during exercise in lean and overweight participants.

Values are expressed as mean ± SEM (n= 40; 30L, 10O). The protocol is designed to provide an energy cost of 750 KJ. A significant difference between lean and overweight (P< 0.001) was found. During Low exercise, mean values were (720 ± 14) KJ for lean and (580 ± 35) KJ for overweight. At High exercise, mean values were (753 ± 20) KJ for lean (567 ± 29) KJ for overweight.

When corrected for body composition (measured through impedance) and then expressed per unit FFM, unpaired t-test showed that there was a significant difference between lean and overweight in LI (P< 0.001) and HI (P< 0.001), with the overweight group, still having lower EE than lean. See Figure 7.2.
Values are presented as mean ± SEM (n= 40; 30L, 10O). A significant difference between lean and overweight (P< 0.001) was found. At Low exercise, mean values were (14 ± 1) KJ/kg for lean and (9 ± 1) KJ/kg for overweight. At High exercise, mean values were (15 ± 1) KJ/kg for lean and (9 ± 1) KJ/kg for overweight.

**7.5.3 Post-exercise measurements:**

**7.5.3.1 Subjective appetite ratings, plasma metabolites and GLP-1**

There were no significant difference between lean and overweight in appetite, TAG, NEFA and insulin post-exercise. However, mean glucose level in lean group was significantly lower than that for overweight (P= 0.005), as shown in Figure 7.3.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are expressed as mean ± SEM (n= 33; 23L, 10O). ANOVA showed that there was a significant difference in terms of overweight alone (P=0.005).

There was no significant mean difference in the satiety hormone GLP-1 level between lean and overweight (P= 0.103). Moreover, no significant difference in (exercise*overweight) interaction (P= 0.336) was observed. However, there was a significant effect of (time *overweight) interaction between lean and overweight participants (P= 0.030), as illustrated in Figure 7.4 GLP-1 was rapidly decreased post-exercise, except in LI for overweight. It was decreased after half an hour post-exercise. Also, at (150 min) one hour post-exercise, GLP-1 started to increase in overweight and decrease in the lean group for both legs.
A 500 kcal breakfast was given immediately after taking fasting measurements (F). Shading indicates the period of exercise intervention. Values are expressed as mean ± SEM (n= 33; 23L, 10O). ANOVA showed that there was a significant effect of (time*overweight) interaction between lean and overweight participants (P= 0.030).

### 7.5.3.2 EE and substrate utilisation

There was a significant difference between lean and overweight regarding the effects of exercise intensity on the mean estimated EE post-exercise (P< 0.001). Overweight showed greater mean EE than lean. Figure 7.5 depicts the mean of EE post-exercise.
Mean Low values were (4.38 ± 0.14) KJ/min for lean and (5.81 ± 0.24) KJ/min for overweight. Mean High values were (4.82 ± 0.19) KJ/min for lean and (5.87 ± 0.33) KJ/min for overweight. Values are expressed as mean ± SEM (n= 40; 30L, 10O). There were no significant effect of (exercise*overweight) and (time*overweight) interaction.

The EE values were corrected for body composition (measured through impedance), then expressed per unit FFM. When expressed in this way, repeated measures ANOVA showed that there was a significant effect of exercise (P= 0.049) and time (P< 0.001). But, the effect of (exercise*time) interaction (P= 0.739) and overweight difference (P= 0.189) was insignificant. Mean LI were (0.084 ± 0.002) KJ/min/FFM for lean and (0.093 ± 0.003) KJ/min/FFM for overweight. Mean HI were (0.092 ± 0.003) KJ/min/FFM for lean and (0.094 ± 0.005) KJ/min/FFM for overweight. Figure 7.6 illustrates EE post-exercise for lean and overweight participants.
Values are expressed as mean ± SEM (n= 40; 30L, 10O). There was no difference in overweight (P= 0.189) and (exercise*time) interaction (P= 0.739). However there was an effect of exercise (P= 0.049) and time (P< 0.001). There were no significant effects of (exercise*overweight) and (time*overweight) interaction. FFM= fat free mass.

There was a significant difference between lean and overweight in terms of the effect of exercise intensity on the mean of RQ (P< 0.001) post-exercise. Overall, lean showed higher RQ than overweight. Figure 7.7 depicts the mean values of RQ post-exercise for lean and overweight.
Values are presented as mean ± SEM (n= 40; 30L, 10O). Low mean values were (0.937 ± 0.02) for lean and (0.866 ± 0.03) for overweight. High mean values were (0.891 ± 0.01) for lean and (0.856 ± 0.02) for overweight. No significant effect of (exercise*overweight) and (time*overweight) interaction were found.

7.5.3.3 Fat oxidation

There was a significant difference between lean and overweight regarding the effect of exercise intensity on the mean of fat oxidation post-exercise (P< 0.001). Overall, overweight volunteers have greater mean of fat oxidation than lean. Also, (exercise*overweight) was close to being significant (P= 0.07). Value of AUC for overweight at LI was (111.32 ± 05.38) g and at HI was (112.86 ± 05.50) g, for lean at LI was (81.75 ± 2.85) g and at HI was (91.26 ± 04.06) g. Figure 7.8 shows the mean values of fat oxidation post-exercise for lean and overweight.
Values are expressed as mean ± SEM (n= 40; 30L, 10O). Low mean values were (1.17 ± 0.04) g/min for lean and (1.59 ± 0.07) g/min for overweight. For High mean (1.31 ± 0.05) g/min for lean (1.61 ± 0.09) g/min for overweight. No significant effect s of (exercise*overweight) and (time*overweight) interaction were found.

The fat oxidation values were corrected for body composition (measured through impedance) then expressed per unit FFM. In this way, repeated measures ANOVA showed that exercise (P= 0.025) and time (P< 0.001) had significant effect. On the other hand, there was no significant effect of (exercise*time) interaction (P= 0.271) and overweight difference (P= 0.110). Value of AUC for overweight at LI was (1.78 ± 0.07) g/kg and at HI was (1.80 ± 0.80) g/kg, for lean at LI was (1.54 ± 0.04) g/kg and at HI was (1.73 ± 0.06) g/kg. See Figure 7.9.
Values are presented as mean ± SEM (n= 40; 30L, 10O). Mean Low values were (0.024 ± 0.001), (0.021 ± 0.001) and (0.022 ± 0.001) g/min/FFM for lean and (0.027 ± 0.001), (0.025 ± 0.001) and (0.025±0.001) g/min/FFM for overweight. Mean High values were (0.028 ± 0.001), (0.023 ± 0.001) and (0.023 ± 0.001) g/min/FFM for lean and (0.027 ± 0.001), (0.025 ± 0.001) and (0.025 ± 0.001) g/min/FFM for overweight. There was no difference in overweight (P= 0.110) and (exercise*time) interaction (P= 0.271). However there was an effect of exercise (P= 0.025) and time (P< 0.001). No significant effects of (exercise*overweight) and (time*overweight) interaction were found. FFM= fat free mass.

7.5.3.3.1 *Ad Libitum* pasta test meal intake and 48 hours intake.

There was no difference between lean and overweight in terms of *ad libitum* pasta meal, EI with macronutrient intake in the 0-24 hour and the 24-48 hour post-exercise at LI and HI (P> 0.05) (data not shown).
7.6 Discussion:

The present study aimed to compare two cross-over studies in (chapters 4 and 6) exploring the effect of HI and energy matched LI on appetite and GLP-1 (an appetite hormone), EE and EI post-exercise for lean and overweight participants. In order to ensure that the energy cost was matched between both intensities, the protocol was calculated by MET’s and it was measured by adjusting the VO$_{2\text{max}}$ for each individual (Ainsworth et al., 1993).

A total of forty volunteers, thirty in the lean range of BMI (20-25 g/m$^2$), and ten in the overweight range of BMI (25> g/m$^2$) were compared. The average VO$_{2\text{max}}$ for the lean group was (36.3 ± 1.1ml/kg/min) and for the overweight group was (28.5 ± 1.1ml/kg/min), the significant difference in VO$_{2\text{max}}$ between the lean and overweight groups might be because the overweight group exhibiting a higher perceived exertion and lower tolerance to HI than the lean group (Hulens et al., 2003).

All participants had the same inclusion criteria i.e. they had the same age range, REE and workload; however, they were different in terms of fat mass and FFM, as expected lean people have lower fat mass and higher FFM than overweight participants. However, REE per FFM was the same between both groups.

In this study, the approximate energy cost of this protocol was 750 KJ (Little et al., 2010), and the result showed no significant difference between LI and HI in terms of the energy cost during either exercise session for both lean and overweight people. However, the mean energy cost for overweight participants was around 200 KJ/day lower than the protocol for both intensities, which was unexpected (Figure 7.2).

The differences in body weight would not be expected to influence the relationship between work output and EE on a bicycle as demonstrated on a treadmill (Kissileff et al., 1990). However, a difference between lean and overweight participants in EE during
exercise was still evident when corrected for body composition and expressed per unit FFM. The difference might be due to overweight participants not reaching their exact VO$_{2\text{max}}$, which was significantly lower than for lean participants. This difference was emphasised by Ekkekakis and Lind (2005) who showed that overweight participants exhibited a lower rate of pleasure in comparison with lean participants during imposed-intensity conditioning. Our findings are also consistent with findings of a previous study by Marzullo et al. (2008), which compared 8 obese with 8 lean males in a cycling test to exhaustion, and it was suggested that obese people achieved a lower exercise performance, output, and lower RER than the lean males. However, their measurements were only taken at rest and at a peak point of the exercise, whilst in our study LI was compared with HI.

Body temperature appears to be an important factor in the fatigue process under conditions of heat-stress (Meeusen et al., 2006), hence, one alternative possibility that could explain the differences between overweight and lean participants is the lower heat loss in the overweight individuals (Kissileff et al., 1990) due to a reduced surface area to volume ratio. However, body temperature was not observed in our study, so these differences cannot be ascertained.

Our results showed that acute HI and LI did not affect insulin and glucose differently when compared lean and overweight participants suggesting there are no changes in insulin and glucose homeostasis between lean and overweight participants in response to the exercise. The molecular mechanisms by which a type of exercise enhances glucose homeostasis are not completely understood, it is assumed that exercise enhances glycaemia partly by improving glucose transport into the contracting muscles fibres (Rose and Richter, 2005), however, the mean glucose level was higher in the overweight group than the lean group. Similarly, Ueda et al. (2009b) showed that there was no significant alteration in glucose level between the LI (50% VO$_{2\text{max}}$) for 60 min and resting conditions throughout the course.
of investigation, when comparing 7 lean vs 7 obese young males. Our study did not find any exercise-associated modifications of insulin and glucose levels in either group in response to exercise intensity, which is also similar to the conclusions shown by Broom et al. (2007) in nine lean males comparing 60 min running at 72 % VO$_{2\text{max}}$ with rest.

Exercise has recently been shown to have an influence on GLP-1 concentrations as a satiety hormone (Ueda et al., 2013), it was shown that one bout of aerobic exercise increases GLP-1 plasma concentration, with a decrease in subsequent EI in lean and obese individuals (Ueda et al., 2009a, Ueda et al., 2009b). There is currently only one study investigating the difference in mean plasma GLP-1 between lean (n = 7) and overweight males (n = 7), which demonstrated that in both groups 1 h cycling at LI (50 % VO$_{2\text{max}}$) significantly increased subsequent GLP-1 in plasma when compared with rest, but there were no significant differences detected between obese and lean people (Ueda et al., 2009b). Their finding was similar to our findings, but in our results there was also a different GLP-1 pattern as a result of a significant difference in (exercise*overweight) and (time*overweight) interactions between lean and overweight groups. This variation might be related to a theory that the excess body weight is linked to a decrease in fasting and post-prandial GLP-1 levels (Holst, 2007). Hence, in our study the change in GLP-1 responses post-exercise may be due to in part a delay in clearance in the overweight group compared with the lean group.

Research to date has focused on acute effects of exercise intensity on appetite; appetite related hormones and EI, without measuring EE and fat oxidation post-exercise between lean and overweight individuals, which was the motivation behind the present comparison study.
A study by Marzullo et al. (2008) that used a cycling test to exhaustion suggested that exercise was linked with the magnitude of the reduction in NEFA concentrations, which was greater in the lean group than in the obese group (Marzullo et al., 2008). However, our study showed no significant difference between the lean and overweight groups in TAG or NEFA plasma levels during the 90 min post-exercise for both intensities, taking into account that overweight participants have less power than the lean group. In fact, fat oxidation rate is not strictly dependent on NEFA availability (Bennard et al., 2005). The results of our investigation showed that overweight people have significantly higher EE and fat oxidation than their lean counterparts during the 75 mins post-HI and LI exercise. This result is in contrast with findings from a study in ten lean and obese healthy males who completed a 1255.2 KJ HI session (Santiworakul et al., 2014). It showed that fat oxidation was increased in both lean and obese groups for 180 min post-HI, with no significant difference in EE and fat oxidation between both groups. However, the protocol was focused on HI only, whereas our study involved both HI and LI exercise in the comparison between overweight and lean subjects. Moreover, our result showed no difference in EE and fat oxidation post-exercise when the values were adjusted for FFM in both groups. Overall these results suggest that fat mass may influence the amount EE and fat oxidation post-exercise.

There is evidence that exercise of a sufficient HI may influence post-exercise EI in young males and females even in the absence of changes in subjective appetite rate (Tremblay et al., 1994). In contrast, suppression of appetite post-HI does not always translate into a reduction in EI in lean males (Deighton et al., 2013b). Hence, one of the primary outcomes of our investigation was to compare the effect of HI with LI on EI post-exercise in both lean and overweight people. Our results can be compared to the study by Kissileff et al. (1990) which investigated the impact of cycling at HI (90 W) and LI (30 W) for 40 min (or
rest) on food intake in 9 lean and 9 obese women. They found food intake post-exercise was significantly less after the HI exercise and rest than the LI exercise in the lean group, but was similar between both legs for the obese women, and feelings of hunger were significantly higher after LI than HI in obese group only. However this is in contrast to our study as there was no significant difference in feelings of hunger and *ad libitum* test meal and EI 48 h post-HI and LI exercise between groups. Despite both groups taking part in the same study, obese individuals may likely to under-report their intake to a greater extent (Schoeller, 1995). Nevertheless, as clearly shown in chapter 6, despite there not being a significant difference in the EI post both legs, the overweight group ate 3000 KJ lower at the first 24 h post-HI. In addition, the similarity in *ad libitum* test meal results between the overweight and lean groups could be argued as each group had been served with the same amount of *ad libitum* pasta test meal post-HI and LI exercise, but a possible difference could be found if both groups were served with an open buffet, as other studies have found that there is a positive relationship between the meal variety and EI (Raynor and Epstein, 2001).

Our findings are in line with a study in twelve obese and four lean participants, cycling-equivalent to nearly 418.4 KJ, which found that there was no significant difference in EI post-exercise or at rest between lean and obese individuals (Durrant et al., 1982). The results also indicated that in both exercise and rest conditions, the overweight women ate significantly more than the lean women, which might be as a result of a need for a greater EI in regards to having a greater BMI and greater overall energy requirements (George and Morganstein, 2003). However this is in contrast with another study that showed that EI was lower after one hour LI (50 % VO$_{2\text{max}}$) than in the rest condition in both obese and lean participants, suggesting that exercise elicits similar appetite and EI response in lean and obese males (Ueda et al., 2009b).
A recent study conducted by Holliday and Blannin (2014) in 14 lean participants (7 males and 7 females) showed no significant difference in EI at one hour post-HI (running at 90 \% \text{VO}_{2\text{max}}) and iso-caloric, (1883 KJ), moderate intensity running (60 \% \text{VO}_{2\text{max}}).

Therefore, there was no solid evidence for the long-lasting post-exercise suppression of appetite, resulting in reduced EI 60 min post-exercise. These findings were in lean participants and showed no effect of intensity on EI, however, we have extended this to both lean and overweight participants where no effect of intensity is again shown.

It should be noted that the role of the gastrointestinal hormones in appetite regulation post-HI was not fully investigated, and whether a similar appetite suppression would be detected in overweight adults while exercising at the same amount of total energy was not known (Sim et al., 2013). There are different mechanisms and consequences of exercise in short-term control of energy balance between groups; however, these mechanisms require further explanation. Our results support the need for longer term interventions to test these mechanisms. Future studies should investigate more quantitative short-term relationships between exercise and EI, as such relationships could be causally probed for their physiological mechanisms and their relation to differences between lean and obese individuals (Kissileff et al., 1990).

In conclusion, overweight participants have a higher absolute of EE and fat oxidation 75 min post-exercise. Despite there being no difference between HI and LI in terms of EI post-exercise in the lean and overweight groups, HI was more beneficial for the lean participants as it increased EE and fat oxidation post-exercise. Also, it may provide overweight individuals with an option to choose between intensities with a possibility to reduce EI if the exercise was frequently performed.
Chapter eight
8 General Discussion:

The rapidly rising number of individuals who are overweight and obese has been called a pandemic, with over 35% of adults worldwide considered to be overweight or obese (Mitchell and Shaw, 2014). By aiming to lower obesity through the concept of energy balance, this helps to recommend special strategies to reduce obesity (Hill et al., 2012). Exercise increases total EE by producing additional EE during the exercise, and also by elevating EE post-exercise which is an important component of the total energy balance (Saris and Schrauwen, 2004). There is no reliable evidence to indicate that in a short time period, exercise induces changes in EI or nutrient preferences to compensate for the energy cost of exercise, therefore exercise does not induce positive energy balance (King et al., 1997b). Moreover, there is some evidence for the role of exercise intensity on hunger, LI does not impact on hunger as much as short-term HI (King et al., 1994). Indeed, King and colleagues reported that within 48 hours, the effect of moderate exercise intensity (70% HRmax) substantially increased EE, although this did not necessarily lead to an increase in hunger or EI in the eight lean males investigated (King et al., 1997a). It has also been suggested that partaking in LI for a long time period is the most appropriate exercise in order to prevent obesity (Erdmann et al., 2007). Despite the evidence that suggests regular HI leads to an improvement in fat oxidation during exercise, the effects of HI on fat oxidation with appetite suppression post-exercise had not yet been examined (Boutcher, 2010).

Based on this information, the aims of this study were to:

1. Examine the effect of HI vs LI exercise on appetite, GLP-1, EI, EE and fat oxidation and utilisation post-exercise in lean and overweight individuals.

2. Investigate whether there is a gender or BMI difference in response to exercise.
To cover these points the discussion will be divided to three sections. **Section one** is the discussion of main findings from chapters four and six; chapter four compared the effect of two types of exercise intensities on appetite, GLP-1, EI, EE and fat oxidation and utilisation in lean people post-exercise, and chapter six compared the same indices in overweight individuals.

**Section two** is the discussion of the findings for chapter five, which compared the gender difference responses to HI and LI exercise post-exercise in order to determine the beneficial intensity for each gender.

**Section three** is the discussion of the findings from chapter seven, which compared the different BMI responses to HI and LI exercise and whether this difference in BMI can play a role in appetite, appetite hormones, EI, EE and fat oxidation and utilisation response to the exercise.

**Section one: effect of exercise intensity on energy balance.**

Chapter four and six examined the effect of exercise intensity on thirty lean (chapter four) and on ten overweight people (chapter six) on appetite, GLP-1, EI, EE and fat oxidation post-exercise.

Generally, chapter four found that in lean people, an acute bout of HI suppressed prospective food consumption, improved EE, NEFA utilisation and fat oxidation, but did not stimulate appetite and EI or influence glucose, insulin and GLP-1 and TAG levels post-exercise in comparison with LI, at the same energy cost. Despite the difference in NEFA, EE and fat oxidation between HI and LI, however, the role of catecholamine during and post-exercise with other appetite hormone such as PYY was not measured in our study.
The general findings from chapter six showed that in overweight participants, the effect of HI did not differ from LI exercise in terms of its influence on appetite, GLP-1, glucose, insulin, TAG and NEFA plasma level. Also, no difference was seen between the intensities in EI, EE and fat oxidation post-exercise, when taking in account the lower amount of EI 24 h post-HI. Despite there being no difference in the effect of exercise between both intensities, this finding could allow overweight individuals the option to choose which type of exercise is convenient for them.

Section two: Investigation into whether there is a gender difference in the response to exercise intensity.

The impact on HI vs LI exercise in lean people was examined in chapter four and chapter five comparing gender response to exercise intensity in lean individuals. Several interesting results indicating the potential of exercise intensity in lean people have been reported (Jan Bilski, 2009). However most of the studies in the literature did not specifically investigate the differences in response to exercise intensity between the genders post-exercise.

Chapter five found that there was no difference in appetite and GLP-1, fat mobilisation, glucose plasma levels between genders post-exercise in lean individuals. However, there was a different pattern in rating for prospective food consumption and NEFA and insulin response post-exercise between genders, whereby women had a lower score for prospective food consumption and a higher level of NEFA and insulin immediately (until 30 min) post-exercise. In addition, a gender difference in EI, EE and fat oxidation was observed; men had a higher EI, EE and fat oxidation than women, with a difference in the pattern of EE and fat oxidation post-exercise. Nevertheless, this effect of exercise on EE and fat oxidation disappeared once corrected for FFM, suggesting more research is needed into the influence of body composition as a factor in the different response to exercise. Observations from
the present investigation were that HI influenced EE and fat oxidation post-exercise for men by increasing EE and fat oxidation post-exercise to a greater extend compared to women. These findings provide insights to be considered when designing future research on gender response to exercise intensity for longer time periods (e.g. training). The difference in the hormonal response between genders such as: catecholamine hormone during and post-exercise should be taken in account in order to design a stable exercise protocol for each gender.

Section Three: Investigation into role of BMI in response to the exercise intensity.

The impact of HI vs LI exercise in lean (chapter four) compared with overweight individuals (chapter six) were examined in chapter seven. Studies have raised several concerns about exercise intensity and fat oxidation, suggesting that the increase in fat oxidation rate was associated with the increase in EE and lean mass, which is why this chapter compared BMI response to exercise intensity (Thupari et al., 2002). Our results found that despite no effect of BMI on appetite, NEFA mobilisation, and insulin plasma levels post-exercise, there was a change in pattern of GLP-1 response post-exercise between lean and overweight groups; GLP-1 level was elevated until 30 min post- LI for overweight group when compared to lean group. There was also an effect of BMI on the plasma glucose level, EE and fat oxidation; with the overweight group having a higher EE and fat oxidation than lean participants post-exercise. This effect of exercise on EE and fat oxidation disappeared when adjusted for FFM, which again suggests there is a need to focus more on the effect of body composition on EE and fat oxidation in response to exercise in all the groups investigated. The second major finding of this comparison was that HI exercise appeared to be more beneficial for the lean group as it elicited a greater increase in EE and fat oxidation post-exercise. HI did not induce an increase in EI in either groups, instead it tended to reduce subsequent 24 h EI by 3000 KJ post-exercise for the
overweight group. This research has highlighted many questions in need of further investigation, such as whether body composition and/or body fat distribution affects the body’s response to exercise intensity in both genders and body types.

8.1 Strength and Limitations

Our study has different strengths and limitations; the main strength of our study is its design (cross-over study) and the fact that we measured several aspects of appetite (subjective feelings, plasma levels of several appetite-related hormone (GLP-1) and EI for 48 h post-exercise). Moreover, our results serve to strengthen previous findings in lean and overweight individuals, specifically that acute exercise, even at HI or at LI, is able to induce a negative energy balance without stimulating physiological compensatory adaptations at the level of the appetite control system (Martins et al., 2014).

Although this thesis has attempted to provide further insights into the effects of exercise on appetite, gut hormones and EE, food intake, the following limitations are associated with different aspects of the studies:

- There is a need to have a larger sample size, especially for the overweight population to increase study power.
- Appetite hormone measurements were limited to GLP-1. Measuring PYY and other anorectic hormones is needed as it is hard to distinguish between the effects of GLP-1 and PYY endogenously stimulated by exercise on appetite and EI by the experimental design alone. GLP-1 and PYY might play differential roles in appetite regulation processes post-exercise, therefore measuring the circulating levels of both hormones with the gastric emptying rate during and post-exercise might provide more clarification of the findings (Ueda et al., 2009a).
Subsequent investigation into the effect of exercise intensity on *ad libitum* food intake should be performed in the free-living situation.

EE and fat oxidation post-exercise needs to be measured for longer, for at least three hours, in order to provide a clearer understanding of the effect of exercise intensity on EE and fat oxidation during exercise recovery.

8.2 Future directions for research

Notwithstanding the above limitations, the findings of the studies reported here indicate some useful avenues for future research as follows:

- Short-term exercise, carried out over 2-3 days, would likely provide a picture of energy balance, but a longer period of time may be needed to accurately estimate the coupling between EI and EE (King et al., 1997b). Also, it has been shown that longer-term changes to appetite, appetite hormone regulation and their influence on EI are unknown (Schubert et al., 2014), therefore in future studies it would also be worthwhile to study the chronic effect of exercise on appetite and the energy balance including EI and EE. It would also be possible to study the consequences over a longer time period following both HI and LI, as further research is required to investigate whether differences in appetite occur in response to repeated bouts of HI or LI (Deighton et al., 2013b). Overall the accumulated effect of exercise needs be examined in the future.

- Examine the chronic effect of exercise in obesity for both genders.

- Measuring EE and fat oxidation for at least three hours post-exercise at each study day or for longer.

- Our results lead to an interest in further studies including other types of exercise (e.g. resistance exercise), other participants characteristics such as: patients who have had
bariatric surgery or after dietary weight-loss, in different ranges of BMI for example overweight, obese and morbidly obese.

- Examine the effect of exercise intensity on appetite, EE and EI with hormonal responses in different age ranges, such as the effect of exercise intensity in pre and post-menopausal women.

- Examine the responses to exercise with different types of pre-meal macronutrient such as: fat, protein and CHO.

- Use a larger sample sizes; despite the small sample size of the overweight participants, our findings have indicated the possibility that circulating GLP-1 levels were differentially linked with exercise intensity (LI or HI), and thereby affect subsequent EI. To confirm the present findings, the study would have to be applied to a larger population (Ueda et al., 2009a).

- More investigation of the physiological responses and roles of each gut hormone on EI in response to exercise intensity will help to create and develop appropriate exercise programs for individual’s needs, especially in overweight or obese subjects.
9 References:


BOWEN, J., NOAKES, M. & CLIFTON, P. M. 2006. Appetite Regulatory Hormone Responses to Various Dietary Proteins Differ by Body Mass Index Status Despite


CHIEF MEDICAL OFFICER 2009. Annual report of the Chief Medical Officer. London: Department of Health


M3 - 10.1017/S00296655108005995.


M3 - 10.1017/S000711450774922X.


197


10 Publications and presentations

10.1 Conferences


- Shamlan, G., Robertson, M. D., & Collins, A. L. The independent effect of exercise intensity on appetite, energy intake and energy expenditure: is there a gender difference? 2013 at 18th Annual Congress at the European College of Sport Science. (Presentation)


- Shamlan, G., Robertson, M. D., & Collins, A. L. Effect of exercise intensity on appetite, energy and fat oxidation in overweight and lean Individuals. Accepted to be presented at ACSM's 62nd Annual Meeting, 6th World Congress on Exercise is Medicine and World Congress on the Basic Science of Exercise Fatigue. San Diego, USA.

10.2 Journals

- Shamlan, G., Williams, P., & Robertson, M. D. Validation PRO-Diary against the traditional pen and paper (P&P) method in a laboratory setting using healthy young adults. (in process)
11 Appendix

11.1 Appendix A: ethics letter

18 May 2011

Miss Ghada Shamian
Nutritional Sciences
Faculty of Health & Medical Sciences

Examining the acute effects of exercise intensity on subsequent appetite, food intake, 24 hour blood pressure and resting energy expenditure EC/2011/20/FHMS

On behalf of the Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the submitted protocol and supporting documentation.

Date of confirmation of ethical opinion: 17 May 2011

The list of documents reviewed and approved by the Committee is as follows:

- Detailed Protocol
- Subject Information Sheet
- Lay summary
- GP Letter/Standard Letters
- Consent Form
- Questionnaire/interview Schedule
- Risk Assessment and Insurance proforma

This opinion is given on the understanding that you will comply with the University's Ethical Guidelines for Teaching and Research, and with the conditions set out as follows:

- That a number of amendments are made to the GP Letter—these include:
  - Line 3—"expenditure" and "being carried out" and "study involves completing".
  - Line 4—"At each visit your patient will complete".

  The letter says that the study will be carried out at the University and then later at the Cedar Centre—please clarify.

  The letter should state that a copy of the Consent Form is enclosed so that the GP can see that their patient has consented to the GP being notified of participation in the study. The letter should also make clear that the letter is for information only and no action (reply) is required.

  'Questionnaire' is spelt incorrectly on the title line of the questionnaire.

  I would be grateful if you would confirm, in writing, your acceptance of the conditions above.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University’s Ethics Committee.
The Committee should be notified of any amendments to the protocol, any adverse reactions suffered by research participants, and if the study is terminated earlier than expected, with reasons. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.

You are asked to note that a further submission to the Ethics Committee will be required in the event that the study is not completed within five years of the above date.

Please inform me when the research has been completed.

Yours sincerely,

[Signature]

Glenn Moutlon
Secretary, University Ethics Committee

Regrettably

cc: Professor S Williamson, Chairman, Ethic Committee
Miss Ghalia Shamlan

PGMS
FHMS

02 July 2012

Dear Miss Shamlan

Examining the acute effects of exercise intensity on subsequent appetite, food intake, 24 hour blood pressure and resting energy expenditure EC/2011/20/FHMS

I am writing to inform you that the Chairman, on behalf of the Ethics Committee, has considered the Amendments requested to the above protocol and has approved them on the understanding that you will comply with the University's Ethical Guidelines for Teaching and Research, and with the conditions set out as below.

- To correct a typo on the Participant Information Sheet page 2 – i.e. ‘warn’ instead of ‘warn’
- The requirements of each visit for participants are not clear as they might be as the text is quite dense so suggest that you include more spacing so potential participants can easily grasp what is required during each visit.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University's Ethics Committee.

Date of confirmation of ethical opinion: 17 May 2011.

Date of approval of amendment to protocol: 2 July 2012.

The list of amended documents reviewed and approved by the Chairman is as follows:-

<table>
<thead>
<tr>
<th>Document</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment advert</td>
</tr>
<tr>
<td>Subject Information Sheet</td>
</tr>
</tbody>
</table>

Yours sincerely

Glenn Moulton
Secretary, University Ethics Committee
Academic Registry
### 11.2 Appendix B: Dutch Eating Behaviour Questionnaire (DEBQ) (van Strien t; 1987)

Participant code_____________________                                     Date___/___/___

Please answer the following questions as carefully and honestly as possible. Read each question and simply fill in the column which best applies to you.

<table>
<thead>
<tr>
<th>Question</th>
<th>Never</th>
<th>Seldom</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
<th>Not relevant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. If you have put on weight, do you eat less than you usually do?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Do you have a desire to eat when you are irritated?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. If food tastes good to you, do you eat more than you usually do?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Do you try to eat less at meal times than you would like to eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Do you have a desire to eat when you have nothing to do?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Do you have a desire to eat when you are depressed or discouraged?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. If food smells and looks good, do you eat more than you usually eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. How often do you refuse food or drink offered because you are concerned about your weight?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Do you have a desire to eat when you are feeling lonely?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. If you see or smell something delicious, do you have a desire to eat it?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Do you watch exactly what you eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Do you have a desire to eat when somebody lets you down?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. If you have something delicious to eat, do you eat it straight away?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Do you deliberately eat foods that are slimming?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Do you have a desire to eat when you are cross?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Do you have a desire to eat when you are approaching something unpleasant to happen?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. If you walk past the baker do you have a desire to buy something delicious?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. When you have eaten too much, do you eat less than usual the following days?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. Do you get a desire to eat when you are anxious, worried or tense?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. If you walk past a snack bar or café, do you have a desire to buy something delicious?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21. Do you deliberately eat less in order not to become heavier?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. Do you have a desire to eat when things are going against you, or things have gone wrong?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. If you see others eating, do you have also the desire to eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. How often do you try not to eat between meals because you are watching your weight?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25. Do you have a desire to eat when you are frightened?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26. Can you resist eating delicious food?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27. How often in the evening do you try not to eat because you are watching your weight?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28. Do you have a desire to eat when you are disappointed?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. Do you eat more than usual when you see other eating?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. Do you take your weight into account when you eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31. Do you have a desire to eat when you are emotionally upset?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32. When preparing a meal are you inclined to eat something?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
11.3 Appendix C: physical activity questionnaire (par-q screening questionnaire)

PAR-Q & YOU

(A Questionnaire for People Aged 13 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. Have you 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. Have you lost your balance because of dizziness or do you ever lose consciousness?

5. Do you have a bone or joint problem (for example, back, knee, 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 NO to any of these questions, you are encouraged to photocopy the PAR-Q but only if you use the entire form.

No changes permitted.

NOTE: If this PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness program, this section may be used by any registered health profession.

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

Name: ____________________________

Signature: _________________________

Nombre: __________________________

Firma: ____________________________

Date: _____________________________

Fecha: ____________________________

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.

Date: _____________________________

Fecha: ____________________________

continued on other side...
11.4 Appendix D:

11.4.1 TAG

As well as, to the amount of the quinoneimine dye, and produced by the reaction as follows:

TAG → glycerol + fatty acid (lipoprotein lipase)

Glycerol + ATP → glycerol -3-P + ADP (Glycerol kinase)

Glycerol -3-P + O₂ → dihydroxyacetone phosphate + H₂O (Glycerol-3-phosphate oxidase)

H₂O + 4-chlorophenol + 4-aminophenazone → quinoneimine dye + H₂O ( Peroxidase)

11.4.2 NEFA

NEFA + ATP + CoA → Acyl CoA synthetises → Acyl CoA + AMP + PPI

Acyl CoA + O₂ → 2,3,-trans-Enoyl-CoA + H₂O

2 H₂O + TOOS + 4-amino antipyrine → quinoneimine dye + H₂O purple adduct

TOOS = N-ethyl-N-(2hydroxy-3-sulphopropyl) m-toluidine

11.4.3 Glucose:

Glucose reacts with oxygen and water in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide.

B-D glucose + O₂ + H₂O → Gluconic acid + H₂O (Glucose oxidase)

Hydrogen peroxide then reacts with phenol and and 4-aminoantipyrene, in the presence of peroxidise with the production of a red quinoneimine and water.

2 H₂O + phenol + 4 aminoantipyrine → red quinoneimine + 4H₂O (peroxidase)