

MEASURING THE EFFECT OF EXERCISE ON APPETITE, FOOD INTAKE AND APPETITE- ASSOCIATED HORMONES

A thesis submitted by

Adrian Holliday

To the

School of Sport, Exercise and Rehabilitation Sciences,

University of Birmingham

For the degree of

DOCTOR OF PHILOSOPHY



Exercise Metabolism Research Group

School of Sport, Exercise and Rehabilitation Sciences

University of Birmingham

September 2013

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

ABSTRACT

The relationship between exercise and appetite has implications for acute energy balance and weight-management. Evidence would indicate that aerobic exercise of a high intensity can transiently suppress appetite, particularly in overweight and recreationally-active, healthy-weight individuals. However, the effect of such a transient appetite suppression on subsequent food intake may be limited. The aim of this thesis was to investigate appetite responses to exercise in highly-trained endurance athletes and to assess the effect of other exercise characteristics, as well as exercise intensity, in mediating these responses.

Chapter 3 introduce a novel tool – The Visual Meal Creator – that is shown to be a valid measure of subjective appetite and may prove a strong predictor of food intake, hence proving a beneficial tool for appetite research. Using this tool alongside established techniques, the findings of Chapters 4 and 5 demonstrate that an exercise-induced suppression of appetite is experienced in highly-trained athletes, although there does appear a blunting to this response. It may be that an elevated fitness level, resulting in reduced physiological and metabolic perturbations during exercise mediates this blunting. Any exercise-induced appetite suppression response would appear independent of the duration, or energy cost of exercise. This is partly supported by findings of Chapter 6: a suppression of appetite after very-low volume sprint interval cycling exercise in overweight and obese individuals. However, in no instance was a suppression enduring (lasting >30 minutes) and feeding was not influenced by prior exercise in any study of this thesis.

The responses to exercise of appetite-associated hormones were also investigated throughout Chapters 4 to 6. PP and PYY appear to respond only modestly to exercise, at least within trained individuals. Acylated ghrelin and GLP-1 exhibited more profound, anorexigenic responses to exercise. The acylated ghrelin response may be entirely intensity-dependent, whereas the GLP-1 response exhibited some degree of duration or energy cost

dependency. Further, an enduring suppression of acylated ghrelin was observed after sprint interval cycling exercise, to an extent that has very rarely been previously observed. However, in all instances, there appeared a dissociation between changes in appetite-associated hormone concentration and changes in both subjective appetite and energy intake. This questions the well-held belief of the importance of appetite-associated hormones in mediating post-exercise appetite regulation.

ACKNOWLEDGEMENTS

On 19th September 2005, I began studying at the University of Birmingham. Eight years and five days later, I conclude this chapter of my life with the submission of this thesis. The journey would not have been surmountable, nor half as enjoyable, had it not been for a number of important, special people. My interest in exercise metabolism research was kindled by Professor Asker Jeukendrup, whose infectious enthusiasm for science I caught during my final year project as an undergraduate. Such was his influence that I became his PhD student the following year. I would like to thank him for passing on the bug to me. When Professor Jeukendrup left the School part way through my PhD, Dr Andrew Blannin stepped in and took the reigns as my Primary Supervisor. I have really enjoyed working with Andy. For his support and guidance, I am eternally grateful.

I must acknowledge the contribution of the undergraduate project students that I have had the pleasure of working with over the past four years. I owe a big thank-you to Chris Batey, without whose computer programming skills developing the Visual Meal Creator would have proved a lot more difficult.

The administration staff and the technical support staff keep the School functioning. I cannot overstate the importance of these people. A special thank you must be paid to Val and Rebecca in the main office and to the “techies” Dave, Ken, Andy, Rob and Steve.

I have been fortunate to have been supported by a fantastic peer group. During my time at the University, I have been an active member of the Birmingham University Athletics Club. Through the club, I have had (but unfortunately not ran) some great times. I have got to know many great people and have formed lifelong friendships. Cheers lads! Within the School of Sport and Exercise Sciences, I would like to thank Oliver Witard (despite him being a United fan), for showing me the ropes in the early days. Thanks also go to all members, past and present of Lunch Club (and of its predecessor, Tea Club). There are too many to name, but you know who you are; you all made coming to work a lot of fun. A

special mention must go to Daniel Crabtree, who I could always bounce ideas off and talk football with, and a special thank you is paid to Oliver Wilson, for making me feel unintelligent but extremely well-organised; we just about got there!

I would like to thank Sergio Agüero, for *that* goal.

I wish to pay a huge thanks to my parents, Keith and Glenis, and my sister Maegan. Not just during my PhD, but throughout my life, in everything that I have done, you have been there to offer limitless love and support (financial, as well as emotional). I could never, ever have asked for more. I love you all dearly. Thank you.

Finally, thank you Lindsay. You are the reason I made it through a troubled, homesick-stricken first year, the reason I am still at this University, the reason that I moved to the School of Sport and Exercise Sciences and the reason I have made it here, to the end. Day in, day out, from my very second week in Birmingham, you have been here for me and put up with me. I couldn't have done this without you. Thank you and I love you.

PUBLICATIONS and COMMUNICATIONS

Peer-review Journal Submission

Holliday, A., Batey, C., Eves, F.F. and Blannin, A.K. A novel tool for the measurement of subjective appetite: The Visual Meal Creator (submitted)

Conference communications

Holliday, A. Introduction to appetite and monitoring. English Institute of Sport Weight Management CPD Day, Cardiff, 2012. Invited speaker.

Holliday, A. and Blannin, A.K. Matching energy intake to expenditure of isocaloric exercise at high and moderate intensities. The 18th Annual Congress of the European College of Sport Science, Barcelona, 2013. Mini oral presentation

Holliday, A. and Blannin, A.K. Matching energy intake to energy expenditure of isocaloric exercise at high and moderate intensities. Treatment of Obesity Conference, University of Birmingham. 2012. Oral presentation

Holliday, A., Higgs, S., Blannin, A.K. and Jeukendrup, A.E. The effect of exercise intensity on subjective appetite, food intake and satiety peptides in highly-trained male athletes. The 58th Annual Meeting of the American College of Sports Medicine, Denver, 2011. Oral presentation

Unrelated publications

Holliday, A. and Jeukendrup, A.E. (2012), "The Metabolic Adaptations to Endurance Training" in Mujika, I. (ed.), *Endurance Training - Science and Practice* (1st Edition), Vitoria-Gasteiz: p143.

ABBREVIATIONS

- % Δ – percentage change from baseline
- 15 min – 15 minutes duration exercise condition
- 30 min – 30 minutes duration exercise condition
- 45 min – 45 minutes duration exercise condition
- ANOVA – analysis of variance
- ArRP – agouti-related peptide
- AUC – area-under-the-curve
- BMI – body mass index
- BPAR – Birmingham Photographic Appetite Rating
- bpm – beats per minute
- CART – cocaine- and amphetamine-regulated transcript
- CCK – cholecystokinin
- CNS – central nervous system
- CV – coefficient of variation
- DEBQ – Dutch Eating Behaviour Questionnaire
- EE – energy expenditure
- EI – energy intake
- EI_{AD LIB.} – *ad libitum* energy intake
- EI_{MATCH} – energy intake at the “matching” task
- ELISA – enzyme-linked immunosorbent assay
- EST – estimate of energy expenditure/intake
- EX – exercise condition
- FAST – fasted state condition
- FED – fed state condition
- GHS-R – growth hormone secretagogue receptor

GLP-1 – glucagon-like peptide 1

GLP-1R – glucagon-like peptide 1 receptor

HIE – high-intensity interval/intermittent exercise

HIGH – high-intensity exercise condition

HIT – high intensity interval/intermittent training

HR/HR_{max} – heart rate/maximum heart rate

INSR – insulin receptor

IPAQ – International Physical Activity Questionnaire

LB – large breakfast condition

LB+S – large breakfast and snacks condition

LEPR – leptin receptor

LOW – low-intensity exercise condition

METS – metabolic equivalent of task

MOD – moderate-intensity exercise condition

NPY – neuropeptide Y

OXM – oxyntomodulin

POST-AB – post-absorptive state condition

POMC – proopiomelanocortin

PP – pancreatic polypeptide

PYY – polypeptide tyrosine tyrosine

REI – relative energy intake

RER – respiratory exchange ratio

REST – resting condition

RPE – rating of perceived exertion

SB – small breakfast condition

SD – standard deviation

SEM – standard error of the mean

SICE – sprint interval/intermittent cycling exercise

SIE – sprint interval/intermittent exercise

VAS – visual analogue scale

VIMEC – Visual Meal Creator

$\text{VO}_2/\text{VO}_{2\text{max}}$ – rate of oxygen uptake/maximum rate of oxygen uptake

W_{max} – maximal power output

YR – Y receptor

CONTENTS

Abstract.....	i
Acknowledgements.....	iii
Publications and communications.....	v
Abbreviations.....	vi
Contents.....	ix
Chapter 1 General introduction.....	1
1.1 Obesity and overweight – definition, prevalence, socio-economic cost and health implications.....	2
1.2 The energy balance equation: the role of exercise and appetite.....	3
1.3 The effect of acute exercise bouts on appetite, food intake and short- to medium-term energy balance.....	5
1.3.1 Is this an oversimplified message? The effect of exercise variables on appetite and the appetite response in different populations.....	7
1.3.1.1 Exercise intensity and the form of exercise.....	7
1.3.1.2 Exercise duration and energy expenditure.....	9
1.3.1.3 Mode of exercise.....	18
1.3.1.4 Study population.....	18
1.4 Peripheral endocrine signals and the relation of appetite.....	21
1.4.1 The effect of acute exercise on circulating concentrations of appetite-associated hormones.....	25
1.4.1.1 Ghrelin.....	25
1.4.1.2 Satiety peptides: PYY, GLP-1 and PP.....	26
1.4.1.3 The relationship between appetite-associated hormones and subjective appetite and food intake in the post-exercise period.....	28

1.5	Methodological issues associated with acute exercise and appetite research.....	32
1.5.1	Lack of continuity in study design.....	32
1.5.2	Problems with isolating variables.....	33
1.5.3	Measurements of appetite and food intake.....	35
1.6	Future direction of research.....	37
1.7	Scope of thesis.....	39
1.8	References.....	40
 Chapter 2 – General methods.....		48
2.1	Pilot testing: The development of The Birmingham Photographic Appetite	
	Rating.....	50
2.1.1	Introduction.....	50
2.1.2	Method.....	52
2.1.2.1	Participants.....	52
2.1.2.2	Study design.....	52
2.1.2.3	Procedure & protocol.....	52
2.1.2.4	Breakfast meal.....	53
2.1.2.5	Measures.....	54
2.1.2.6	Statistical analysis.....	56
2.1.3	Results.....	60
2.1.3.1	Energy intake at the test meal.....	60
2.1.3.2	Subjective appetite scores.....	60
2.1.3.2.1	VAS.....	60
2.1.3.2.2	BPAR.....	60
2.1.3.3	Correlation between subjective appetite measures.....	62
2.1.3.4	Ability to predict between-subject differences in energy intake.....	62
2.1.3.4.1	VAS.....	62
2.1.3.4.2	BPAR.....	63

2.1.3.5. Ability to predict within-subject differences in energy intake.....	65
2.1.3.5.1 VAS.....	65
2.1.3.5.2 BPAR.....	65
2.1.4 Discussion.....	67
2.2 General methods of Chapters 4, 5 and 6.....	70
2.2.1 Study design.....	70
2.2.2 Pre-testing sessions.....	70
2.2.2.1 Chapters 4 and 5.....	70
2.2.2.2 Chapter 6.....	71
2.2.3 Measures.....	72
2.2.3.1 Eating restraint.....	72
2.2.3.2 Food intake.....	73
2.2.3.3 Subjective appetite.....	74
2.2.3.4 Analysis of exhaled gas.....	75
2.2.3.5 Blood sampling and analysis.....	76
2.2.3.5.1 Sampling.....	76
2.2.3.5.2 Analysis.....	76
2.2.3.5.2.1 Glucose.....	76
2.2.3.5.2.2 Appetite-associate hormone.....	76
2.2.4 Statistical analysis.....	77
2.3 References.....	78

Chapter 3 – The development of a novel tool for the measurement of subjective

appetite. Part B: The Visual Meal Creator.....	80
3.1 Abstract.....	81
3.2 Introduction.....	83
3.3 Method.....	87
3.3.1 Participants.....	87

3.3.2 Study design.....	87
3.3.3 Procedures & protocol.....	88
3.3.4 Breakfast meals and snacks.....	89
3.3.5 Measures.....	90
3.3.6 Statistical analysis.....	92
3.4 Results.....	95
3.4.1 Energy intake at the test meal.....	95
3.4.2 Subjective appetite scores.....	95
3.4.2.1 VAS.....	95
3.4.2.2 VIMEC.....	96
3.4.3 Correlation between subjective appetite scores obtained with VAS and VIMEC....	98
3.4.4 Ability to predict between-subject differences in energy intake.....	98
3.4.4.1 VAS.....	98
3.4.4.2 VIMEC.....	98
3.4.5 Ability to predict within-subject differences in energy intake.....	99
3.4.5.1 VAS.....	99
2.4.5.2 VIMEC.....	99
3.4.6 Reliability and reproducibility of subjective appetite.....	99
3.4.6.1 Day-to-day measures.....	99
3.4.6.2 Test-retest measures.....	100
3.5 Discussion.....	101
3.6 References.....	107

Chapter 4 – The effect of exercise intensity on subjective appetite, food intake and appetite-associated hormone in highly trained male endurance athletes.....	110
4.1 Abstract.....	111
4.2 Introduction.....	113
4.3 Method.....	115

4.3.1	Participants.....	115
4.3.2	Experimental trial conditions.....	115
4.3.3	Procedure & protocol.....	116
4.3.4	Blood sampling and analysis.....	116
4.3.5	Statistical analysis.....	117
4.4	Results.....	118
4.4.1	Exercise trials.....	118
4.4.2	Subject appetite.....	118
4.4.2.1	VAS.....	118
4.4.2.2	BPAR.....	120
4.4.3	Energy intake at the <i>ad libitum</i> test meal.....	121
4.4.4	Satiety peptide concentration.....	122
4.4.4.1	PP.....	122
4.4.4.2	PYY.....	123
4.4.5	Relationship between hormones, subjective appetite and food intake.....	124
4.4.5.1	Between-condition correlation of change in hormone concentration with exercise and change in appetite with exercise.....	124
4.4.5.2	Between-condition correlation of percentage change in hormone concentration with percentage change in energy intake, and of percentage change in appetite score with percentage change in energy intake.....	125
4.5	Discussion.....	126
4.6	References.....	134

Chapter 5 – The effect of exercise duration on appetite, food intake and appetite-associated hormones.....	138
5.1 Abstract.....	139
5.2 Introduction.....	141

5.3 Method.....	144
5.3.1 Participants.....	144
5.3.2 Experimental trial conditions.....	144
5.3.3 Procedure & protocol.....	145
5.3.4 Blood sampling and analysis.....	145
5.3.5 Statistical analysis.....	146
5.4 Results.....	148
5.4.1 Exercise trials.....	148
5.4.2 Subjective appetite.....	148
5.4.2.1 VAS.....	148
5.4.2.2 VIMEC.....	149
5.4.3 Food intake at the <i>ad libitum</i> test meal.....	151
5.4.4 Plasma glucose and appetite-associated hormones concentrations.....	153
5.4.4.1 Glucose.....	153
5.4.4.2 Acylated ghrelin.....	153
5.4.4.3 PYY.....	156
5.4.4.4 GLP-1.....	156
5.4.5 Relationship between hormones, subjective appetite and food intake.....	159
5.4.5.1 Between-condition correlation of change in hormone concentration with exercise and change in appetite with exercise.....	159
5.4.5.2 Between-condition correlation of percentage change in hormone concentration with percentage change in energy intake, and of percentage change in appetite score with percentage change in energy intake.....	159
5.5 Discussion.....	161
5.6 References.....	168

Chapter 6 – The effect of sprint interval cycling exercise on appetite, food intake

and appetite-associated hormones in overweight and obese individuals.....173

6.1 Abstract.....	174
6.2 Introduction.....	176
6.3 Method.....	180
6.3.1 Participants.....	180
6.3.2 Experimental trial conditions.....	181
6.3.3 Procedure & protocol.....	181
6.3.4 Blood sampling and analysis.....	183
6.3.5 Statistical analysis.....	183
6.4 Results.....	185
6.4.1 Resting and exercise conditions.....	185
6.4.2 Subjective appetite.....	185
6.4.2.1 VAS.....	185
6.4.2.2 VIMEC.....	186
6.4.3 Plasma glucose and appetite-associated hormones.....	188
6.4.3.1 Glucose.....	188
6.4.3.2 Acylated ghrelin.....	188
6.4.3.3 PYY.....	188
6.4.3.4 GLP-1.....	189
6.4.4 Food intake and energy balance.....	191
6.4.5 The relationship between appetite-associated hormones, subjective appetite and energy intake.....	193
6.5 Discussion.....	194
6.6 References.....	201

Chapter 7 – Matching energy intake to expenditure of isocaloric exercise at high

and moderate intensities.....206

7.1 Abstract.....	207
7.2 Introduction.....	209
7.3 Method.....	211
7.3.1 Participants.....	211
7.3.2 Study design.....	211
7.3.3 Preliminary testing.....	211
7.3.4 Procedure & protocol.....	213
7.3.5 Measures.....	215
7.3.6 Statistical analysis.....	215
7.4 Results.....	217
7.4.1 Exercise trials.....	217
7.4.2 Energy expenditure, energy intake of the matching task and verbal estimate.....	217
7.4.3 <i>Ad libitum</i> energy intake.....	218
7.4.4 Food selection: energy density and macronutrient intake.....	219
7.5 Discussion.....	221
7.6 References.....	227
 Chapter 8 – General discussion.....	231
8.1 General discussion.....	232
8.2 A novel tool for the measurement of subjective appetite.....	233
8.3 The effect of exercise on appetite in highly-trained athletes.....	241
8.4 The effect of different exercise characteristics on appetite responses to exercise..	245
8.5 Questioning the regulatory role of appetite-associated hormones on appetite and food intake in the post-exercise period.....	248
8.6 What does regulate post-exercise appetite and food intake?.....	254
8.7 Summary of thesis findings.....	259
8.8 Future research.....	260

8.9 Thoughts, speculation and practical implications.....	262
8.9.1 Can an exercise-induced suppression of appetite be used as a tool to promote acute energy deficit and assist weight-loss?.....	262
8.9.2 Losing weight is difficult; the key for society is to prevent weight-gain.....	263
8.9.3 Exercise for the avoidance of weight-gain.....	264
8.10 References.....	266
Appendices.....	272
Appendix 1 – Written consent form, Chapter 2.....	272
Appendix 2 – General Health Questionnaire.....	273
Appendix 3 – Food item, with nutritional information, of the Birmingham Photographic Appetite Rating.....	276
Appendix 4 – The Dutch Eating Behaviour Questionnaire.....	277
Appendix 5 – Food and drink items, with nutritional information, of the <i>ad libitum</i> breakfast meal for Chapter 5.....	279
Appendix 6 – Food and drink items, with nutritional information, of the buffet meal for Chapter 6.....	280
Appendix 7 – Food items, with nutritional information, of the Visual Meal Creator.....	281
Appendix 8 – The International Physical Activity Questionnaire.....	282
Appendix 9 – Food and drink items, with nutritional information, of the buffet meal for Chapter 7.....	290

General introduction: The effect of acute exercise on subjective appetite, appetite-associated hormones, food intake and short-term energy balance

1.1 Obesity and overweight – definition, prevalence, socio-economic cost and health implications

The prevalence of obesity and overweight has escalated to pandemic proportions. Recent estimates state that obesity, defined by the World Health Organisation as “abnormal or excessive fat accumulation that may impair health” and classified as possessing a body mass index (BMI) value of $\geq 30 \text{ kg}\cdot\text{m}^{-2}$, afflicts over 500 million people globally, with 1.4 billion adults either obese or overweight (BMI 25-29 $\text{kg}\cdot\text{m}^{-2}$) (Finucane *et al.* 2011). This equates to 35% of the world’s population. In England, statistics indicate that 24% of men and 26% of women are obese, with a further 41% of men and 32% of women being overweight (Health and Social Care Information Centre, 2013). Perhaps more worryingly for the future, three in ten children are now overweight or obese (Health and Social Care Information Centre, 2013)

The implications of an obesity pandemic are far-reaching, with economic and health costs. In 2007, it was estimated that the total financial burden of obesity and obesity-related illness on the National Health Service of the UK was £3.2 billion per year (Allender and Rayner, 2007). Projections suggest that the total annual cost to the UK economy could be as high £50 billion by 2050 (Butland *et al.*, 2007). Obesity increases the risk of a number of serious and potentially fatal diseases, including type II diabetes (Chan *et al.*, 1994; Colditz *et al.*, 1990), cardiovascular disease (Hubert *et al.*, 1983), stroke (Suk *et al.*, 2003), some cancers (Carroll, 1998) and psychiatric disorders, such as depression (Onyike *et al.*, 2003; Simon *et al.*, 2006). The gravity of this increased risk of disease is perhaps best highlighted in a study published in the *Lancet* in 2009 (Prospective Studies, 2009). Collaborative analysis of 57 prospective follow-up studies, with a minimum follow-up period of 20 years, showed that, for every 5 $\text{kg}\cdot\text{m}^{-2}$ an individual’s BMI was above 25 $\text{kg}\cdot\text{m}^{-2}$, the risk of mortality increased approximately 30%. Further, obese individuals died, on average, 10 years earlier than healthy-weight counterparts. It was found that approximately 8% of deaths could be

attributed to obesity, making obesity the UK's second largest contributor to mortality (Prospective Studies, 2009).

The obesity pandemic will most probably worsen before it gets better. It has been forecast that by 2030, there will be a further 11 million obese adults in the UK (Wang *et al.*, 2011). This is likely to result in 8.5 million more cases of diabetes, 5.7-7.3 million more cases of heart disease and stroke and around half a million more incidences of cancer. Hence, the economic burden of obesity is expected to rise by £2 billion a year (Wang *et al.*, 2011). In light of the current pandemic and these alarming projections, successful, cost-effective treatments and preventative measures for obesity must be sought.

1.2 The energy balance equation: the role of exercise and appetite

Weight-gain, leading to overweight and obesity, arises from prolonged, repeated periods of positive energy balance. This occurs when total energy consumption exceeds total energy expenditure. While sounding over-simplistic, the manipulation of this equation over prolonged periods in favour of either a positive balance or a negative balance will lead to weight-gain or weight-loss, respectively.

Those seeking to shift their energy balance in favour of an energy deficit are often advised to increase their amount of physical activity and undertake exercise (Donnelly *et al.*, 2009; Jakicic *et al.*, 2001). While exercise has been shown to effectively increase energy expenditure (Poehlman and Horton, 1989), both sides of the equation must be considered. From a homeostatic point of view it may be considered intuitive that any imbalance in energy status will trigger a mechanism that will restore equilibrium, ensuring the tight regulation of energy balance. This belief in energy balance being a tightly regulated homeostatic system would lead to the acknowledgement of fast-acting compensatory increases in energy intake, through an increase in appetite and drive to eat, in response to energy deficit. This view is supported by evidence that energy deficit caused by a reduction in food intake (through meal skipping and fasting) resulted in a subsequent upregulation of appetite and food intake

(Hubert *et al.*, 1998; Johnstone *et al.*, 2002). However, such a notion was challenged as long ago as the early 1970s by findings of Edholm and colleagues (Edholm *et al.*, 1970), who showed an absence of day-to-day coupling of energy intake and energy expenditure. They did, however, demonstrate that there may be a delayed compensatory response that allowed a tighter coupling over a seven day period. Subsequent research would indicate that a compensatory increase in energy intake, in response to exercise initiation is absent (Stubbs *et al.*, 2002a), minimal (Staten, 1991; Stubbs *et al.*, 2004; Woo *et al.*, 1982) or at most, incomplete, accounting for ~30% of the exercise-induced increase in energy expenditure (Stubbs *et al.*, 2002b; Whybrow *et al.*, 2008). Nevertheless, the persistence of a negative energy balance is commonly seen during the early stages (1 – 3 weeks) of exercise training commencement (Staten, 1991; Stubbs *et al.*, 2002b; Whybrow *et al.*, 2008; Woo *et al.*, 1982). Further, it has been shown that the appetite and food intake response may differ between a food-restriction and an exercise-induced energy deficit (Hubert *et al.*, 1998; King *et al.*, 2011a). This not only suggests that exercise may influence appetite independent of alterations in energy balance, but also highlights the benefit of exercise within a weight-management strategy.

With this in mind, the relationship between exercise, appetite and food intake is clearly of importance. This review will primarily address the effect of acute exercise on short-term appetite, food intake and energy balance. The effects of chronic exercise training on long-term changes in appetite, food intake and bodyweight is beyond the scope of this review and will not be discussed. (See Caudwell *et al.*, 2009 and Hopkins *et al.*, 2010 for reviews of the effect of chronic exercise on appetite. See Curioni and Lourenco, 2005 and Donnelly *et al.*, 2009 for a systematic review and meta-analysis of the success of exercise in weight-management and for recommendations for exercise interventions to promote weight-loss). Furthermore, while the cognitive regulatory mechanisms of eating behaviour are both intriguing and of considerable importance, this review will focus on metabolic components of acute appetite regulation. For a review of the cognitive regulation of eating behaviour, the reader is directed to two comprehensive review articles (King, 1999; Stubbs, 1998).

1.3 The effect of acute exercise bouts on appetite, food intake and short- to medium-term energy balance

The weight of evidence would suggest that completing an acute bout of exercise does not result in an increase in appetite and food intake. A 1999 systematic review carried out by John Blundell and Neil King (Blundell and King, 1999) showed that, when reviewing the findings of 48 intervention studies, 19% showed an increase in energy intake after exercise, 65% showed no change and 16% showed a decrease. It is now generally accepted that there is only a weak short-term coupling of energy intake and expenditure, opposing the concept of a fast-acting compensatory mechanism to regulate energy balance (King *et al.*, 1997a; King *et al.*, 1996; Lluch *et al.*, 2000; Staten, 1991). Contrary to early intuitive belief, it has been widely reported that vigorous exercise can in fact transiently suppress appetite (Broom *et al.*, 2009; Broom *et al.*, 2007; Burns *et al.*, 2007; Kawano *et al.*, 2013; King *et al.*, 2011b; King *et al.*, 1994; King *et al.*, 1996; Kissileff *et al.*, 1990; Thompson *et al.*, 1988; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b; Westerterp-Plantenga *et al.*, 1997). This much publicised phenomenon was substantiated by the work of John Blundell's research group, leading them to coin the term "anorexia of exercise" to explain this exercise-induced suppression of appetite after high intensity ($\geq 60\%$ $\text{VO}_{2\text{max}}$) exercise (King *et al.*, 1994). This response is not observed after low- or moderate-intensity exercise (George and Morganstein, 2003; Kissileff *et al.*, 1990). Any suppression is also rather short-lived. Hence, while such a subjective hunger suppression can lead to reductions in food intake in the immediate post-exercise period (~ 10 minutes, (Westerterp-Plantenga *et al.*, 1997); ~ 15 minutes, (Kissileff *et al.*, 1990)), food intake is largely unaffected when a meal is consumed ≥ 60 minutes after exercise (King *et al.*, 2010; King *et al.*, 2011b; King *et al.*, 1997b; Schubert *et al.*, 2013; Thompson *et al.*, 1988).

Such a transient suppression of hunger, it could be argued, is unlikely to lead to reductions in food intake in free living conditions when we consider that a major eating episode (i.e., lunch or dinner meal) is unlikely to be consumed within 60 minutes of the

cessation of exercise (given the time taken for immediate recovery, to possibly warm-down, to shower, change clothes, possibly return home from the site of exercise, e.g., the gym, and to cook or prepare food).

The effect of an acute exercise bout on more medium-term (hours to days post-exercise) energy intake is perhaps more relevant for assessing whether a compensatory mechanism will act to re-establish energy balance after exercise-induced energy deficit. Evidence would indicate that a single bout of aerobic exercise has little impact on medium-term hunger and energy intake. Broom *et al.* (Broom *et al.*, 2009) found that while aerobic exercise (60 minutes of treadmill running at $\sim 70\%$ $\text{VO}_{2\text{max}}$) did suppress hunger during the two-hour period from the cessation of exercise to a standardised meal (versus a control condition), subjective hunger was no different for the remaining 5 hour period after this meal. Further work from this research group confirmed this response in the hours after exercise (King *et al.*, 2011a; King *et al.*, 2011b).

King and co-workers, (King *et al.*, 2010) investigated the effect of prolonged treadmill running (90 minutes at $\sim 70\%$ $\text{VO}_{2\text{max}}$) on appetite and energy intake, with measures obtained for the 24 hour period after exercise, in 9 healthy males. Subjective appetite was transiently suppressed during exercise, but did not differ from the control condition post-exercise. The lack of significant difference in appetite between the exercise and control condition was reflected by very similar energy intakes over the 24 hour trial period (4109 kcal during the control conditions vs. 4208 kcal during the exercise condition). When considering the increased energy expenditure of the exercise bout, the exercise condition resulted in participants remaining in energy deficit for the 24 hour period. These findings were substantiated with evidence of no compensatory increase in energy intake over a 5-day period following 2 days of exercise in healthy-weight and overweight young girls (Dodd *et al.*, 2008).

Such findings suggest that while prolonged aerobic exercise may not suppress appetite to such an extent, or for long enough, to result in a reduction in post-exercise energy intake, neither does it trigger a compensatory response by which appetite and

therefore energy intake is upregulated, even when the energy cost of exercise is very high. Hence, this would advocate that exercise can be an effective means of creating a medium-term energy deficit that can be maintained for at least 24 hours.

1.3.1 Is this an over-simplified message? The effect of exercise characteristics on appetite and the appetite response in different populations.

While these findings appear relatively conclusive, it is likely that the issue is not quite so clear cut. The appetite response to exercise could be dependent on a number of exercise characteristics and differ in different populations.

1.3.1.1 Exercise intensity and the form of exercise

The importance of the intensity of exercise for a post-exercise appetite response, as just discussed, is well established. However, only recently has the effect of supramaximal, intermittent exercise on appetite and food intake been investigated. It has been consistently shown that high-intensity interval exercise (HIE) and high-intensity interval training (HIT) can elicit numerous physiological adaptations (Bartlett *et al.*, 2012; Burgomaster *et al.*, 2006; Burgomaster *et al.*, 2008; Burgomaster *et al.*, 2005) and health benefits (Babraj *et al.*, 2009; Little *et al.*, 2011; Whyte *et al.*, 2010) usually observed with more traditional, longer duration, continuous aerobic exercise. Thus, HIT is considered by some to be a preferable, time-effective form of exercise. A criticism of such low-volume exercise bouts, however, is a low energy cost. HIT is considered by many to be an ineffective form of exercise for inducing weight-loss, with the low energy cost being unlikely to yield an energy deficit sufficient to promote weight-loss. Typically, only modest weight-loss is achieved in long-term HIT studies (Burgomaster *et al.*, 2008; Helgerud *et al.*, 2007), even in overweight and obese individuals (Whyte *et al.*, 2010), although significant reductions in both body weight (Trapp *et al.*, 2008) and fat mass have been achieved (see Boutcher, 2011).

Despite a number of chronic HIT studies, there has been a dearth of research into the effect of HIE on appetite and acute food intake. To date, only three studies have investigated the effect of low-volume, high-intensity exercise, or sprint interval exercise (SIE) on appetite and food intake (Deighton *et al.*, 2012; Deighton *et al.*, 2013; Sim *et al.*, 2013). Deighton *et al.* (Deighton *et al.*, 2012) found that just 6 x 30 second Wingate tests transiently suppressed appetite. This response was achieved with an estimated mean energy cost of exercise of just 142 kcal. However, food intake at an *ad libitum* buffet meal, provided 45 minutes after the cessation of exercise was no different to that of a control condition (Deighton *et al.*, 2012). In a follow-up study, 10 x 4 minutes of cycling at 85% $\text{VO}_{2\text{max}}$ suppressed appetite to a greater extent than isocaloric continuous exercise at 60% $\text{VO}_{2\text{max}}$ (Deighton *et al.*, 2013). Most recently, Sim *et al.* (Sim *et al.*, 2013) addressed the appetite response to high-intensity intermittent exercise (30 minutes of 1 minute cycling at 100% $\text{VO}_{2\text{peak}}$, 4 minutes recovery at 50% $\text{VO}_{2\text{peak}}$) and very-high-intensity exercise (30 minutes of 15 seconds cycling at 170% $\text{VO}_{2\text{peak}}$, 60 seconds recovery at 32% $\text{VO}_{2\text{peak}}$), compared with continuous exercise (30 minutes cycling at 60% $\text{VO}_{2\text{peak}}$) and rest, in overweight men. Measures of subjective appetite did not differ between any of the conditions during or post-exercise. However, *ad libitum* energy intake, measured 70 minutes post-exercise (and after a standardised liquid meal was provided immediately upon exercise cessation) was lower after high- and very-high-intensity intermittent exercise, compared with rest, and rest and continuous exercise respectively. *Ad libitum* energy intake was monitored for the remainder of the experimental day and the following day. Intake during this period was significantly lower after very-high-intensity exercise, compared with rest and continuous exercise. This data suggests that exercise of a sufficiently high intensity may provide a strong enough stimulus to influence food intake in the hours and days post-exercise, even in the absence of changes in subjective appetite. However, further work is certainly required to substantiate this curious finding.

While the intensity of exercise is evidently a key factor in regulating appetite responses, it may well not be the only characteristic that influences post-exercise appetite. **Table 1.1** shows a number of studies that have addressed the effect of acute exercise on appetite and energy intake, with the study design and findings summarised. The table is ordered chronologically. As well as highlighting the importance of the intensity as an exercise characteristic that influences the appetite response, the contention surrounding the role of other characteristics should also become clear.

1.3.1.2 Exercise duration and energy expenditure

The duration of exercise is a much less common primary characteristic under investigation. When studies have utilised exercise conditions of varying duration, duration is rarely isolated as a variable (Deighton *et al.*, 2012; Imbeault *et al.*, 1997; Pomerleau *et al.*, 2004). Similarly, exercise energy expenditure is rarely an isolated variable under investigation (Deighton *et al.*, 2012; Kissileff *et al.*, 1990; Sim *et al.*, 2013; Ueda *et al.*, 2009a).

The weight of evidence would suggest that the appetite response post-exercise is independent of the duration of exercise and energy expenditure. Suppressions in post-exercise appetite have been observed after high-intensity continuous exercise of a duration as little as 30 minutes (Ueda *et al.*, 2009a) and after supramaximal intermittent exercise of total duration as low as 3 minutes of intense exercise (23 minutes, if recovery period is included) (Deighton *et al.*, 2012). The former study observed an appetite suppression accompanied by a reduction in energy intake, compared with a resting control condition, while the latter study did not see a reduced energy intake after sprint interval exercise. With regards to energy expenditure, the sprint interval bout of Deighton *et al.* (Deighton *et al.*, 2012) yielded an energy cost of just 142 kcal. In a separate study, a 240 kcal bout of “hard” cycling also suppressed appetite, as was evidenced by a decreased energy intake in an *ad libitum* test meal administered 15 minutes post-exercise (Kissileff *et al.*, 1990). At the other

end of the spectrum, a 90 minute bout of treadmill running at an intensity of 70% $\text{VO}_{2\text{max}}$ was not sufficient to elicit an appetite suppression or to influence food intake at *ad libitum* test meals 1 hour and 4 hours post-exercise (King *et al.*, 2010; King *et al.*, 2011a). Further, the exercise bouts in these two studies of King and co-workers yielded very large energy costs of ~1100 kcal (King *et al.*, 2011a) and ~1300 kcal (King *et al.*, 2010). However, other high-intensity, continuous exercise bouts of longer duration and large energy costs have resulted in appetite suppression (Broom *et al.*, 2009; Broom *et al.*, 2007; Burns *et al.*, 2007).

These findings, collectively, show that a suppression of appetite can be experienced after very low-duration and low-energy cost bouts, but conversely, no suppression of appetite has been observed with very long, energetic bouts. This would indicate that the duration and energy cost of exercise do not play a major regulatory role in the appetite and food intake response to exercise. It would appear that, if the exercise is of sufficient intensity, post-exercise appetite can be suppressed, regardless of the duration of the exercise bout and the energy expended. However, it is possible that other factors and study design issues could contribute to the differing findings discussed in the previous paragraph, such as differing study populations and different modes of exercise. These issues will be discussed later in this section. While indicating the minimal influence of exercise duration and energy cost, these collective findings do not conclusively prove that neither variable influences post-exercise appetite. For this, direct investigation of the effect of exercise duration and energy cost are required, within the same study or a series of studies following the same study design (see section 1.5.2, p33).

Study	Population			Exercise Trial				Outcome Measures			Findings	
	Activity level / Bodyweight	Gender	Age	Mode	Intensity	Duration	EE	What	When	How	Subjective appetite	Food intake
(Kissileff <i>et al.</i> , 1990)	Inactive, Ob and HW	F	23 ± 5 yrs	Cycle	a) 30W (mod) b) 90W (hard ~65.5% VO _{2max})	40 min.	a) ~130 kcal b) ~240 kcal	EI, hunger	15 min. after exercise	Yoghurt test meal	↑ hunger after test meal with moderate ex. vs. rest and strenuous ex. in obese only	EI ↓ after hard ex. vs. mod ex. and rest for non-obese.
(Imbeault <i>et al.</i> , 1997)	Moderately active, HW	M	24 ± 3 yrs	Walk/run	a) 35% VO _{2max} (low) b) 70% VO _{2max} (high)	a) 72 ± 14 min. b) 34 ± 6 min.	490 kcal for both trials	EI, REI, hunger	“immed. after the shower following exercise”	<i>Ad libitum</i> buffet meal	↔	EI ↔ after ex. REI ↓ after high intensity ex. vs. low and rest. REI ↓ after low vs. rest.
(Westerterp-Plantenga <i>et al.</i> , 1997)	Untrained – Ob & non-Ob.	M	25 ± 7 yrs	Cycle	60% W _{max}	120 min.	-	EI	Before ex and 10min. after ex.	<i>Ad libitum</i> buffet meal	-	EI ↓ before and after ex vs. rest. ↑CHO component after ex.
(Lluch <i>et al.</i> , 2000)	Active, HW. Restrained and non-restrained eaters	F	22 ± 2 yrs	Cycle	70% VO _{2max}	50 min.	384 ± 54 kcal	EI, hedonic rating of food.	Immed. Post-ex, allowed to eat for 30 min.	<i>Ad libitum</i> lunch meal		Trend for ↑ EI in unrestrained eaters post-ex. Trend for ↓ EI in restraint eaters post-ex. REI ↓ post-ex. vs. rest.
(George and Morganstein, 2003)	Inactive HW vs. OW	F	35± yrs	Walk/run	60% HR _{max}	60 min.	~150-200 kcal	EI, nutrient intake	~ 30 min. post-ex	<i>Ad libitum</i> buffet meal	-	EI ↔ after ex.

Study	Population			Exercise Trial				Outcome Measures			Findings	
	Activity level / Bodyweight	Gender	Age	Mode	Intensity	Duration	EE	What	When	How	Subjective appetite	Food intake
(Pomerleau <i>et al.</i> , 2004)	Moderately active, HW	F	22 ± 2 yrs	Walk/run	a) 40% VO _{2peak} (LIE) b) 70% VO _{2peak} (HIE)	65 min. and 37 min.	~84 kcal	EI; REI; appetite	60 min. post-ex (lunch); 6.5 h post-ex (diner); Total EI 9 h post ex	<i>Ad libitum</i> Buffet meal & snack bags	↔	EI ↑ (lunch) post HIE, vs. control. REI ↓ (lunch) post HIE & LIE vs. control
(Burns <i>et al.</i> , 2007)	HW	M and F	25 ± 1 yrs	Run	75% VO _{2max}	60 min.	896 ± 49 kcal	Hunger		15-point scale	↓ during and after ex vs. rest	-
(Martins <i>et al.</i> , 2007)	<1h ex. per day, HW	M and F	26 ± 5 yrs	Cycle	65% HR _{max} Intermitt.	60 min.	492 kcal	EI; REI; appetite	60 min. post-ex.	<i>Ad libitum</i> buffet meal	↓ during ex. ↔ after ex.	EI ↑ REI ↓ with exercise
(Broom <i>et al.</i> , 2007)	Phys. Active / competitive sports people (VO _{2max} , 63 ± 2 ml/kg/min), HW	M	21 ± 1 yrs	Run	75% VO _{2max}	60 min.	~930 kcal	Hunger	Hunger: every 30mins. for 5h, every hour thereafter.	Hunger: 16-point scale	↓ with exercise during and for 2hours post ex.	-
(Broom <i>et al.</i> , 2009)	Phys. Active / competitive sports people (VO _{2max} , 62 ± 2 ml/kg/min), HW	M	21 ± 0 yrs	a)Run b)W. lifting	a) 70% VO _{2max} b) 80% of 12 rep max.	a) 60 min. b) 3 sets, 10 exercise (90min.)	a) 915 kcal b) ~350 kcal	Hunger	Hunger: pre, during & immed. Post-ex + every 30 min. thereafter for 7h.	Hunger: 16-point scale	Hunger ↓ during exercise and remained so until meal consumed. Greater hunger suppression with aerobic vs. resistance.	-

Study	Population			Exercise Trial				Outcome Measures			Findings	
	Activity level / Bodyweight	Gender	Age	Mode	Intensity	Duration	EE	What	When	How	Subjective appetite	Food intake
(Ueda <i>et al.</i> , 2009a) Comparable	HW	M	23 ± 3 yrs	Recum. cycling	a) Mod. intensity: 50% VO _{2max} b) High intensity: 70% VO _{2max}	Both 30 min.	-	EI, appetite	30 min. post-ex	<i>Ad libitum</i> pasta meal	Hunger ↓ during and immediately post-ex in mod. and high intensity conditions	EI ↓ after mod. and high intensity exercise
(Ueda <i>et al.</i> , 2009b) changes	Ob vs. HW	M	23 ± 4 yrs	Recum. cycling	50% VO _{2max}	60 min.	707 kcal for obese. 496 kcal for healthy-weight	EI, REI, hunger	60 min. post ex.	Pasta-based test meal	↔ vs. baseline and rest trial	EI & REI ↓ post-ex. vs. post-rest. EI and REI ↓ after ex. in obese vs. HW.
(King <i>et al.</i> , 2010)	Physically active (VO _{2max} , 60.5 ± 1.5 ml/kg/min), HW	M	22 ± 1 yrs	Running	70 % VO _{2max}	90 min.	1273 ± 45 kcal	EI, REI, appetite.	1 hour and 4 hours post-exercise	<i>Ad libitum</i> cold buffet and hot buffet	Hunger, prospective intake ↓ during exercise only	EI ↔ vs. resting control trial. REI ↓ after ex
(Unick <i>et al.</i> , 2010)	OW/Ob	F	28 ± 8 years	Walking	70-75% predicted HR _{max}	42 min.	354 kcal	EI, REI, hunger	60-120 min. post-exercise	<i>Ad libitum</i> snack buffet	↔ vs. baseline ↔ vs. rest.	EI ↔ vs. resting control trial. REI ↓ after ex.
(King <i>et al.</i> , 2011a)	Physically active (VO _{2max} , 57 ± 1 ml/kg/min), HW	M	23 ± 1 yrs	Running	70% VO _{2max}	90 min.	1127 kcal	EI, appetite.	6.5 hours after exercise, preceded by 2 standard. meal	<i>Ad libitum</i> buffet meal	↔ vs. baseline ↔ vs. rest	EI ↔ vs. resting control trial.

Study	Population			Exercise Trial				Outcome Measures			Findings	
	Activity level / Bodyweight	Gender	Age	Mode	Intensity	Duration	EE	What	When	How	Subjective appetite	Food intake
(King <i>et al.</i> , 2011b)	HW,	M	22 ± 1 yrs	Swim.	“moderate -intensity” – between 12 and 14 on rating of perceived exertion.	6 x 7 min. intermitt. with 3 min. recovery	Net EE (ex. minus resting) = 459 ± 20 kcal	EI, REI, macron. intake, appetite	1 hour and 5 hours after exercise	2 x <i>ad libitum</i> buffet meals	↓ during and immediately after ex vs. rest after ex. vs. rest during recovery	EI ↔ vs. resting control trial. REI ↓ after ex.
(Deighton <i>et al.</i> , 2012)	Predom. HW	M	23 ± 3 yrs	a) cont. cycling b) intermitt. sprint cycling	a) 65% VO _{2max} b) maximal effort	a) 60 min. b) 6 x 30 sec with 4 min. recovery	a) 631 ± 100 kcal b) 142 ± 12kcal	EI, REI, appetite	45 min. and 4.25 hours after exercise	<i>Ad libitum</i> cold buffet and hot buffet	↓ immediately after intermittent sprint cycling vs. rest but ↑ in hours post-ex.	EI ↔ after either continuous or intermittent sprint vs. rest ↓ REI after continuous vs. rest.
(Deighton <i>et al.</i> , 2013)	HW	M	22 ± 3 yrs	a) cont. b) high-intensity intermit.	a) 60% VO _{2max} b) 85% VO _{2max}	a) 60 min. b) 10 x 4 min with 2 min recovery	-	EI, REI, appetite	4 hours post-ex., preceded by standard. meal, 45 min. post-ex.	<i>Ad libitum</i> hot meal.	↓ immediately post-ex. after continuous and intermittent vs. rest. ↓ after intermittent vs. continuous	EI ↔ after either continuous or intermittent vs. rest ↓ REI after continuous and intermittent vs. rest.
(Larson-Meyer <i>et al.</i> , 2012)	Endurance-trained, HW vs. habitual walkers, HW	F	24 ± 5 yrs	Running /walking	70% VO _{2max}	60 min.	Runners: 483 ± 50 kcal Walkers: 325 ± 138 kcal	EI, REI, hunger and satiety	2 hours post-exercise	<i>Ad libitum</i> meal, offering hot and cold items	↔ vs. baseline and vs. rest in both runners and walkers.	Runners: ↔ EI vs. rest. ↓ REI Walkers: trend for ↓ EI vs. rest. ↓ REI

Study	Population			Exercise Trial				Outcome Measures			Findings	
	Activity level / Bodyweight	Gender	Age	Mode	Intensity	Duration	EE	What	When	How	Subjective appetite	Food intake
(Oh and Taylor, 2012)	Predom. HW	M and F	25 ± 8 yrs	Walk	“brisk”	15 min.	-	Grams of choc. snack eaten	“through-out trial”	<i>Ad libitum</i> access to choc. Snack	-	↓ consumption of chocolate snack.
(Wasse <i>et al.</i> , 2012)	Physically active, HW	M	23 ± 3 yrs	a) Cycling b) Running	Both 70% mode-specific VO _{2max}	60 min.	a) 919 ± 208 kcal b) 779 ± 201 kcal	Hunger	Hunger: every 30 min.	Hunger: 100mm VAS	↓ vs. rest for both cycling and running	-
(Sim <i>et al.</i> , 2013)	OW	M	30 ± 8 yrs	a) Cont. b) HIE c) VHIE	a) 60% VO _{2peak} b) 1 min @ 100% VO _{2peak} , 4 min. @ 50% VO _{2peak} c) 15 sec @ 170% VO _{2peak} , 60sec @ 32% VO _{2peak}	All 30 min.	Mech. work: a) 69 ± 11 kcal b) 55 ± 10 kcal c) 54 ± 11	Appetite, acute EI, EI for rest of day and following day (free-living)	70 min post-exercise. Standard. snack meal on cessation of exercise	<i>Ad libitum</i> B’fast meal of porridge and milk	↔ between conditions and vs. baseline	Acute EI ↓ HIE and VHIE vs. rest. VHIE ↓ continuous. Free-living EI ↓ VHIE vs. rest.

Study	Population			Exercise Trial				Outcome Measures			Findings	
	Activity level / Bodyweight	Gender	Age	Mode	Intensity	Duration	EE	What	When	How	Subjective appetite	Food intake
(Heden <i>et al.</i> , 2013)	HW and Ob	M and F	26 ± 6 yrs	Walking	55-60% VO _{2peak}	60 min.	430 ± 116 kcal for HW; 519 ± 148 for Ob	Hunger and fullness	Hunger and fullness: every 30min. for 4 hours, after standard. b'fast, morning after evening exercise.	Hunger and fullness: 100 mm VAS	↔ hunger ↓ fullness after ex. in Ob only.	-
(Holmstrup <i>et al.</i> , 2013)	Ob	M and F	25 ± 1 yrs	Running a) cont. b) intermit.	a) 60-65% VO _{2peak} b) 60-65% VO _{2peak}	a) 60 min. b) 60 min. total. 12 x 5min. bouts, hourly	-	Subjec. hunger and satiety	Hunger and satiety: every 20 min.	Hunger and satiety: 100 mm VAS	↔ hunger and satiety between continuous and rest, during and immediately after ex. ↓ hunger for intermittent vs. rest.	-
(Kawano <i>et al.</i> , 2013)	HW	M	24 ± 2 yrs	a) rope skip b) cycling	a) 65% VO _{2max} b) 64% VO _{2max}	a) 3 x 10 min. with 5 min. recovery b) until EE matches skipping bout.	a) 295 kcal b) 288 kcal	Hunger	Hunger and desire to eat: before, during, and every 15 min after ex. for 2hrs.	Hunger and desire to eat: 100 mm VAS	↓ for cycling and rope skip vs. rest during and <30min. post-ex. ↓ for rope skip vs. cycling during ex. ↑ for cycling vs. rest 105min post-ex.	-

Table 1.1 – Review of a number of studies investigating the effect of exercise on appetite and food intake. Ob, obese; OW, overweight; HW, healthy weight; Predom., predominantly; M, male; F, female; ex, exercise; W. lifting, weight lifting; recum., recumbent; swim., swimming; cont., continuous; intermitt., intermittent; LIE, low intensity exercise; HIE, high intensity exercise; Mech., mechanical; macron., macronutrient; subec., subjective; immed., immediately; standard., standardised; b'fast., breakfast; EE, energy expenditure; EI, energy intake; REI, relative energy intake.. “When” and “How” refers to the measures of energy intake, unless otherwise stated. Hunger/appetite measured using VAS unless stated.

1.3.1.3 Mode of exercise

It remains unclear as to whether the appetite response to exercise is dependent on the mode of exercise undertaken. Commonly, ergometer cycling and treadmill running are the modes utilised in appetite research, with suppression of subjective appetite observed with both (see **table 1.1**). In addition, swimming (King *et al.*, 2011b) and weight-lifting (Broom *et al.*, 2009) have also been shown to suppress appetite. As it has been postulated that the “up and down” movement of the trunk during certain types of exercise can cause disturbance to the GI tract, leading to greater secretion of appetite-associate hormones, direct comparisons of modes of exercise that do and do not cause excessive trunk movement have been made, with contrasting findings. Kawano and colleagues (Kawano *et al.*, 2013) compared the appetite response to energy- and intensity-matched cycling and rope skipping. Both modes elicited a suppression of appetite during, and immediately after exercise, compared with baseline and a resting control condition. However, the suppression was greater with rope skipping. Furthermore, appetite rebounded in the two-hour post-exercise period in the cycling condition, but not after rope skipping. Conversely, Wasse *et al.* (Wasse *et al.*, 2012) found no difference between the effect of cycling and running on post-exercise appetite, with both modes reducing hunger scores, compared with rest. Neither study addressed post-exercise food intake.

In light of the majority of the current literature, it would appear unlikely that mode of exercise influences post-exercise appetite responses. Data would suggest that exercise of a sufficient intensity can transiently suppress appetite, regardless of mode. However, further direct comparisons of different modes are required to fully clarify any remaining contention.

1.3.1.4 Study population

It is clear from **table 1.1** that studies have utilised a wide range of study populations, specifically with regard to activity level and bodyweight. It is difficult to elucidate whether different populations respond differently with regard to post-exercise appetite. Transient

suppressions of appetite after high-intensity exercise have been observed with highly-active (Broom *et al.*, 2009; Broom *et al.*, 2007), moderately- active, healthy-weight individuals (Burns *et al.*, 2007; Kawano *et al.*, 2013; Wasse *et al.*, 2012) and overweight/obese individuals (Holmstrup *et al.*, 2013). No such response is commonly seen with low- to moderate-intensity exercise in both healthy-weight (Imbeault *et al.*, 1997; Pomerleau *et al.*, 2004; Ueda *et al.*, 2009b) and overweight/obese (George and Morganstein, 2003; Ueda *et al.*, 2009b; Unick *et al.*, 2010) populations. However, differential responses have been observed in studies directly comparing healthy-weight individuals with overweight and obese counterparts (Heden *et al.*, 2013; Kissileff *et al.*, 1990), with obese exhibiting increased hunger (Kissileff *et al.*, 1990) and decreased fullness (Heden *et al.*, 2013) post-moderate-intensity exercise, compared with healthy-weight individuals.

There is evidence that those familiar with high-intensity exercise may exhibit some degree of blunting to the exercise-induced suppression of appetite, or may require a higher-intensity exercise bout than less active individuals, to experience an appetite suppression. While non-habitual intense exercisers have been seen to experience appetite suppression after exercise of an intensity $\sim 60\%$ VO_{2max} (King *et al.*, 1994; Martins *et al.*, 2007; Ueda *et al.*, 2009a), exercise of a similar intensity (60-70% VO_{2max}) has failed to elicit any suppression in those more used to regular exercise (King *et al.*, 2011a; King *et al.*, 1997a; Larson-Meyer *et al.*, 2012), or suppression was observed but was only present during, but not after exercise (King *et al.*, 2010). It is possible that, by exercising regularly, and at a relatively high intensity, some adaptation leads to the blunting of a post-exercise suppressive appetite response. This may be a necessary adaptation, needed to promote post-exercise refuelling or a mechanism by which energy deficit is opposed. It is possible that such a mechanism is physiological in nature, governed by changes in appetite-associated hormones (see section 1.4, p21), or purely behavioural; regular exercisers are likely aware of potential benefits of immediate post-exercise feeding, so cognition may override the physiological regulation of appetite. Hence, it is possible that any “intensity threshold” for transient appetite suppression is dependent on the degree to which an individual is

accustomed to exercise, or on their fitness level. While some studies have utilised physically active individuals, no studies to date have extensively and thoroughly investigated the effects of exercise on appetite in highly-trained, athletic populations. If a “threshold intensity” for post-exercise appetite suppression does exist, and if it is perhaps different for different individuals based on their activity and training status, it would follow that highly-trained athletes would be further resistant to this suppression effect, requiring an even greater exercise intensity in order to experience a suppression of appetite.

Further evidence for a differential response of appetite and food intake to exercise, based of habitual activity level is provided by a recent study of Rocha *et al.* (2013). They observed that 60 minutes of moderate intensity cycling did not alter hunger ratings, nor *ad libitum* lunch intake 60 minutes post-exercise in either active or inactive, healthy-weight males. However, when monitoring food intake over the remainder of the test day and the 3 days following, active males increased intake on the exercise experimental day, compared with the resting control experimental day. This indicates a compensatory response to the exercise energy expenditure, resulting in a small over compensation of 27% over the course of the test day. In contrast, inactive males exhibited a latent response, with an apparent increased compensatory intake 3 days post-exercise. The cumulative percentage of energy compensation over the entire 4 days of monitoring did not differ significantly between active and inactive males. This data would suggest that a compensatory response of increased food intake, to the energy expenditure of an acute bout of exercise, occurs in both active and inactive healthy-weight males. However, this response appears to occur at different rates, with active males possessing a more rapid response, suggesting a tighter regulation of energy homeostasis.

As well as studies utilising participant populations of varying bodyweight and activity level, there is variability in the gender of participants studied. Some studies investigated effects in males and females combined, some in exclusively one gender while others directly compared responses between males and females (see **table 1.1**). Acutely, the collective data from **table 1.1** would indicate that a gender difference in the appetite response to

exercise is not present, with findings from male only and female only studies following the same patterns. Further, studies that have compared males with females have also not observed a difference (Burns *et al.*, 2007). However, there are a limited number of such direct comparisons and investigations with female populations are rare.

From this discussion, it is clear that contention remains regarding the effect of a single exercise bout on acute appetite. The exercise intensity-dependency of an appetite suppression response would appear unquestionable. However, the largely-accepted “intensity threshold” for the effect may be different for different populations and may be governed by activity level or fitness level. The mode of exercise undertaken is an unlikely regulator of any appetite suppression and it would seem probable that both genders respond similarly to acute bouts of exercise, although direct comparisons are desired to confirm these observations. What is less clear is the role of the duration and energy cost of exercise. Further, direct assessment of the effect of differing exercise duration and energy cost of exercise is necessary before these characteristics can be confirmed as regulatory factors or not.

Despite the need for further clarity regarding the effect of different exercise characteristics on post-exercise appetite and food intake in differing populations, it would appear from the extensive literature that a single exercise bout does not result in an acute compensatory increase in appetite and food intake, regardless of the intensity, duration, energy cost, or mode of exercise, or the bodyweight, activity status or gender of the individual.

1.4 Peripheral endocrine signals and regulation of appetite

If exercise does elicit responses in appetite and food intake, the influence of physiological regulatory mechanisms in exercise-induced changes are likely to be of importance. The secretion of hormones from the gastrointestinal tract has long been

identified as an effective peripheral feedback mechanism, allowing activity at the gut, such as the digestion of food or gastric emptying, to be relayed to the central nervous system (CNS). Via direct or indirect action upon the hypothalamus - the predominant site within the CNS for the regulation of appetite - these hormones provide the link between gastrointestinal activity and food intake. Released from a number of organs and tissue, these hormones exhibit their effect via various pathways. These are summarised in diagrammatical form by **figure 1.1**. The arcuate nucleus of the hypothalamus can be considered the command centre of appetite regulation, integrating signals from the periphery and initiating orexigenic and anorexigenic drive via two different subsets of neurons. The orexigenic subset contains the peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP), which initiate the drive to eat. The anorexigenic subset contains the peptides cocaine-and amphetamine-regulated transcript (CART) and proopiomelanocortin (POMC), which signal satiety and promote the cessation of eating. Both the NPY/AgRP and CART/POMC neuron subsets act via common downstream second-order neurons and neuropeptides, largely in the lateral hypothalamic area and the paraventricular nucleus of the hypothalamus (Berthoud, 2002; Zheng and Berthoud, 2008). Signals from the brain stem and spinal cord also contribute to appetite control at the arcuate nucleus, acting via stimulation of the vagus nerve and the nucleus of the solitary tract. (See Williams *et al.*, 2001 and Schwartz *et al.*, 2000 for reviews of the central regulatory pathways of appetite control).

For an in depth review of gastrointestinal hormones and their regulatory effect on appetite, the reader is directed to a number of excellent review articles (Gardiner *et al.*, 2008; Murphy *et al.*, 2006; Wren and Bloom, 2007). This section will focus on the response to acute exercise seen in the circulating concentrations of the hormones ghrelin, polypeptide tyrosine tyrosine (PYY), glucagon-like peptide 1 (GLP-1) and pancreatic polypeptide (PP). These hormones have been shown to exhibit interesting, if not entirely conclusive responses to exercise in the existing literature.

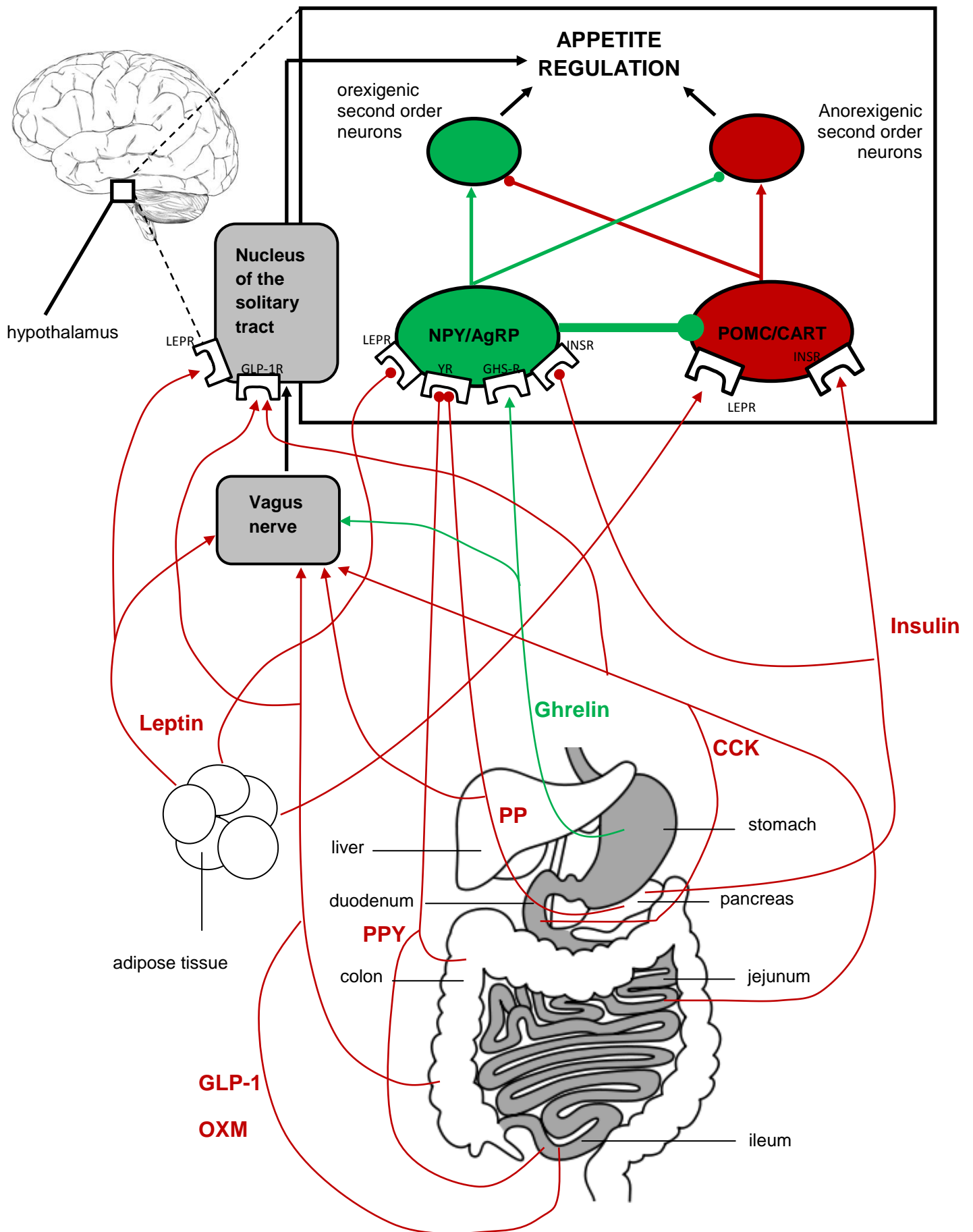


Figure 1.1. The hormonal regulation of appetite. Green colour = orexigenic effect. Red colour = anorexigenic effect. Arrowhead = stimulatory effect. Roundhead = inhibitory effect.

PP = pancreatic polypeptide

PYY = polypeptide tyrosine tyrosine

GLP-1 = glucagon-like peptide 1

OXM = oxyntomodulin

CCK = cholecystokinin

NPY = neuropeptide Y neurons

AgRP = agouti-related protein neurons

POMC = proopiomelanocortin neurons

CART = cocaine- and amphetamine-regulated transcript

LEPR = leptin receptor

GLP-1R = GLP-1 receptor

YR = Y receptors

GHS-R = growth hormone secretagogue receptor

INSR = insulin receptor

1.4.1 The effect of acute exercise on circulating concentrations of appetite-associated hormones

1.4.1.1 Ghrelin

Ghrelin, a hormone secreted predominantly from the stomach has a strong orexigenic effect in humans (Wren *et al.*, 2001). It is unique in that it is the only gut hormone that stimulates appetite. The effect of exercise on plasma ghrelin levels remains unclear, despite widespread investigation. There is substantial data suggesting that ghrelin is unresponsive to acute bouts of exercise (Dall *et al.*, 2002; Kraemer *et al.*, 2004; Schmidt *et al.*, 2004), although an exercise-induced suppression of ghrelin has also been reported (Hagobian *et al.*, 2008).

However, only the acylated form of ghrelin can bind to the growth hormone secretagogue receptor (GHS-R) and cross the blood-brain barrier, where it can exhibit the orexigenic effect on the arcuate nucleus of the hypothalamus via NPY/agouti-related protein (AgRP) neurons (Murphy *et al.*, 2006). Thus, it was deemed necessary to isolate the acylated form and investigate response to exercise. This research has shown differing responses in these different forms (Broom *et al.*, 2009; Broom *et al.*, 2007; Mackelvie *et al.*, 2007; Marzullo *et al.*, 2008; Shiiya *et al.*, 2011).

A decrease in circulating acylated ghrelin with exercise was first observed by Broom and co-workers (Broom *et al.*, 2007). Plasma concentrations were lower immediately after 60 minutes of treadmill running at 75% $\text{VO}_{2\text{max}}$, compared with a resting trial, and area under the curve for acylated ghrelin concentration remained lower during a 2-hour recovery period. Hunger ratings mimicked this response. Marzullo *et al.* (Marzullo *et al.*, 2008) directly assessed differing responses of acylated and total ghrelin to exhaustive incremental exercise. They observed a significant decrease in acylated ghrelin concentration in both lean and overweight individuals, while total ghrelin concentration remained unchanged with exercise. This differential response has been confirmed by others (Shiiya *et al.*, 2011), while a short-lived, transient suppression of acylated ghrelin with high-intensity aerobic exercise has been substantiated by a number of studies (King *et al.*, 2010; King *et al.*, 2011a; King *et*

al., 2011b; Wasse *et al.*, 2012), with suppression not enduring into the early recovery period, post-exercise.

Recently, focus has expanded to measures of a number of episodic, anorexigenic gut hormones. These satiety peptides are released predominantly from the gastrointestinal tract in rapid response to food ingestion. Investigation into the effect of exercise upon the release of a range of such hormones has yielded interesting, if not conclusive findings.

1.4.1.2 Satiety peptides: PYY, GLP-1 and PP

Much of this investigation has focussed on the peptides PYY and GLP-1 as these have both demonstrated strong anorexigenic effects (Batterham *et al.*, 2003). GLP-1, secreted from the small and large intestine, is released into the circulation postprandially and exerts its effects upon the arcuate nucleus of the hypothalamus, acting via the GLP-1 receptor and GLP-1 neurons within the nucleus of the solitary tract (NST) and the vagus nerve (Murphy *et al.*, 2006). Its anorexigenic effects have been demonstrated in humans, with a meta-analysis of the effects of GLP-1 showing that acute administration results in, on average, a 11.7%, (727 kJ) decrease in energy intake, compared with a control administration of saline, with similar results observed in lean and obese individuals (Verdich *et al.*, 2001). PYY is also released from the large and small intestine with concentrations within the circulation peaking after food intake (Batterham *et al.*, 2002). While the mechanism, or mechanisms, for the action of PYY remains a subject of some contention, it is thought that a likely mechanism is the inhibition of NPY/AgRP neurons and subsequent orexigenic pathways (Murphy *et al.*, 2006). While fewer studies have addressed exercise responses in PP, a peptide produced by endothelial cells of the pancreas and released into the circulation postprandially, it too and has been shown to have an anorexigenic effect in humans when administered peripherally (Batterham *et al.*, 2003). The mechanism by which PP exerts this anorexigenic effect is still unknown. It has been postulated that it acts centrally, binding to Y4 and Y5 receptors, with possible direct activation of neurons in the

area postrema, where there is a high density of Y4 receptors. Also, it has been suggested that PP's primary action is to slow gastric emptying (Schmidt *et al.*, 2005), however, there is also evidence that disputes this (Adrian *et al.*, 1981).

Early investigation into appetite responses would suggest that PYY, PP and GLP-1 can all be increased transiently with exercise. Martins and colleagues (Martins *et al.*, 2007) demonstrated elevations in the plasma concentrations of all three hormones after 60 minutes of intermittent cycling (3 x 17 minutes, with 3 minutes recovery, preceded and followed by a 2 minute warm-up and warm-down) at 65% of predicted maximal heart rate. Increases in PYY concentration were short-lived, while increases in PP, and to a lesser extent GLP-1, were maintained into the recovery period. This transient PYY response has been substantiated by others (Broom *et al.*, 2009; King *et al.*, 2011a; Ueda *et al.*, 2009b), while the PP response is in agreement with earlier previous findings (Gingerich *et al.*, 1979; Greenberg *et al.*, 1986; Hilsted *et al.*, 1980) of post-exercise increases in plasma PP after moderate- and low-intensity exercise in healthy males; increases in PP with exercise, unlike exercise-induced suppression of appetite and perturbations in other appetite-associated hormones, would appear not restricted to high-intensity exercise.

Ueda and colleagues (Ueda *et al.*, 2009a) investigated the effect of exercise intensity upon changes in the plasma levels of the anorexigenic hormones PYY₃₋₃₆ (the most abundant form of PYY) and GLP-1. As the post-exercise suppression of appetite phenomenon appears to be exercise intensity dependent, it was necessary to attempt to understand the role that intensity plays in changes in appetite-associated hormones. Ten healthy young males of low physical activity levels completed three trial conditions: high intensity recumbent cycling exercise (75% $\text{VO}_{2\text{max}}$), moderate-intensity recumbent cycling exercise (50% $\text{VO}_{2\text{max}}$) and rest, all of a duration of 30 minutes. Both exercise conditions produced increases in plasma PYY and GLP-1 during exercise (vs. baseline measures and rest condition). While this increase in plasma concentration was independent of exercise intensity for GLP-1, the increase in plasma PYY was greater during high intensity exercise. However, only GLP-1 plasma levels remained elevated when measurements were taken 30

minutes post-exercise. In addition, energy intake at a test meal administered 30 minutes post-exercise, was significantly lower after high- and moderate-intensity exercise compared to the resting condition, with no difference between the two exercise conditions. Correlation analysis showed strong, negative correlations between elevations in plasma GLP-1 and energy intake after both high- and moderate-intensity exercise ($r = -0.893$, $p < 0.001$ and $r = 0.816$, $p = 0.002$ respectively).

These findings suggest that these anorexigenic hormones may be secreted and act via different mechanisms (it must be acknowledged that observed changes in circulating levels of any hormone or metabolite could be a result of changes in rates of appearance or disappearance, or both and that the endocrinological effect of hormones is also dependent on tissue sensitivity as well as delivery). In addition, this brings into question the role of (at least preprandial) PYY concentration in the regulation of energy intake, due to the weak correlation between plasma PYY and energy intake. Strong correlation between GLP-1 and energy intake, and a prolonged GLP-1 suppression, lasting to the time of the test meal would suggest that GLP-1 may play a more powerful post-exercise regulatory role in appetite and food intake.

1.4.1.3 The relationship between appetite-associated hormones and subjective appetite and food intake in the post-exercise period

The role of appetite-associated hormones in regulating post-exercise appetite and food intake is unclear. As well as the aforementioned findings of Ueda *et al.* (Ueda *et al.*, 2009a), a weak coupling between changes in appetite-associated hormone levels and energy intake has been reported by others (Broom *et al.*, 2009; Erdmann *et al.*, 2007; Martins *et al.*, 2007; Wasse *et al.*, 2012). In studies where correlation or regression analysis was conducted, these relationships, between varying hormones and both subjective appetite and energy intake are shown in **table 1.2**. To summarise, significant relationships have been observed between changes in GLP-1 concentration with exercise and corresponding changes in energy intake at an *ad libitum* meal, although this relationship was not observed

for changes in PYY (Ueda *et al.*, 2009a). A lack of a correlation has also been observed between acylated ghrelin and *ad libitum* energy intake (King *et al.*, 2010; King *et al.*, 2011b). Larsen-Meyer *et al.* (Larson-Meyer *et al.*, 2012) showed that GLP-1 and PYY concentration were significant predictors of hunger in runners, post-exercise, but not for walkers. Both total and acylated ghrelin were observed to not be significant predictors. In contrast, Broom and colleagues (Broom *et al.*, 2007) showed a significant positive relationship between acylated ghrelin concentration and hunger immediately post-exercise, yet failed to observe a significant correlation between acylated ghrelin and hunger at any time point in a follow up study (Broom *et al.*, 2009). However, **table 1.2** also highlights the infrequency in which this analysis is carried out in exercise and appetite research. To fully understand the regulatory role of appetite-associated hormones on subjective appetite and food intake post-exercise, correlation or regression analysis should be included in studies of this nature.

Study	Hormone	Relationship with subjective appetite	Relationship with energy intake
(Broom <i>et al.</i> , 2007)	Acylated ghrelin	Immediately post-ex: $r = 0.781$, $p = 0.013^*$. No sig. correl. at any other time post-ex.	-
(Mackelvie <i>et al.</i> , 2007)	Total ghrelin (TG)	Δ hunger vs. Δ TG with ex.: $r = -0.19$	-
	Acylated ghrelin (AG)	Δ hunger vs. Δ AG with ex.: $r = 0.43$, $p = 0.013^*$	-
	Deacylated ghrelin (DG)	Δ hunger vs. Δ DG with ex.: $r = -0.07$	-
(Broom <i>et al.</i> , 2009)	Acylated ghrelin	No significant correlations	-
	PYY	No significant correlations	-
(Ueda <i>et al.</i> , 2009a)	GLP-1		Δ GLP-1 AUC (compared with resting condition) vs. Δ EI: $r = -0.893$, $p < 0.001^*$ (high intensity exercise) $r = -0.816$, $p = 0.002^*$ (moderate intensity exercise).
	PYY		No sig. correlation for Δ PYY AUC (compared with resting condition) vs. Δ EI
(King <i>et al.</i> , 2010)	Acylated ghrelin		No significant correlation between immediate pre-meal acylated ghrelin and <i>ad libitum</i> energy intake at the meal.
(King <i>et al.</i> , 2011b)	Acylated ghrelin		No significant correlation between immediate pre-meal acylated ghrelin and <i>ad libitum</i> energy intake at the meal.

Study	Hormone	Relationship with subjective appetite	Relationship with energy intake
(Larson-Meyer <i>et al.</i> , 2012)	Total ghrelin	B = 0.038, p = 0.45 (runners) B = 0.033, p = 0.22 (walkers)	-
	Acylated ghrelin	B = 0.077, p = 0.60 (runners) B = 0.63, p = 0.12 (walkers)	-
	GLP-1	B = -0.36, p = 0.008* (runners) B = -0.14, p = 0.09 (walkers)	-
	PYY	B = -1.02, p < 0.001* (runners) B = -0.034, p = 0.78 (walkers)	-
(Wasse <i>et al.</i> , 2012)	Acylated ghrelin	No significant correlation with hunger throughout trial.	-

Table 1.2. Relationships between appetite-associated hormones and both subjective appetite and energy intake. B = unstandardised coefficient.

1.5 Methodological issues associated with acute exercise and appetite research

Despite a large body of literature regarding the effect of acute exercise on appetite, food intake and appetite-associated hormones, contention remains. It is possible that the lack of consistency in study findings and the difficulty in comparing and contrasting findings can be attributed to issues associated with methodology. This section will briefly discuss these issues.

1.5.1 Lack of continuity in study design

A vast number of studies have utilised a wide range of study designs, with little inter- and intra-research group continuity. **Table 1.1** shows a number of studies that have investigated the effect of acute bouts of exercise on appetite and food intake. Expressed in the table are detailed study designs and study findings. Looking at the study findings, there is considerable variety and disagreement. When this is the case, it is then necessary to try to decipher the reasons for disagreement, primarily looking at differences in methodologies and critiquing study design. However, due to the lack of continuity in methodologies throughout the literature, this is fraught with difficulty and a reasonable attempt to explain the disagreement in study findings is very tricky.

As a crude example, Ueda *et al* (Ueda *et al.*, 2009b) demonstrated a decrease in energy intake after exercise, Pomerleau and colleagues (Pomerleau *et al.*, 2004) found that exercise increased energy intake, while Imbeault and co-workers (Imbeault *et al.*, 1997) showed that energy intake was unaffected by exercise. However, when we look at the study designs in an attempt to highlight reasons for this disagreement, we find that many aspects of the designs differ. These include: bodyweight-status, activity level and gender of the study population; the intensity, duration and mode of exercise and the techniques used to obtain appetite measures. With such lack of continuity, it is almost unfeasible to attribute the different study findings to any single confounding variable. This makes drawing firm

conclusions from the existing literature very difficult and attempting to formulate hypotheses of mechanisms underpinning observations all but impossible.

Closer inspection of **table 1.1** unveils a number of similar discrepancies in both findings and study design. Such observations highlight the need for a more consistent, systematic approach to the study question, to allow for a true understanding of differing study findings and effects.

1.5.2 Problems with isolating variables

As discussed in 1.3.1, the role of different exercise characteristics in the effect of exercise on appetite responses has yet to be fully established. This is partly due to issues associated with the isolation of variables under investigation. Often, when investigating the effects of different exercise conditions, the intensity of exercise is the primary focus. As discussed in section 1.3.1.2 (p9), the duration and energy expenditure of the exercise bout are not often the variables under investigation. However, the nature of the relationship between intensity, duration and energy expenditure means that it is impossible to isolate just one of these characteristics as an independent variable in a single investigation. Hence, from a single study, it is not possible to completely attribute study findings to the single primary variable that is being investigated. For example, Kissileff *et al.* (Kissileff *et al.*, 1990) found a reduced food intake 15 minutes after high intensity exercise, but not moderate intensity exercise and attributed this difference purely to the difference in exercise intensity. However, as both bouts lasted for 40 minutes, the energy expenditure of the exercise also varied. The differing responses in food intake after continuous moderate-intensity, intermittent hard and intermittent very hard observed by Sim and colleagues (Sim *et al.*, 2013) could also be, to some extent, governed by differences in energy cost as well as intensity. Similarly, studies that controlled for energy expenditure when assessing the influence of different exercise intensity on appetite responses unavoidably have exercise trials of differing duration (Imbeault *et al.*, 1997; Pomerleau *et al.*, 2004).

A solution to this issue is to conduct two separate studies or two separate comparisons within the same study, using the same study design and, ideally, the same participants. **Figure 1.2** shows, in diagrammatical form, an example of this approach, allowing for a thorough investigation of the effect of exercise intensity, duration and energy cost on appetite. With this study design, any effects that persist through both comparisons can be completely attributed to differences in exercise intensity with confidence. A thorough, systematic approach such as this would be a welcomed addition to the extensive existing literature.

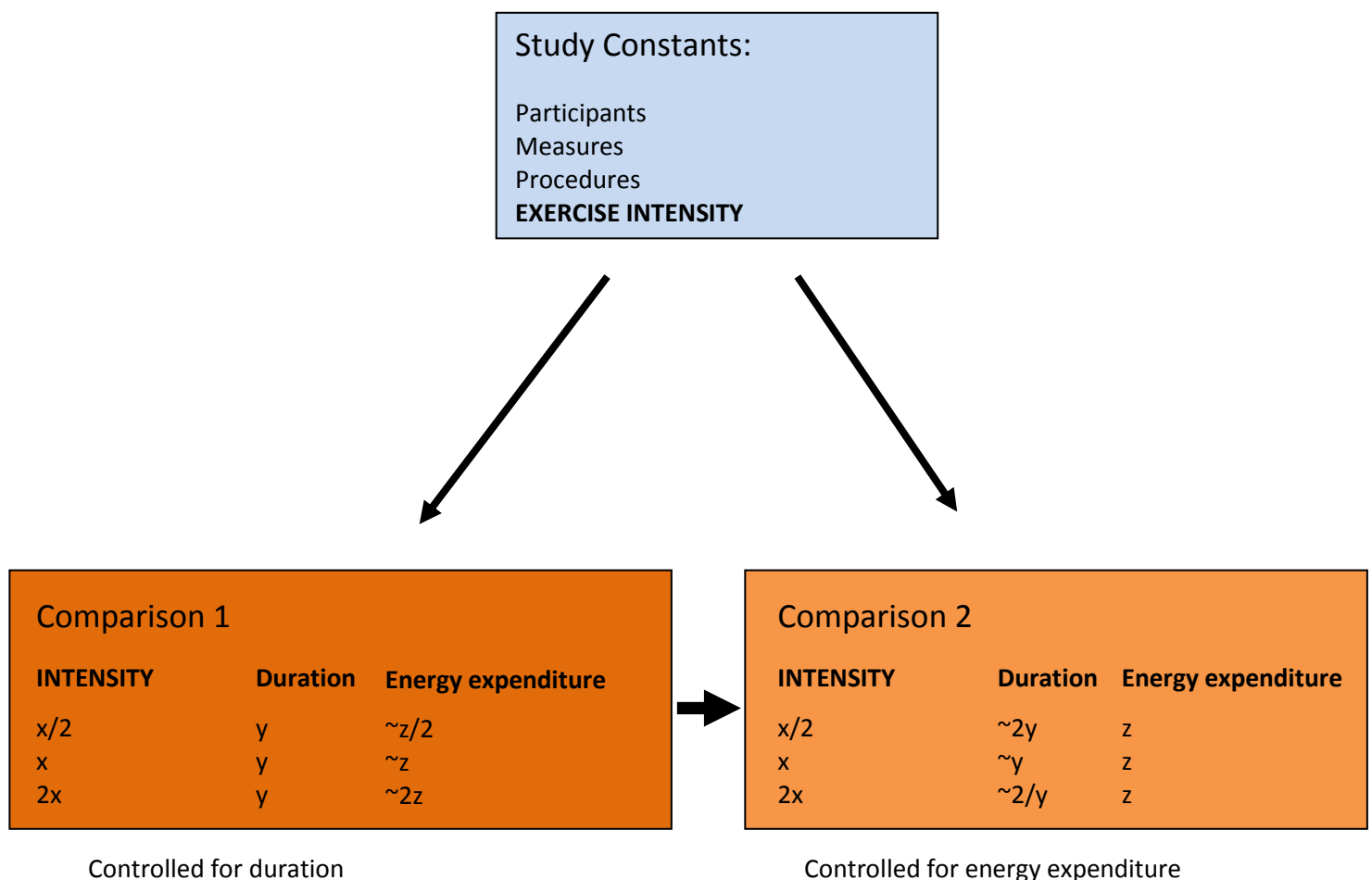


Figure 1.2. Example of progressive, systematic approach to investigating effect of exercise variables on post-exercise appetite responses.

It remains necessary to conduct a number of well-controlled studies, utilising the same study design, in order to address the effect of a number of exercise characteristics on the acute effect of exercise on appetite, food intake and appetite-associated hormones in a range of study populations in a progressive, systematic manner. Such an approach should lead to full elucidation of this study question.

1.5.3 Measurements of appetite and food intake

A number of different measures, using different techniques and tools have been used to address post-exercise appetite. Subjective appetite is usually measured using visual analogue scales to answer questions regarding hunger, fullness, desire to eat and expected intake. The answer is given by making a mark on an ungraded line, typically 100mm or 150mm in length, anchored on either end with extreme answers to the question. A score is denoted by measuring how far along the line the mark is made. These are quick and relatively easy for the participant to complete and are inexpensive to administer. They provide a valid measure of subjective appetite that proves reliable for within-subject, repeated measures and scores correlate with subsequent food intake (Blundell *et al.*, 2010; Flint *et al.*, 2000; Stratton *et al.*, 1998; Stubbs *et al.*, 2000). Visual analogue scales also allow independent repeat measures within close proximity, so are successful at detecting changes in appetite over time, which is commonly an important measure of interest within exercise and appetite research. Test-retest reproducibility of VAS is good (Flint *et al.*, 2000; Stratton *et al.*, 1998), but day-to-day reproducibility has been shown to be weak (Flint *et al.*, 2000; Raben *et al.*, 1995).

However, this method does have a number of limitations. VAS provides only a surrogate of energy intake, exhibiting questionable ability to predict eating behaviour. The correlation between immediately pre-meal VAS score and food intake at an *ad libitum* test meal have been observed to be weak-to-moderate in strength in some instances (Flint *et al.*, 2000; Parker *et al.*, 2004). The between-subject reliability is considered poor (Stubbs *et al.*, 2000). This is likely because the VAS is in effect a measure of *relative* appetite, offering no

indication of absolute appetite or prediction of absolute food intake and not taking into account habitual food intake. For instance, a VAS score for hunger of 130 on a 150mm scale for participant A and participant B would indicate that they are both very hungry. However, consider that participant A is perhaps a large individual who is active and with considerable lean muscle mass (see (Blundell *et al.*, 2012) for the relevance of body composition and lean mass for energy intake) and an unrestrained eater (Lluch *et al.*, 2000), while participant B is of much smaller body mass, with little lean muscle mass and is a restrained eater. The likelihood is that participant A habitually eats more than participant B at a meal and throughout a day. Therefore, despite the same score on the VAS, suggesting the same degree of hunger, there is likely to be a large variation in their respective intakes at an *ad libitum* intake measure.

Other limitations also persist. VAS offers no indication of aspects of eating behaviour such as food preference and likely macronutrient intake. The nature of the measure, with a vague question and line format can be considered a rather abstract assessment of appetite, with it possible that some participants struggle to conceptualise the constructs of hunger and fullness. This may particularly be the case with certain populations, such as children.

Subjective measures of appetite are often accompanied by a more objective measure such as measures of appetite-associated hormones, or proxy measures of eating behaviour, typically in the form of *ad libitum* food intake. An extensive review of these measures and the methodological issues associated was conducted by Stubbs *et al.* (1998) and the reader is directed to this work for a thorough discussion. The current piece will provide a more succinct overview.

These *ad libitum* intake measures can take the form of homogeneous meals, with no aspect of food choice or in the form of buffet-style meals, offering a wide range of food options (see **table 1.1**). The buffet-style option can be considered advantageous due to it allowing for a measure of food preference and macronutrient intake. It also ensures that eating cessation does not occur prematurely due to the participants not being particularly fond of the food offered or due to boredom of consuming the same food. Conversely, buffet-

style meals offer a non-habitual eating environment and style, which may lead to overeating, due to a large degree of food options. It is possible that the more quantitative a measure is, the greater the disruption to normal eating behaviour (Stubbs *et al.*, 1998). Also, the make-up of these test meals across different studies varies dramatically, making comparisons between study findings with regard to energy intake and food choice difficult.

An important limitation of the *ad libitum* test meal for measures of appetite is that it is not possible to take independent repeated measures within close proximity. Prior food intake will always impact upon the eating behaviour exhibited at a later eating episode several hours later. So, changes in appetite over time are not easily measured with any sensitivity. In addition, such test meals are highly labour intensive and can be expensive, especially if a large food choice is provided and food is made available in excess to ensure true *ad libitum* feeding. This can also lead to a large degree of food wastage, which can be considered undesirable or unethical.

With limitations seen with the common methods used for both subjective appetite and food intake measures, it may be considered desirable and prove beneficial to come up with a solution.

1.6 Future directions of research

Future studies should focus on methodological issues, as well as research questions. The methods used for the measure of appetite parameters are not perfect. Seeking an alternative method or methods for the measure of appetite, maintaining the strengths of existing measures but addressing their limitations may prove beneficial for use within exercise and appetite research.

A more systematic, progressive approach to the research area would prove useful, especially for fully elucidating questions for which contention remains. One of these issues is whether duration of exercise and the energy cost of exercise influence appetite and the

response of appetite-associated hormones, as these questions have not been addressed directly. While the effect on exercise intensity of appetite and appetite-associated hormones has been extensively investigated, what remains to be assessed is whether this effect differs with different populations, based on habitual exercise levels. For this reason, it may be of interest to study the appetite response to high-intensity exercise in highly-trained athletic populations. Using such a population will also allow for the completion of a more strenuous exercise bout, with a greater exercise load; it would be of interest to see whether a greater load could augment a post-exercise suppression of appetite and hence result in a greater influence on food intake in the hours after exercise. As well as studies within athletic populations being both useful and of interest, research assessing appetite responses to exercise should investigate responses in the population that the issues of energy balance and weight-management are most clinically relevant: the overweight and obese. Improving the understanding of responses of appetite parameters to manageable, sustainable bouts of exercise in overweight and obese individuals is necessary.

The research of this thesis will attempt to address these issues, with the following primary aims:

1. To attempt to develop and validate a novel tool for the measurement of subjective appetite
2. To investigate the effect of exercise on appetite in athletic populations, and in doing so, assess the degree to which appetite responses to exercise may vary in different populations
3. Shed further light on the influence of exercise characteristics, such as the duration, energy cost and mode of exercise, on appetite responses to exercise.

A secondary aim was to investigate the relationship between changes in appetite-associated hormones and changes in both subjective appetite and *ad libitum* energy intake, with these relationships being indicative of the likely regulatory role of these hormones in post-exercise appetite responses.

1.7 Scope of thesis

This thesis consists of five experimental chapters. Chapter 2 comprises of a General methods section, which includes the findings of pilot testing that preceded the investigation of Chapter 3. Chapter 3 cover the development and validation of a novel tool for the measurement of subjective appetite. Chapters 4 and 5 address the effect of exercise intensity and exercise duration on subjective appetite, food intake and circulating concentrations of appetite-associated hormones in highly trained athletes. An almost identical study design is utilised, simply changing the independent variable in order to ensure study continuity and allow for sound comparisons of findings. Chapter 6 differs slightly by addressing the effect of sprint interval cycling exercise on appetite, food intake and appetite-associated hormones in overweight and obese individuals. Chapter 7 approaches post-exercise feeding from a slightly different perspective and assesses individuals' ability to consciously match energy intake with the energy expenditure of exercise. Finally, Chapter 8 discusses the findings of Chapters 2 to 7. The implications and practical relevance of these findings are addressed and possible future directions of study are proposed.

1.8 References

- Adrian, T.E., Greenberg, G.R., Fitzpatrick, M.L., and Bloom, S.R. (1981). Lack of effect of pancreatic polypeptide in the rate of gastric emptying and gut hormone release during breakfast. *Digestion* 21, 214-218.
- Allender, S., and Rayner, M. (2007). The burden of overweight and obesity-related ill health in the UK. *Obesity Reviews* 8, 467-473.
- Babraj, J., Vollaard, N., Keast, C., Guppy, F., Cottrell, G., and Timmons, J. (2009). Extremely short duration high intensity interval training substantially improves insulin action in young healthy males. *BMC Endocrine Disorders* 9, 3.
- Bartlett, J.D., Hwa Joo, C., Jeong, T.-S., Louhelainen, J., Cochran, A.J., Gibala, M.J., Gregson, W., Close, G.L., Drust, B., and Morton, J.P. (2012). Matched work high-intensity interval and continuous running induce similar increases in PGC-1 α mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. *Journal of Applied Physiology* 112, 1135-1143.
- Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatei, M.A., *et al.* (2002). Gut hormone PYY₃₋₃₆ physiologically inhibits food intake. *Nature* 418, 650-654.
- Berthoud, H.-R. (2002). Multiple neural systems controlling food intake and body weight. *Neuroscience & Biobehavioral Reviews* 26, 393-428.
- Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., Van Der Knaap, H., *et al.* (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity Reviews* 11, 251-270.
- Blundell, J.E., Caudwell, P., Gibbons, C., Hopkins, M., Näslund, E., King, N.A., and Finlayson, G. (2012). Body composition and appetite: fat-free mass (but not fat mass or BMI) is positively associated with self-determined meal size and daily energy intake in humans. *British Journal of Nutrition* 107, 445-449.
- Blundell, J.E., and King, N.A. (1999). Physical activity and regulation of food intake: current evidence. *Medicine & Science in Sports & Exercise* 31(11) *Supplement*, S573.
- Boutcher, S.H. (2011). High-intensity intermittent exercise and fat loss. *Journal of Obesity* 2011.
- Broom, D.R., Batterham, R.L., King, J.A., and Stensel, D.J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 296, R29-R35.
- Broom, D.R., Stensel, D.J., Bishop, N.C., Burns, S.F., and Miyashita, M. (2007). Exercise-induced suppression of acylated ghrelin in humans. *J Appl Physiol* 102, 2165-2171.
- Burgomaster, K.A., Heigenhauser, G.J.F., and Gibala, M.J. (2006). Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. *Journal of Applied Physiology* 100, 2041-2047.
- Burgomaster, K.A., Howarth, K.R., Phillips, S.M., Rakobowchuk, M., MacDonald, M.J., McGee, S.L., and Gibala, M.J. (2008). Similar metabolic adaptations during exercise after

low volume sprint interval and traditional endurance training in humans. *The Journal of Physiology* 586, 151-160.

Burgomaster, K.A., Hughes, S.C., Heigenhauser, G.J.F., Bradwell, S.N., and Gibala, M.J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *Journal of Applied Physiology* 98, 1985-1990.

Burns, S.F., Broom, D.R., Miyashita, M., Mundy, C., and Stensel, D.J. (2007). A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of Sports Sciences* 25, 635-642.

Butland, B., Jebb, S., Kopelman, P., McPherson, K., Thomas, S., Mardell, J., and Parry, V. (2007). Foresight. Tackling obesity: future choices. Project report, pp. 155. <http://www.bis.gov.uk/foresight/our-work/projects/published-projects/tackling-obesity/reports-and-publications>

Carroll, K.K. (1998). Obesity as a risk factor for certain types of cancer. *Lipids* 33, 1055-1059.

Caudwell, P., Hopkins, M., King, N.A., Stubbs, R.J., and Blundell, J.E. (2009). Exercise alone is not enough: weight loss also needs a healthy (Mediterranean) diet? *Public Health Nutr* 12, 1663-1666.

Chan, J.M., Rimm, E.B., Colditz, G.A., Stampfer, M.J., and Willett, W.C. (1994). Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 17, 961-969.

Colditz, G.A., Willett, W.C., Stampfer, M.J., Manson, J.E., Hennekens, C.H., Arky, R.A., and Speizer, F.E. (1990). Weight as a risk factor for clinical diabetes in women. *American Journal of Epidemiology* 132, 501-513.

Curioni, C.C., and Lourenco, P.M. (2005). Long-term weight loss after diet and exercise: a systematic review. *Int J Obes Relat Metab Disord* 29, 1168-1174.

Dall, R., Kanaley, J., Hansen, T., Moller, N., Christiansen, J., Hosoda, H., Kangawa, K., and Jorgensen, J. (2002). Plasma ghrelin levels during exercise in healthy subjects and in growth hormone-deficient patients. *Eur J Endocrinol* 147, 65-70.

Deighton, K., Barry, R., Connon, C., and Stensel, D. (2012). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*, 1-10.

Deighton, K., Karra, E., Batterham, R.L., and Stensel, D.J. (2013). Appetite, energy intake and PYY₃₋₃₆ responses to energy-matched continuous exercise and submaximal high intensity exercise. *Applied Physiology, Nutrition, and Metabolism*.

Dodd, C.J., Welsman, J.R., and Armstrong, N. (2008). Energy intake and appetite following exercise in lean and overweight girls. *Appetite* 51, 482-488.

Donnelly, J.E., Blair, S.N., Jakicic, J.M., Manore, M.M., Rankin, J.W., Smith, B.K., and null (2009). American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Medicine and science in sports and exercise* 41, 459-471.

Edholm, O.G., Adam, J.M., Healy, M.J.R., Wolff, H.S., Goldsmith, R., and Best, T.W. (1970). Food intake and energy expenditure of army recruits. *British Journal of Nutrition* 24, 1091-1107.

Erdmann, J., Tahbaz, R., Lippl, F., Wagenpfeil, S., and Schusdziarra, V. (2007). Plasma ghrelin levels during exercise -- Effects of intensity and duration. *Regulatory Peptides* 143, 127-135.

Finucane, M.M., Stevens, G.A., Cowan, M.J., Danaei, G., Lin, J.K., Paciorek, C.J., Singh, G.M., Gutierrez, H.R., Lu, Y., Bahalim, A.N., *et al.* (2011). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *The Lancet* 377, 557-567.

Flint, A., Raben, A., Blundell, J.E., and Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 24, 38-48.

Gardiner, J.V., Jayasena, C.N., and Bloom, S.R. (2008). Gut Hormones: A Weight Off Your Mind. *Journal of Neuroendocrinology* 20, 834-841.

George, V.A., and Morganstein, A. (2003). Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite* 40, 43-46.

Gingerich, R.L., Hickson, R.C., Hagberg, J.M., and Winder, W.W. (1979). Effect of endurance exercise training on plasma pancreatic polypeptide concentration during exercise. *Metabolism* 28, 1179-1182.

Greenberg, G.R., Marliss, E.B., and Zinman, B. (1986). Effect of exercise on the pancreatic polypeptide response to food in man. *Horm Metab Res* 18, 194,196.

Hagobian, T.A., Sharoff, C.G., and Braun, B. (2008). Effects of short-term exercise and energy surplus on hormones related to regulation of energy balance. *Metabolism* 57, 393-398.

Health and Social Care Information Centre (2013). Health Survey for England. Statistics on Obesity, Physical Activity and Diet – England, 2013. <http://www.hscic.gov.uk/searchcatalogue?productid=11194&q=obesity&sort=Relevance&size=10&page=1#top>

Heden, T.D., Liu, Y., Park, Y., Dellsperger, K.C., and Kanaley, J.A. (2013). Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology*.

Helgerud, J., Hoydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., Simonsen, T., Helgesen, C., Hjorth, N., Bach, R., and Hoff, J. (2007). Aerobic high-intensity intervals improve vo2max more than moderate training. *Medicine & Science in Sports & Exercise* 39, 665-671.

Holmstrup, M.E., Fairchild, T.J., Keslacy, S., Weinstock, R.S., and Kanaley, J.A. (2013). Satiety, but not total PYY, Is increased with continuous and intermittent exercise. *Obesity, n/a-n/a*.

Hopkins, M., King, N.A., and Blundell, J.E. (2010). Acute and long-term effects of exercise on appetite control: Is there any benefit for weight control? *Current Opinion in Clinical Nutrition and Metabolic Care* 13, 635-640.

Hubert, H.B., Feinleib, M., McNamara, P.M., and Castelli, W.P. (1983). Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 67, 968-977.

Hubert, P., King, N.A., and Blundell, J.E. (1998). Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity. *Appetite* 31, 9-19.

Imbeault, P., Saint-Pierre, S., Almeras, N., and Tremblay, A. (1997). Acute effects of exercise on energy intake and feeding behaviour. *British Journal of Nutrition* 77, 511-521.

Jakicic, J.M., Clark, K., Coleman, E., Donnelly, J.E., Foreyt, J., Melanson, E., Volek, J., Volpe, S.L., and null (2001). American College of Sports Medicine position stand. Appropriate intervention strategies for weight loss and prevention of weight regain for adults. *Medicine and science in sports and exercise* 33, 2145-2156.

Johnstone, A.M., Faber, P., Gibney, E.R., Elia, M., Horgan, G., Golden, B.E., and Stubbs, R.J. (2002). Effect of an acute fast on energy compensation and feeding behaviour in lean men and women. *Int J Obes Relat Metab Disord* 26, 1623-1628.

Kawano, H., Mineta, M., Asaka, M., Miyashita, M., Numao, S., Gando, Y., Ando, T., Sakamoto, S., and Higuchi, M. (2013). Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite* 66, 26-33.

King, J.A., Miyashita, M., Wasse, L.K., and Stensel, D.J. (2010). Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* 54, 492-498.

King, J.A., Wasse, L.K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R.L., and Stensel, D.J. (2011a). Differential acylated ghrelin, peptide YY₃₋₃₆, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology & Metabolism* 96, 1114-1121.

King, J.A., Wasse, L.K., and Stensel, D.J. (2011b). The acute effects of swimming on appetite, food intake, and plasma acylated ghrelin. *Journal of Obesity* 2011.

King, N.A. (1999). What processes are involved in the appetite response to moderate increases in exercise-induced energy expenditure? *Proceedings of the Nutrition Society* 58, 107-113.

King, N.A., Burley, V.J., and Blundell, J.E. (1994). Exercise induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition* 48, 715-724

King, N.A., Lluch, A., Stubbs, R.J., and Blundell, J.E. (1997a). High dose exercise does not increase hunger or energy intake in free living males. *Eur J Clin Nutr* 51, 478-483.

King, N.A., Snell, L., Smith, R.D., and Blundell, J.E. (1996). Effects of short-term exercise on appetite responses in unrestrained females. *European Journal of Clinical Nutrition* 50, 663-667.

- King, N.A., Tremblay, A., and Blundell, J.E. (1997b). Effects of exercise on appetite control: implications for energy balance. *Medicine & Science in Sports & Exercise* 29, 1076-1089.
- Kissileff, H.R., Pi-Sunyer, F.X., Segal, K., Meltzer, S., and Foelsch, P.A. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition* 52, 240-245.
- Kraemer, R.R., Durand, R.J., Acevedo, E.O., Johnson, L.G., Kraemer, G.R., Hebert, E.P., and Castracane, V.D. (2004). Rigorous running increases growth hormone and insulin-like growth factor-i without altering ghrelin. *Experimental Biology and Medicine* 229, 240-246.
- Larson-Meyer, D.E., Palm, S., Bansal, A., Austin, K.J., Hart, A.M., and Alexander, B.M. (2012). Influence of running and walking on hormonal regulators of appetite in women. *Journal of Obesity* 2012, 15.
- Little, J.P., Gillen, J.B., Percival, M.E., Safdar, A., Tarnopolsky, M.A., Punthakee, Z., Jung, M.E., and Gibala, M.J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *Journal of Applied Physiology* 111, 1554-1560.
- Lluch, A., King, N.A., and Blundell, J.E. (2000). No energy compensation at the meal following exercise in dietary restrained and unrestrained women. *British Journal of Nutrition* 84, 219-225.
- Mackelvie, K.J., Meneilly, G.S., Elahi, D., Wong, A.C.K., Barr, S.I., and Chanoine, J.-P. (2007). Regulation of appetite in lean and obese adolescents after exercise: role of acylated and desacyl ghrelin. *J Clin Endocrinol Metab* 92, 648-654.
- Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 193, 251-258.
- Marzullo, P., Salvadori, A., Brunani, A., Verti, B., Walker, G.E., Fanari, P., Tovaglieri, I., Medici, C.D., Savia, G., and Liuzzi, A. (2008). Acylated ghrelin decreases during acute exercise in the lean and obese state. *Clinical Endocrinology* 69, 970-971.
- Murphy, K.G., Dhillo, W.S., and Bloom, S.R. (2006). Gut peptides in the regulation of food intake and energy homeostasis. *Endocr Rev* 27, 719-727.
- Oh, H., and Taylor, A.H. (2012). Brisk walking reduces *ad libitum* snacking in regular chocolate eaters during a workplace simulation. *Appetite* 58, 387-392.
- Onyike, C.U., Crum, R.M., Lee, H.B., Lyketsos, C.G., and Eaton, W.W. (2003). Is obesity associated with major depression? Results from the third national health and nutrition examination survey. *American Journal of Epidemiology* 158, 1139-1147.
- Parker, B.A., Sturm, K., MacIntosh, C.G., Feinle, C., Horowitz, M., and Chapman, I.M. (2004). Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr* 58, 212-218.
- Poehlman, E.T., and Horton, E.S. (1989). The impact of food intake and exercise on energy expenditure. *Nutrition Reviews* 47, 129-137.
- Pomerleau, M., Imbeault, P., Parker, T., and Doucet, E. (2004). Effects of exercise intensity on food intake and appetite in women. *Am J Clin Nutr* 80, 1230-1236.

Prospective Studies, C. (2009). Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *The Lancet* 373, 1083-1096.

Raben, A., Tagliabue, A., and Astrup, A. (1995). The reproducibility of subjective appetite scores. *The British journal of nutrition* 73, 517-530.

Schmidt, A., Maier, C., Schaller, G., Nowotny, P., Bayerle-Eder, M., Buranyi, B., Luger, A., and Wolzt, M. (2004). Acute exercise has no effect on ghrelin plasma concentrations. *Horm Metab Res* 36, 174-177.

Schmidt, P.T., Näslund, E., Grybäck, P., Jacobsson, H., Holst, J.J., Hilsted, L., and Hellström, P.M. (2005). A role for pancreatic polypeptide in the regulation of gastric emptying and short-term metabolic control. *Journal of Clinical Endocrinology & Metabolism* 90, 5241-5246.

Schubert, M.M., Desbrow, B., Sabapathy, S., and Leveritt, M. (2013). Acute exercise and subsequent energy intake. A meta-analysis. *Appetite* 63, 92-104.

Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., and Baskin, D.G. (2000). Central nervous system control of food intake. *Nature* 404, 661-671.

Shiia, T., Ueno, H., Toshinai, K., Kawagoe, T., Naito, S., Tobina, T., Nishida, Y., Shindo, M., Kangawa, K., Tanaka, H., and Nakazato, M. (2011). Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocrine journal* 58, 335-342.

Sim, A.Y., Wallman, K.E., Fairchild, T.J., and Guelfi, K.J. (2013). High-intensity intermittent exercise attenuates *ad-libitum* energy intake. *International Journal of Obesity advance online publication*.

Simon, G.E., Von Korff, M., Saunders, K., Miglioretti, D.L., Crane, P.K., van Belle, G., and Kessler, R.C. (2006). Association between obesity and psychiatric disorders in the US adult population. *Archives of general psychiatry* 63, 824-830.

Staten, M.A. (1991). The effect of exercise on food intake in men and women. *American Journal of Clinical Nutrition* 53, 27-31.

Stratton, R.J., Stubbs, R.J., Hughes, D., King, N., Blundell, J.E., and Elia, M. (1998). Comparison of the traditional paper visual analogue scale questionnaire with an Apple Newton electronic appetite rating system (EARS) in free living subjects feeding *ad libitum*. *European Journal of Clinical Nutrition* 52, 737-741.

Stubbs, R.J. (1998). Nutrition Society Medal Lecture. Appetite, feeding behaviour and energy balance in human subjects. *Proc Nutr Soc* 57, 341-356.

Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., and Blundell, J.E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* 84, 405-415.

Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Whybrow, S., Horgan, G.W., King, N., and Blundell, J. (2004). Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. *Am J Physiol Regul Integr Comp Physiol* 286, R350-358.

Stubbs, R.J., Johnstone, A.M., O'Reilly, L.M., and Poppitt, S.D. (1998). Methodological issues relating to the measurement of food, energy and nutrient intake in human laboratory-based studies. *Proc Nutr Soc* 57, 357-372.

Stubbs, R.J., Sepp, A., Hughes, D.A., Johnstone, A.M., Horgan, G.W., King, N., and Blundell, J. (2002a). The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. *Eur J Clin Nutr* 56, 129-140.

Stubbs, R.J., Sepp, A., Hughes, D.A., Johnstone, A.M., King, N., Horgan, G., and Blundell, J.E. (2002b). The effect of graded levels of exercise on energy intake and balance in free-living women. *Int J Obes Relat Metab Disord* 26, 866-869.

Suk, S.-H., Sacco, R.L., Boden-Albala, B., Cheun, J.F., Pittman, J.G., Elkind, M.S., and Paik, M.C. (2003). Abdominal obesity and risk of ischemic stroke: The Northern Manhattan Stroke Study. *Stroke* 34, 1586-1592.

Thompson, D.A., Wolfe, L.A., and Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medicine & Science in Sports & Exercise* 20, 222-227.

Trapp, E.G., Chisholm, D.J., Freund, J., and Boutcher, S.H. (2008). The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *Int J Obes* 32, 684-691.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., and Fujimoto, S. (2009a). Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J Endocrinol* 203, 357-364.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., and Fujimoto, S. (2009b). Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J Endocrinol*, JOE-08-0500.

Unick, J.L., Otto, A.D., Goodpaster, B.H., Helsel, D.L., Pellegrini, C.A., and Jakicic, J.M. (2010). Acute effect of walking on energy intake in overweight/obese women. *Appetite* 55, 413-419.

Verdich, C., Flint, A., Gutzwiller, J.-P., Naslund, E., Beglinger, C., Hellstrom, P.M., Long, S.J., Morgan, L.M., Holst, J.J., and Astrup, A. (2001). A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on *ad libitum* energy intake in humans. *J Clin Endocrinol Metab* 86, 4382-4389.

Wang, Y.C., McPherson, K., Marsh, T., Gortmaker, S.L., and Brown, M. (2011). Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet* 378, 815-825.

Wasse, L.K., Sunderland, C., King, J.A., Miyashita, M., and Stensel, D.J. (2012). The influence of vigorous running and cycling exercise on hunger perceptions and plasma acylated ghrelin concentrations in lean young men. *Applied Physiology, Nutrition, and Metabolism* 38, 1-6.

Westerterp-Plantenga, M.S., Verwegen, C.R., Ijeda, M.J., Wijckmans, N.E., and Saris, W.H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* 62, 1345-1354.

Whybrow, S., Hughes, D.A., Ritz, P., Johnstone, A.M., Horgan, G.W., King, N., Blundell, J.E., and Stubbs, R.J. (2008). The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding *ad libitum*. *Br J Nutr* 100, 1109-1115.

Whyte, L.J., Gill, J.M.R., and Cathcart, A.J. (2010). Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. *Metabolism* 59, 1421-1428.

Williams, G., Bing, C., Cai, X.J., Harrold, J.A., King, P.J., and Liu, X.H. (2001). The hypothalamus and the control of energy homeostasis: Different circuits, different purposes. *Physiology & Behavior* 74, 683-701.

Woo, R., Garrow, J., and Pi-Sunyer, F. (1982). Effect of exercise on spontaneous calorie intake in obesity. *Am J Clin Nutr* 36, 470-477.

Wren, A.M., and Bloom, S.R. (2007). Gut hormones and appetite control. *Gastroenterology* 132, 2116-2130.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillon, W.S., Ghatei, M.A., and Bloom, S.R. (2001). Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 86, 5992-.

Zheng, H., and Berthoud, H.-R. (2008). Neural systems controlling the drive to eat: mind versus metabolism. *Physiology* 23, 75-83.

General methods

This section will firstly introduce developmental and pilot investigation that preceded the work of Chapter 3. Secondly, methods that are common to experimental Chapters 4, 5 and 6 will be described. Deviations from these general methods, along with specific protocol and procedures, will be described in the individual chapters. The methods implemented in Chapter 7 will be described within Chapter 7 itself.

2.1 Pilot testing: The development of The Birmingham Photographic Appetite Rating.

2.1.1 INTRODUCTION

With the most recent figures showing that 65% of adult males and 58% of adult females in England are now overweight or obese (Health and Social Care Information Centre, 2013), the need for a greater understanding of energy balance and weight management is necessary. In an attempt to address the “energy in” side of the energy balance equation, the regulation of appetite has been extensively investigated (the reader is directed to a number of comprehensive review articles (Bellisle, 1999; King *et al.*, 2009; King *et al.*, 1997; Martins *et al.*, 2008). In conducting such research, a wide range of techniques have been utilised to obtain both subjective and objective measures of appetite and measures of eating behaviour.

The strengths and limitations of some of these techniques have been discussed previously (General introduction, section 1.5.3, p35) and will again be discussed in greater depth in the following chapter (section 3.2, p83). Briefly, measures of subjective appetite are commonly obtained with the use of visual analogue scales (VAS) (Blundell *et al.*, 2010), with these measures often used in conjunction with objective appetite measure, such as measures of appetite-associated hormones and surrogates of eating behaviour, usually in the form of a food intake or energy intake measure. Typically, *ad libitum* energy intake is measured from buffet-style or constant composition test meals. However, both the VAS and *ad libitum* intake methods are not without limitation. The VAS approach does not provide any indication of desired portion size or food choice and, therefore, important aspects of eating behaviour are neither assessed nor predicted. The *ad libitum* food intake technique provides a valid quantitative proxy of eating behaviour, but does not allow for independent, repeated measures of appetite within a short time period. Hence, with this approach alone, it is not possible to monitor time course changes in appetite, which is often an important interest of

researchers. Further, the *ad libitum* intake method can be expensive, time-consuming and labour-intensive, and can result in a large degree of food wastage. Due to these limitations, an investigation into the possibility of developing a new tool and technique for the measure of appetite was undertaken. An attempt was made to tackle the limitations of the VAS and *ad libitum* intake methods, while maintaining the desirable attributes of both. The concept of food photography was used to create a tool that would allow for independent, repeated measures of subjective appetite, while allowing for an indication of desired food choice and a prediction of portion size selection.

The purpose of this exploratory, pilot investigation was to develop and assess the validity of a novel photographic images tool for the measurement of subjective appetite – The Birmingham Photographic Appetite Rating (BPAR) – with respect to commonly used, validated measures of subjective appetite and eating behaviour. This was achieved by attempting to manipulate appetite state through acutely controlling diet from breakfast until an *ad libitum* lunch meal, obtaining appetite measures throughout. It was hypothesised that the BPAR score would correlate strongly with VAS scores, producing a very similar appetite profile. Further, we hypothesised that the BPAR score would correlate with *ad libitum* food intake from a constant composition test meal, to a greater extent than VAS.

2.1.2 METHOD

2.1.2.1 Participants

Five recreationally active participants (3 female, 2 male; mean age 23 ± 3 years) were recruited from The School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. Those suffering from illness such as cold or flu, those taking medication that was likely to affect appetite or that needed to be taken with food more frequently than once a day, those with food allergies and those suffering from diabetes were excluded from taking part. Ethical approval was obtained from the Ethics Subcommittee of the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham.

2.1.2.2 Study design

A within-subject, randomised crossover study design was utilised. Participants were randomly assigned to each of the three experimental conditions; fasted (FAST), post-absorptive (POST-AB) and fed (FED) states.

2.1.2.3 Procedure & protocol

Participants arrived at the Exercise Metabolism Laboratory within the School of Sport, Exercise and Rehabilitation Science, University of Birmingham between 07.00 and 09.00, after a ten-hour overnight fast. Upon arrival at the laboratory for the first time, a written consent form was signed (Appendix 1), prior to the completion of a health questionnaire (Appendix 2). Participants were then randomly allocated to one of the three trial conditions; fasted state, post-absorptive state or fed state. Trials were separated by a minimum of 3 days. The study protocol for each condition is shown in diagrammatical form (**figure 2.1**). Between feeding and between the taking of appetite measures, participants remained sedentary, free to read, watch television or work at a computer.

Prior to the first trial, participants were supplied with a food diary and asked to complete the diary on the day before their first trial. They were then asked to replicate this diet on the day prior to each subsequent trial.

2.1.2.4 Breakfast meal

The standardised breakfast meal consisted of cereal (40g) with semi-skimmed milk (125ml), toast (2 medium slices of white bread) with margarine (10g) and jam (12g) and pure orange or apple juice (200ml). This breakfast provided approximately 506 kcal. A choice of two cereals, with similar energy density and macronutrient content (Kellogg's cornflakes (all per 100g): 372 kcal, 84g CHO, 0.9g fat, 7g protein or Kellogg's rice krispies (all per 100g): 383 kcal, 87g CHO, 1g fat, 6g protein) was provided to allow for individual preferences and dislikes. The meal was consumed within 15 minutes. In the FED condition, three snack items were administered at 2, 3 and 4 hours. These were, in order, a 50g flapjack bar (Sainsbury's, 223 kcal), a 25g cereal bar (oat and raisin, Sainsbury's, 98 kcal) and a medium sized banana (~152g, ~98 kcal). This provided an additional 419 kcal (67.1g carbohydrate, 13.9g fat, 5.4g protein, 6.6g fibre), resulting in a total intake in the FED condition of 1182 (kcal, 200.6g carbohydrate, 29.2g fat, 27.9g protein, 17.2g fibre). In FAST participants were provided with neither breakfast nor snacks, remaining fasted until the *ad libitum* lunch meal.

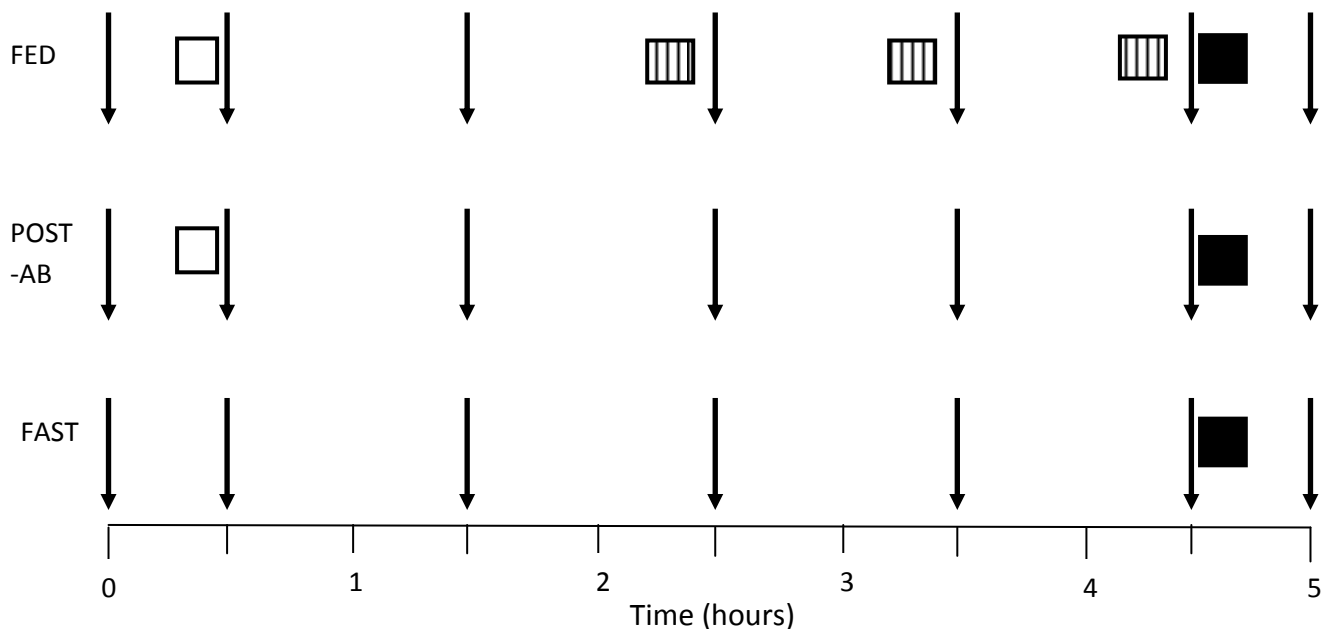


Figure 2.1. Study protocol. Arrow = appetite measure (VAS and BPAR). Clear squares = breakfast. Striped rectangle = snack. Black square = *ad libitum* lunch meal.

2.1.2.5 Measures

Subjective appetite was measured using the BPAR and the widely used VAS technique. The BPAR is a computer programme test, in which the participant views photographic images of 20 different food items (See Appendix 3 for list of food items, with macronutrient information). Each food item is shown, using a continuum of many images, with varying portion sizes. The portion size is altered by the participant using a sliding bar scale, controlled by use of the computer mouse. The participant is asked, “If you were to consume a meal at this moment, consisting of only the food item shown, how much would you expect to eat in order to feel satisfyingly full? Move the sliding bar to select the appropriate portion size.” The participant follows this instruction, selects the desired portion size and moves on to the next food item and repeats the procedure for all 20 food items (See **figure 2.2** for an example of the BPAR in use). The number of images for each food item typically ranged from 10 to 40, allowing for a high resolution. The participant was asked to complete the trial within four minutes. Once completed, the result was analysed and the investigator was able to see which portion size was selected and what number photograph this selection corresponded to. As the weight of food in each photograph was known and recorded, as was the nutritional information, it was possible to calculate the energy content (kcal) of each food item selection, along with macronutrient content. A mean energy content and mean macronutrient content was calculated from all food items rated. Although this information alone is somewhat arbitrary, indicators of changes in macronutrient preference were provided.

Results were derived with three of the 20 food items in the BPAR (porridge, tomato soup and strawberry yoghurt) excluded from the analysis. This was because it was felt that the size of the bowl that they were displayed in was not perceived accurately and therefore portion size was also not accurately perceived. This led to extreme portion selections for these food items. The amorphous nature of the food items is likely to have contributed to the poor perception of portion size, as this has been seen previously with other estimations of

portion size when food is displayed (Weber *et al.*, 1999) and in techniques whereby photographic images of food were used (Venter *et al.*, 2000).

The VAS test consisted of the participant being asked four questions relating to their subjective appetite, based on perception of “hunger,” “fullness,” “desire to eat,” and “expected food intake.” For each question, a 150mm line was presented, anchored at each end by the two extreme answers (for example, the question, “how hungry do you feel at the present moment?” had a line anchored with “the hungriest I have ever been” and “not hungry at all”). The participant was asked to make a mark on this line using a pen. The distance along the line that the mark was made was measured by the investigator using a ruler, with this measure denoting a score for this question. A total VAS test score was calculated as, hunger score + desire score + expected intake score + (150-fullness score). It was decided to use this single score for ease of data analysis and presentation. It has been shown that, with the original 6 question VAS technique of Hill & Blundell (Hill and Blundell, 1982), the scores for each question co-vary to a large extent (Stubbs *et al.*, 2000) and that the first principal component of the questions is the mean value of the scores (Reid, *et al.*, 1998).

Energy intake was measured with the use of an *ad libitum* test meal of pasta and sauce. A choice of two sauces was available, to allow for individual preference. Both sauces had similar nutritional content (Bolognese sauce – 80 kcal•100g⁻¹; Carbonara sauce – 85 kcal•100g⁻¹) and the same sauce was used for all three trials. Participants were accompanied to the Research Kitchen within the School of Sport, Exercise and Rehabilitation Sciences, where they were provided with a large bowl of cooked penne pasta and a separate bowl of sauce, along with an empty plate. They were instructed to serve the pasta and sauce onto the plate as desired and eat as much as they wanted, until they felt satisfyingly full. They were also told that further pasta and sauce were available, should they empty either bowl. The content of each bowl was weighed prior to the meal commencing and again at the cessation of eating; the difference between the two indicating the amount consumed. Subtracted from this was food left remaining on the plate, which was also

weighed before and after the meal. Energy density of the pasta and sauce were known, allowing for the calculation of energy intake.

2.1.2.6 Statistical analysis


The mean energy intake values of the test meal for each condition were compared using a one-way, repeated measures ANOVA. To test for sensitivity to change in appetite, appetite scores from the BPAR and the VAS were both assessed using a 3 (condition: FAST, POST-AB, FED) x 7 (time: 0, 30, 90, 150, 210, 270, 300) factorial, repeated-measures ANOVA. Significant main effects and interactions from ANOVA were further assessed by pairwise comparisons using Bonferroni post-hoc analysis. Also, appetite scores from the BPAR were compared with VAS test scores, with the use of Pearson product moment correlation analysis. This was done for within-condition, between-subject comparisons and for between-condition, within-subject comparison, by assessing percentage difference between the conditions (FAST – FED, FAST – POST-AB and POST-AB – FED).

To assess the ability to predict between-subject differences in energy intake, within-condition correlations of appetite scores and energy intake at the test meal were conducted, for both VAS and BPAR scores. The ability to predict within-subject differences in energy intake with different dietary manipulations was assessed by correlating between-condition percentage changes (FAST – FED; FAST – POST-AB; POST-AB – FED) in VAS and BPAR scores immediately prior to the test meal with percentage changes in energy intake separately for each participant. This within-subject analysis was conducted, as it was felt that an appetite tool should be efficacious at detecting changes in appetite that correspond with changes in energy intake for an individual; as an intervention causes an increase in energy intake for a person, so should it cause a similar increase in appetite score. Differences in correlation coefficients were investigated for statistical significance using t-tests for non-independent correlation coefficients.

A statistical significance level of $p < 0.05$ was used for all analyses of variance and for correlation analysis. All statistical analysis was carried out using the SPSS software programme (SPSS inc., Chicago, Illinois, USA).

Photographic Stimuli Test of Subjective Appetite

If you were to consume a meal at this moment, consisting of this food item alone, how much would you expect to eat in order to feel satisfyingly full?
Move the sliding bar scale to select the appropriate portion size.
When you have selected your desired portion size, click "next/finish."




Chicken

Next

Photographic Stimuli Test of Subjective Appetite

If you were to consume a meal at this moment, consisting of this food item alone, how much would you expect to eat in order to feel satisfyingly full?
Move the sliding bar scale to select the appropriate portion size.
When you have selected your desired portion size, click "next/finish."



Chicken

Next

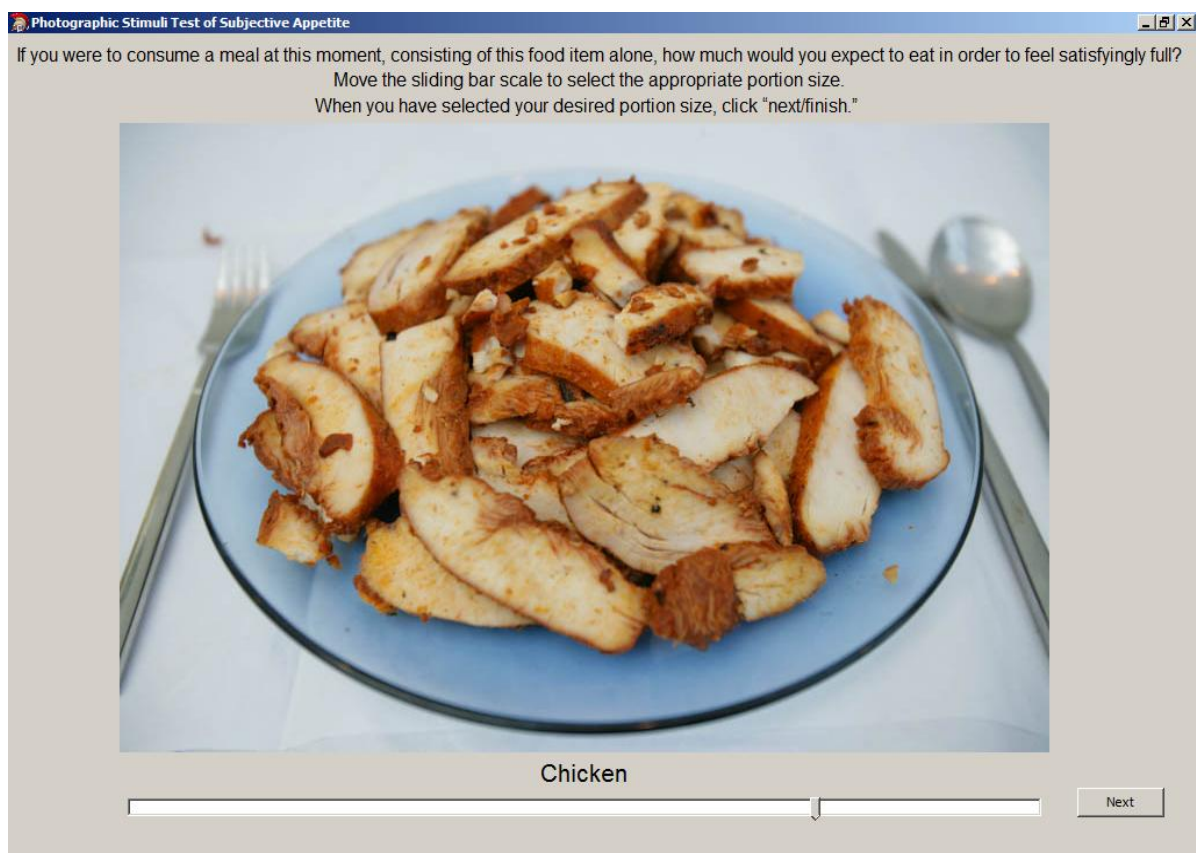
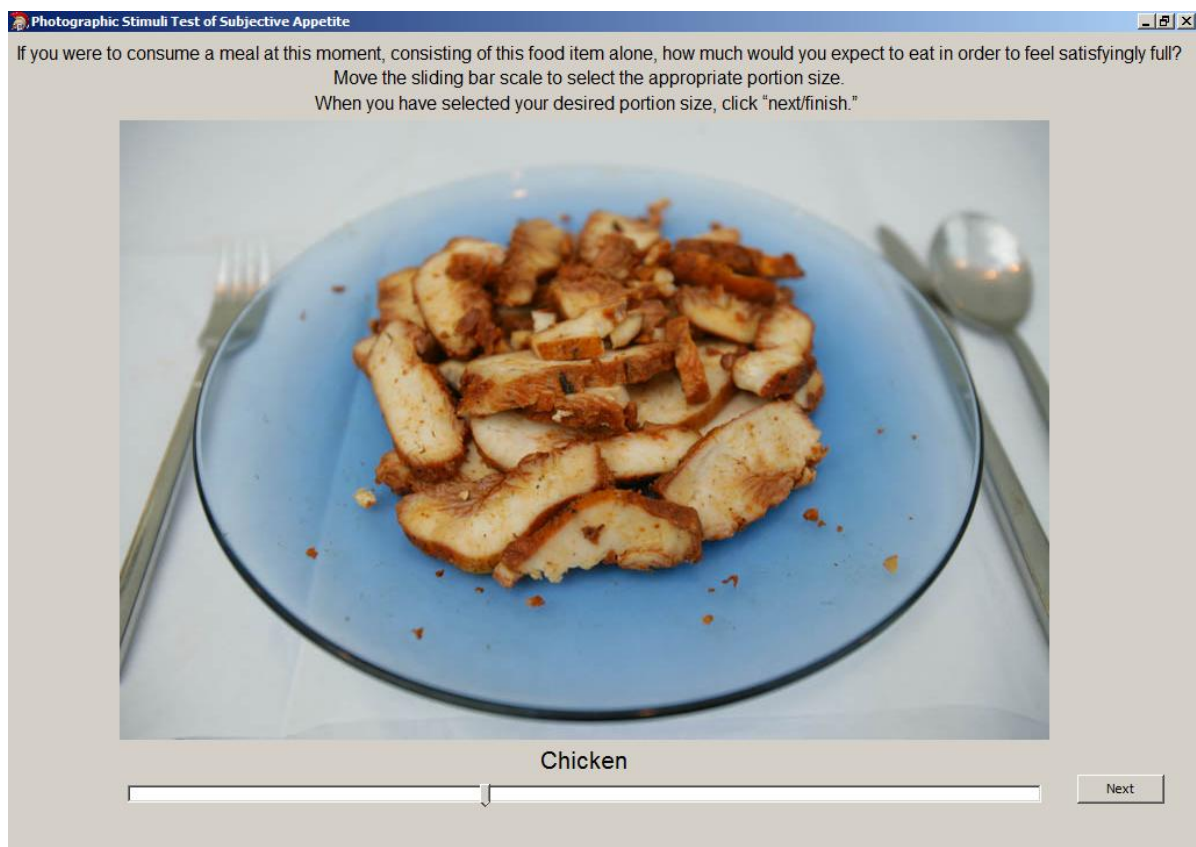


Figure 2.2. Example of BPAR. Figure shows progressive portions of chicken.

2.1.3 RESULTS

2.1.3.1 Energy intake at the test meal

Figure 2.3 shows the mean energy intake values for each of the three trial conditions. The results of a one-way ANOVA with repeated measures, shows that there was a significant effect of condition on energy intake at the test meal ($F(1) = 16.660$, $p = 0.015$). Post-hoc pairwise comparisons, highlighted that the energy intake in FED (374 ± 118 kcal) was significantly lower than during the POST-AB (723 ± 131 kcal, $p = 0.022$) and FAST (757 ± 316 kcal, $p = 0.045$) conditions.

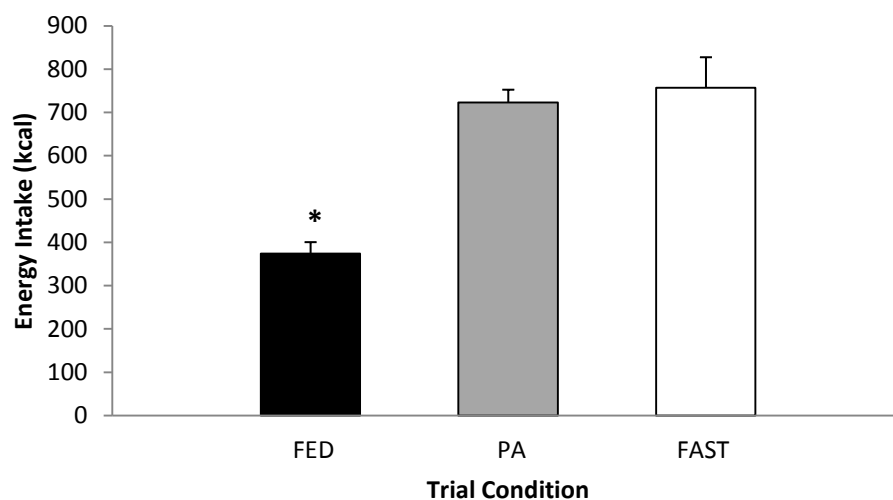


Figure 2.3 – Energy intake at the test meal. Values are mean \pm SEM. * = significantly different from other conditions.

2.1.3.2 Subjective appetite scores

2.1.3.2.1 VAS

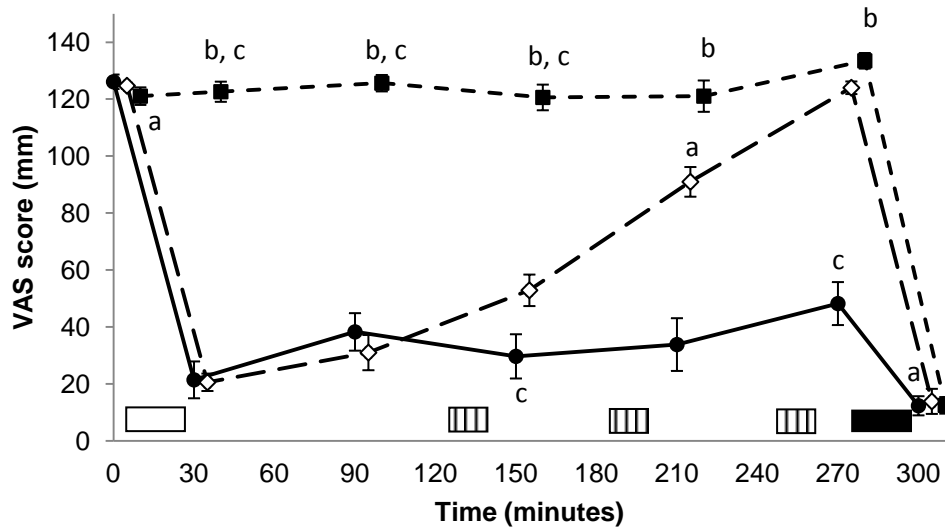
There were no significant differences in VAS scores at baseline. There was a significant condition \times time interaction for VAS score ($F(12) = 22.920$, $p < 0.001$; **figure 2.4a**). Post hoc pairwise comparisons showed significant within-condition effects in all three conditions and between-condition effects throughout (highlighted in **figure 2.4a**).

2.1.3.2.2 BPAR

No significant differences in BPAR scores were observed at baseline. There was a significant condition \times time interaction for BPAR score ($F(12) = 9.033$, $p < 0.001$; **figure**

2.4b). There were no significant within-condition differences for any of the three trial conditions, but between-condition differences were observed, as denoted in **figure 2.4b**.

(a)



(b)

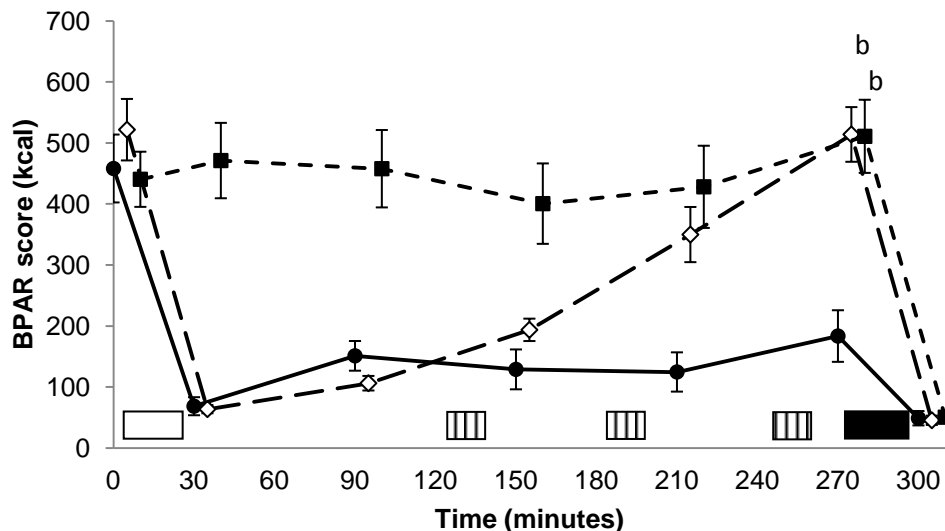


Figure 2.4. Mean appetite scores (\pm SEM) for VAS (a) and BPAR (b), for FED (●), POST-AB (◇) and FAST (■) conditions. Solid line indicates FED, dashed line indicates POST-AB, dotted line indicates FAST. Hollow rectangle = standardised breakfast meal; striped rectangle = snacks; solid, black rectangle = *ad libitum* test meal. a = within-condition effect, significantly different to t=30. b = between-condition effect, significantly different to FED. c = between-condition effect, significantly different to POST-AB.

2.1.3.3 Correlation between subjective appetite measures

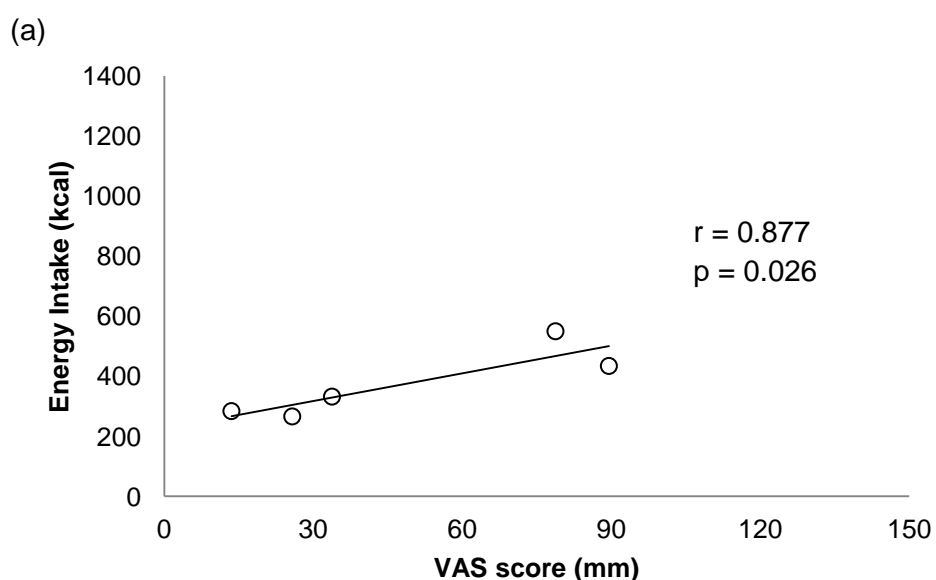
Pearson correlation analysis showed medium strength correlation between VAS and BPAR appetite scores, in the FED ($r = 0.658$, $p = 0.114$), POST-AB ($r = 0.413$, $p = 0.245$) and FAST ($r = 0.604$, $p = 0.140$) conditions.

Between-condition percentage differences in VAS scores and BPAR scores correlated strongly and significantly between all conditions (FAST – FED, $r = 0.941$, $p < 0.001$; FAST – POST-AB, $r = 0.894$, $p < 0.001$; POST-AB – FED, $r = 0.883$, $p < 0.001$).

2.1.3.4 Ability to predict between-subject difference in energy intake

2.1.3.4.1 VAS

Figure 2.5 shows the correlation of the VAS scores obtained immediately prior to the test meal with the energy intake (EI) at the test meal during each of the three trial conditions. The correlation between VAS scores and EI at the test meal was significant only in one trial condition (FED), with that of the FAST condition exhibiting strong positive correlation ($r = 0.801$) that approached significance ($p = 0.052$).



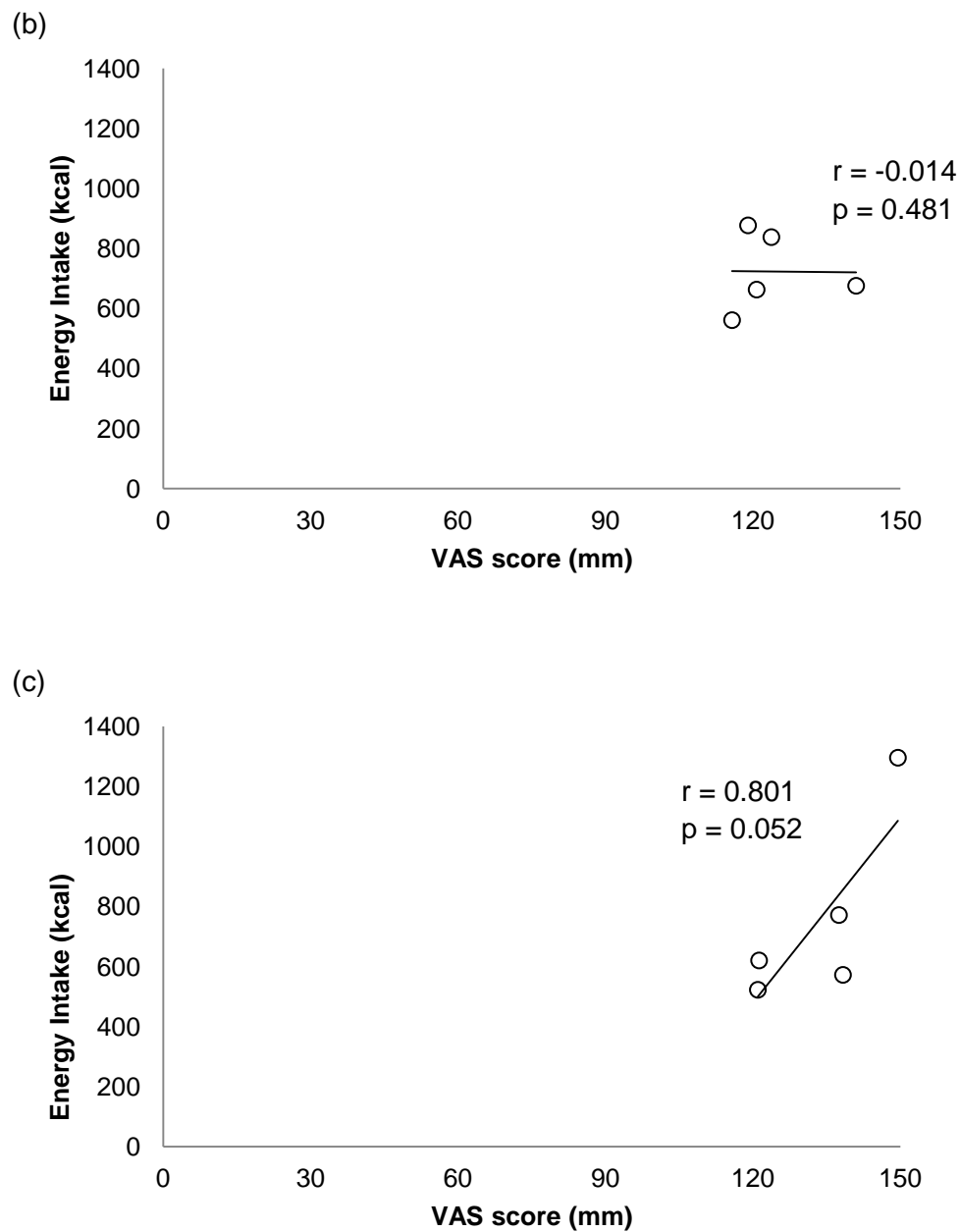


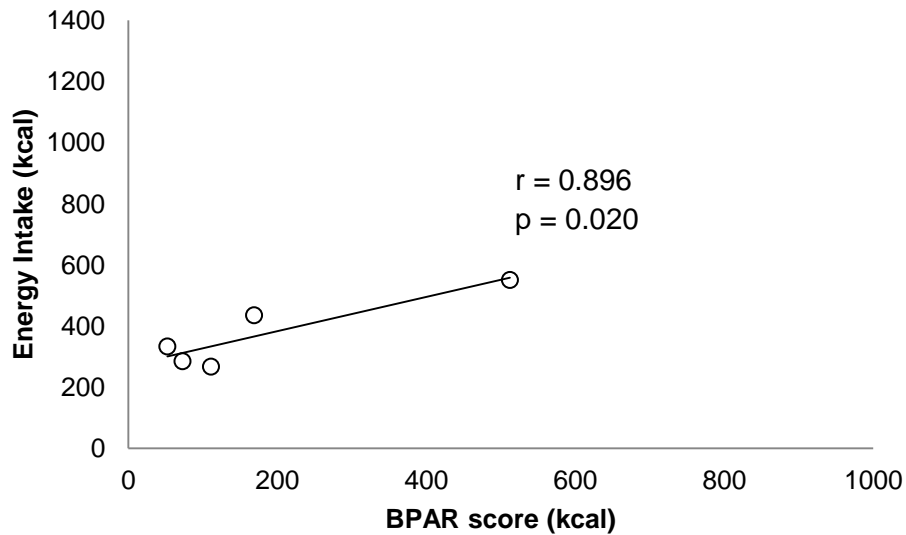
Figure 2.5 – Scatter plot for comparisons of appetite score from the VAS with EI at test meal, in FED (a), POST-AB (b) and FAST (c) conditions.

2.1.3.4.2 BPAR

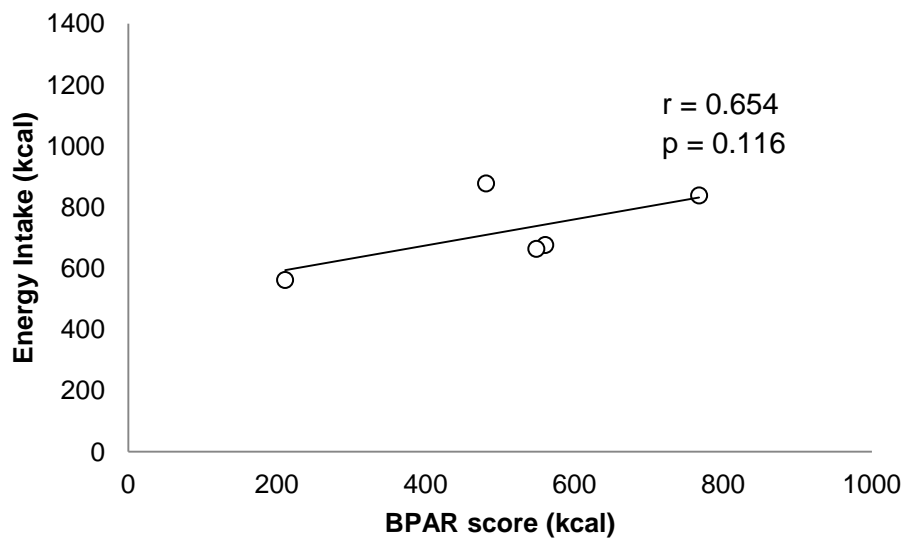
Figure 2.6 shows the correlation of the BPAR scores immediately prior to the test meal with the EI at the test meal during each of the three trial conditions. A significant, strong positive correlation was seen between BPAR score and EI at the test meal in the FED ($r = 0.896$, $p = 0.02$; figure 3a) and FAST ($r = 0.857$, $p = 0.032$; figure 3c) conditions. For the

POST-AB condition, a moderate strength correlation was observed ($r = 0.654$, $p = 0.116$; figure 3b). Differences between correlation coefficients for VAS score vs. energy intake and BPAR score vs. energy intake were not statistically significant.

(a)



(b)



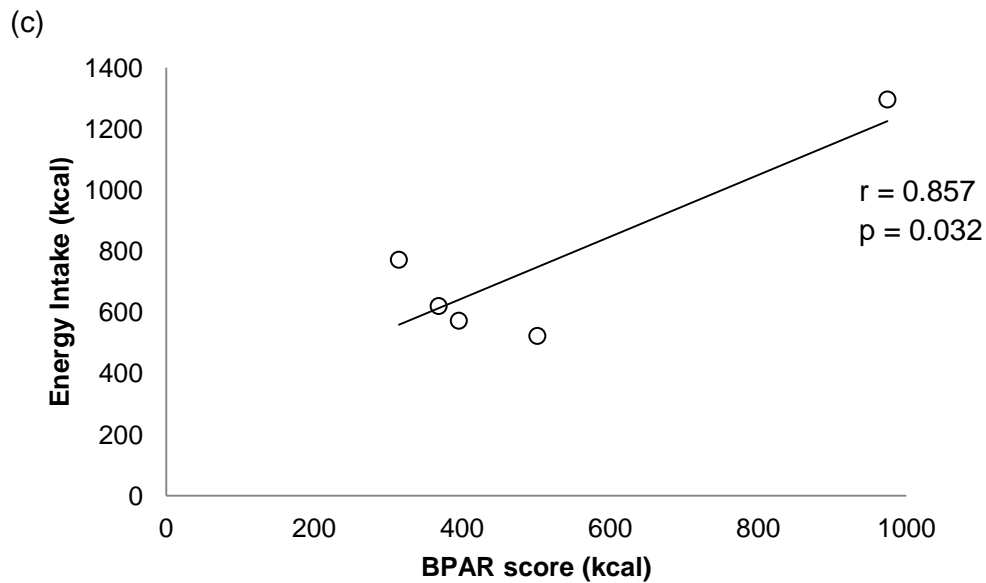


Figure 2.6 – Scatter plot for comparisons of appetite score from the BPAR with EI at test meal, in FED (a), POST-AB (b) and FAST (c) conditions.

2.1.3.5 Ability to predict within-subject differences in energy intake

2.1.3.5.1 VAS

When comparing percentage differences in VAS score between conditions for each participant with percentage differences in EI, a significant, very strong correlation was observed for POST-AB - FED difference (**Table 2.1**).

2.1.3.5.2 BPAR

When comparing percentage differences in BPAR score between conditions for each participant with percentage differences in EI, significant, strong positive correlation was observed for POST-AB - FED differences, with the correlation for FAST – POST-AB differences approaching significance, (**Table 2.1**).

The correlation coefficients for EI vs. VAS and for EI vs. BPAR were compared using t-tests for non-independent correlation coefficients. No significant differences between correlations were observed.

	FAST - FED	FAST – POST-AB	POST-AB - FED
EI vs. VAS	-0.097	0.401	0.912 (p = 0.016)
EI vs. BPAR	-0.445	0.770 (p = 0.064)	0.978 (p = 0.002)

Table 2.1. Product moment correlation coefficients for comparison of changes in EI with changes in VAS score and changes in BPAR score between the three trial conditions. p values are shown when there is a trend for a significant correlation ($p < 0.1$).

2.1.4 DISCUSSION

The aim of this pilot study was to assess the validity of a novel tool - The Birmingham Photographic Appetite Rating (BPAR) – for the measurement of subjective appetite. BPAR appetite scores appeared to be sensitive to changes in appetite. The expected changes in appetite with the manipulations of diet were detected with the BPAR and the appetite profiles for each trial condition reflected the profile obtained from a validated technique for the measure of subjective appetite – the VAS method. Significant within-condition differences, identified with Bonferroni post-hoc pairwise comparisons, were identified with VAS but not BPAR. It is possible that this indicates a greater degree of sensitivity with VAS, compared with BPAR. Within-condition, between-subject subjective appetite scores obtained with the BPAR exhibited moderate strength correlation with those obtained with the VAS technique. Within-subject, between-condition percentage differences in VAS scores and BPAR scores were very strongly correlated. This would indicate that the BPAR is effective at measuring changes in appetite, as VAS is an accepted, validated method for obtaining this measure.

A limitation of VAS is that it has been shown to exhibit a questionable ability to predict food intake (Parker *et al.*, 2004; Raben *et al.*, 1995). Hence, it was desirable to produce a tool that could prove a stronger predictor of food intake than VAS. The BPAR technique appeared to be a strong predictor of food intake, when assessing between- and within-subject differences. BPAR appetite scores immediately prior to the test meal correlated strongly with energy intake at the *ad libitum* test meal. BPAR scores correlated strongly with energy intake in the FED and FAST conditions, while in the POST-AB condition, a moderate strength correlation was observed. This highlighted a stronger correlation between BPAR score and energy intake for between-subject differences than between VAS and energy intake, suggesting that BPAR is the stronger predictor of between-subject differences in food intake. However, it should be acknowledged that the correlation of BPAR score energy intake was not statistically greater than that of VAS and energy intake, as assessed by t-tests for non-independent correlation coefficients. It is possible that

this was due to a very small sample size of 5 participants and a subsequent low statistical power. Nevertheless, such a relationship between an appetite score and energy intake was not observed in the only previous study that has assessed the ability of a photographic images tool to predict *ad libitum* energy intake (Farah *et al.*, 2012).

Within-subject, between-condition percentage difference in BPAR score prior to the test meal correlated strongly with percentage difference in EI at the test meal for two of the three differences calculated (FAST – POST-AB and POST-AB – FED). VAS has previously been shown to be particularly efficacious at detecting within-subject changes (Stubbs *et al.*, 2000), but the ability to predict within-subject changes in energy intake was strong in only one of the differences (POST-AB – FED). BPAR exhibited greater correlation than VAS in each of the three differences calculated (FAST – FED, FAST – POST-AB and POST-AB – FED), although the differences in correlation coefficients was again not statistically significant.

These initial findings, from a pilot investigation of only five subjects are encouraging and suggest that the BPAR is a useful tool for the measurement of subjective appetite. However, further investigation is required. The reliability and reproducibility of the measures must be assessed and the tool's ability to indicate food preference, as well as predict food intake, has not been addressed in this preliminary study. It is likely that such investigation will be conducted after further development of the BPAR that is necessary in order to improve the tool and address current weaknesses; the main one of which is that the BPAR asks participants to select a meal consisting of a single food item. This is an unnatural eating behaviour and using this approach is likely to lead to difficulty in conceptualising appetite.

A limitation of the current validation study design is that the fasted state condition may have proved too severe. From the point of view of assessing the ability of the BPAR to track changes in appetite, it would appear that this condition proved ineffective, as appetite remained very high and unchanged throughout, until the lunch meal. Further, some participants reacted adversely to the long period without food (15 hours by the time of the

lunch meal). Severe “hunger pains” and light-headedness was reported by two of the five participants.

It is considered desirable to develop the BPAR in order to allow the participant to construct a virtual meal, consisting of numerous food items. This would be an improvement on the current restrictive format, whereby participants are asked to “imagine you are to consume a meal at this moment, consisting of this food item alone”. It is hoped that with future developments, an improved format will allow for an easier conceptualisation of subjective appetite, into a meaningful measure of desired food intake, hence further improving on a major limitation associated with the use of VAS. If this is achieved, an improved version of the BPAR could prove a stronger predictor of energy intake and food preference, while maintaining the advantage of being able to allow independent, repeated measures of subjective appetite.

2.2 General methods of Chapters 4, 5 and 6

2.2.1 Study designs

A within-subject, counterbalanced, crossover study design was utilised for the studies of Chapters 4, 5 and 6. Participants were randomly assigned to each trial condition, with measures of subjective appetite and circulating concentrations of appetite-associated recorded throughout each trial.

In each case, experimental trials were preceded by one pre-testing trial session. Experimental trials then commenced a minimum period of 3 days after pre-testing, with trials also separated by a minimum wash out period of 3 days. However, typically, this period between trials was 7 days.

To limit between test variability, participants in each of Chapters 4, 5 and 6 were asked to refrain from moderate or high intensity exercise during the 24h prior to each trial. In addition, a food diary was completed for the 24h prior to the first trial. The participant was then asked to replicate their food intake as closely as possible for the 24h prior to subsequent trials.

2.2.2. Pre-testing sessions

2.2.2.1 Chapters 4 and 5

A single pre-testing session was completed prior to study trials in order to calculate specific exercise intensities to be used for each participant. After refraining from strenuous exercise during the 2 days prior, and abstaining from food for a minimum of 2 hours, participants reported to the Exercise Metabolism Laboratory, in the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, where the participant information pack was administered and explained and the participant was given the opportunity to ask any questions regarding the study. A written consent form was completed prior to the completion of a health questionnaire and the Dutch Eating Behaviour Questionnaire (DEBQ,

Appendix 4). Height and weight were recorded. An incremental exhaustive exercise test was then completed to obtain VO_{2max} , $Watt_{max}$ and HR_{max} . The incremental exercise test was carried out on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands), with the exercise protocol controlled automatically by the computer software programme accompanying the ergometer. The test, preceded by a ten minute warm-up at a self-selected power output, consisted of three minute stages, starting at a power output of 95W and increasing in increments of 35W. Breath-by-breath measures of exhaled gas, were recorded using Oxycon Pro (Jaeger, Wuerzburg, Germany) apparatus (see section 2.2.3.4, p75). Participants were adjudged to have reached the end of the test when they voluntarily stopped pedalling, if their cadence dropped to <60 rpm or if VO_2 ceased to increase with increasing workload. Heart rate (HR) was recorded using a heart rate monitor (Polar S625X; Polar Electro Oy, Kempele, Finland), along with cadence and rating of perceived exertion (RPE), using the Borg Scale (Borg, 1973) during the final 30 seconds of each stage. HR_{max} was noted from the values obtained during the final moments of the test. VO_{2max} was calculated as the highest average value obtained for any one minute period. Submaximal VO_2 values were obtained for each stage by disregarding data from the first 2 minutes of the stage. $Watt_{max}$ was also calculated as: final stage completed (Watts) + ((time completed in final stage (seconds)/180) x 35). From the VO_{2max} value obtained, linear regression was used to calculate the work outputs (in Watts) for each exercise session that would equate to the desired exercise intensity (i.e., target percentage of VO_{2max}).

2.2.2.2 Chapter 6

A single session of pre-testing preceded the study protocol. Participants reported to the Exercise Metabolism Laboratory, in the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. The participant information pack was administered and explained and the participants were given the opportunity to ask any questions regarding the study. Consent forms were signed, prior to the completion of a health questionnaire, as a means of a health screening procedure, and the Dutch Eating Behaviour Questionnaire (DEBQ), which was used to assess eating behaviour. Height and weight were recorded,

before the participant rested for a short period, prior to the measure of resting blood pressure. Upon completion of the blood pressure measure, a 10-lead resting ECG was conducted. The ECG trace was recorded and assessed for any abnormality in cardiac activity by a qualified physician. Once all measures were completed, a short familiarisation bout of exercise was undertaken. Participants mounted a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) and ensured that the handlebars and seat were positioned to their liking. They then cycled for three minutes at a power of 40 Watts, which replicated the three minute warm-up of the exercise trial bout. At the end of this 3 minute period, they immediately commenced a 30 second “flat-out” sprint. The sprint was a modified Wingate anaerobic power test, using a constant torque, relative to the participant’s body weight (torque factor 0.6: braking torque = torque factor x participant’s bodyweight, Nm). Verbal encouragement was provided throughout in an attempt to elicit maximal effort. Visual feedback was provided in the form of a countdown timer and traces of both cadence and power output, using the Wingate Software programme. The pre-testing session concluded with the administering of a food diary and instructions on how to accurately record dietary intake for the 24 hour period prior to the participant’s first trial. It was made clear that they would be expected to replicate this dietary intake for the 24 hour period prior to their second trial.

2.2.3 Measures

2.2.3.1 Eating restraint

Eating restraint was assessed using the Dutch Eating Behaviour Questionnaire (van Strien *et al.*, 1986a) (DEBQ, Appendix 4). The DEBQ has been shown to exhibit moderate to good predictive validity of eating restraint (van Strien *et al.*, 1986b) and proved preferable to alternative scales and questionnaires, with regards test-retest reliability, internal consistency and homogeneity with food intake measures (Allison *et al.*, 1992).

2.2.3.2 Food intake

In Chapters 4, 5 and 6 food intake measures were obtained using the *ad libitum* meal technique. In Chapter 4 this was a homogeneous meal consisting of cornflakes with semi-skimmed milk. This food, representative of a breakfast meal, was chosen due to the time of the day that it would be consumed. Food choice was limited, as it has been previously shown that allowing excessive food choice, such as in a “cafeteria diet” can lead to overfeeding (Larson *et al.*, 2005a; Larson *et al.*, 1995b). The food was pre-weighed and presented as follows; pre-measured milk in a clear, non-graded jug; pre-measured cornflakes in a clear, non-graded cereal container; a clear, glass cereal bowl and metallic spoon. Non-graded apparatus was used to ensure that participants could not estimate measures of their intake. In Chapter 5, the *ad libitum* meal consisted of a small buffet, offering the following food: cornflakes, semi-skimmed milk, sugar, bread, margarine, strawberry jam, orange juice and apple juice (nutritional information shown in Appendix 5). All food was pre-weighed and the meal was presented as follows; milk in a opaque, non-graded bottle; cornflakes in cereal box; four slices of bread; sugar in a small sugar bowl; margarine in a clear tub; jam in its jar; juices in their carton. In Chapter 6, an *ad libitum* lunch buffet was provided (see Appendix 6 for food options and nutritional information). All food was pre-weighed and presented on plates, in opaque bowls or in ungraded jugs.

It was decided to change to buffet-style meals, offering some degree of food choice, in Chapters 5 and 6 for two reasons. Firstly, it was observed in Chapter 4 that some participants ceased eating prematurely, due to boredom of consuming the same food, prior to satiation. An attempt was made to avoid this in the latter two studies in order to assess the measure of relative energy intake (energy intake – energy expenditure) and compare this variable across conditions. Secondly, it was deemed beneficial to address food choice, obtaining measures of the energy density and macronutrient content of the meal consumed. The *ad libitum* buffet technique has been shown to demonstrate high reproducibility for such measures (Arvaniti *et al.*, 2000). It is acknowledged that an increased food choice can lead to an increase in food intake and overeating (Arvaniti *et al.*, 2000; Larson *et al.*, 2005a;

Larson *et al.*, 1995b), however, the buffet-style approach was deemed preferable after much deliberation.

In each experiment, after volitional satiation was reached, all remaining food was weighed and subtracted from the known quantity provided, allowing for determination of consumed food. From this, energy intake was calculated. In Chapters 5 and 6, when there was an element of food choice, macronutrient content of the meal consumed was also calculated, as the nutritional information of each food item available was known.

In all instances, the manner of presentation of the *ad libitum* meal was constant throughout the experiment. The meal options were presented, in the same portions for each trial, on serving surface. On a separate table, cutlery, plate, bowl and cup were laid. The participant was instructed to serve their food at the serving surface and return to the table to consume. The same cutlery and crockery were used throughout the experiment.

2.2.3.3 Subjective appetite

Subjective appetite was assessed using the 4-question VAS test for subjective appetite, as adapted from Hill & Blundell (Hill and Blundell, 1982) and a unique Birmingham Photographic Appetite Rating (BPAR) tool. The VAS addressed “hunger,” “fullness,” “desire to eat,” and “expected food intake”. A composite VAS test score was calculated as, hunger score + desire score + expected intake score + (150-fullness score). It was decided to use this single score for ease of data analysis and presentation and it has been shown that, with the original 6 question VAS technique of Hall & Blundell, the scores for each question co-vary to a large extent (Stubbs *et al.*, 2000) and that the first principal component of the questions is the mean value of the scores (Reid *et al.*, 1998).

Subjective appetite was also measured using the BPAR tool in Chapter 4 and the VIMEC tool in Chapters 5 and 6. The BPAR techniques have been described previously in this chapter of the thesis (section 2.1.2.5, p54). In Chapter 4, three of the 20 food items in the BPAR (porridge, tomato soup and strawberry yoghurt) were disregarded from the results. This was because we felt that the portion size was not perceived accurately and led to extremely large portion selections for these food items, relative to the portion selections of

other foods. The amorphous nature of the food items is likely to have contributed to the poor perception of portion size, as this has been seen previously with other estimations of portion size when real food is displayed (Weber *et al.*, 1999) and in techniques whereby photographic images of food were used (Venter *et al.*, 2000). The VIMEC technique is described in detail in Chapter 3 (section 3.3.5, p90).

The two subjective appetite measure techniques (VAS and BPAR in Chapter 4 and VAS and VIMEC in Chapters 5 and 6) were completed in alternating order throughout each trial condition, to control for any possible influence one technique may have on the other.

2.2.3.4 Analysis of exhaled gas

For the determination of rates of oxygen consumption and carbon dioxide production, exhaled gas was analysed using the online automated gas analysis system, Oxycon Pro (Jaeger, Wuerzburg, Germany). This system has previously shown to be a valid and reliable apparatus for the measure of ventilation rate, rates of oxygen consumption and rates of carbon dioxide production, over a range of exercise intensities (Rietjens *et al.*, 2001; Carter & Jeukendrup, 2002). Participants wore a nose clip and breathed through a mouthpiece. The mouthpiece unit consisted of a sample tube and turbine volume transducer. Expired gas was sampled, through the sample tube, and analysed using oxygen (O₂) and carbon dioxide (CO₂) analysers. The system specifications for the O₂ analyser were as follows: range: 0 to 25%, accuracy: 0.05%, resolution: 0.01%, stability 0.02%/hour. The specifications of the CO₂ analyser were: range: 0 to 15%, accuracy: 0.05%, resolution: 0.01%, stability: 0.02%/hour. The specification of the volume sensor were: range: 0 to 10L, accuracy: 25% or 50ml, resolution: 3ml, flow range: 0 to 15 L/sec, flow accuracy: 3% or 70ml/sec. Prior to data collection, the gas analysers were calibrated using a calibration gas (BOC Gases, Guildford, Surrey, UK) of mixed, known concentrations of O₂ (14.99%) and CO₂ (5.04%) and volume was calibrated using a 3 litre calibration syringe (Jaeger, Wuerzburg, Germany). Values obtained were mean values of 8 breaths.

2.2.3.5 Blood sampling and analyses

2.2.3.5.1 Sampling

Blood samples were obtained via a venous cannula inserted into the antecubital vein of the arm by a qualified member of staff. Blood was immediately transferred to disodium EDTA-treated tubes for analysis of hormones. In Chapters 5 and 6, the disodium EDTA-treated tubes were also pre-treated with the protease inhibitors DPP IV inhibitor (Millipore, MA, USA – 0.01ml per millilitre of blood) and 4- (2 – Aminoethyl) benzenesulfonylfluoride hydrochloride (AEBSF, Alexis Biochemicals, Lausen, Switzerland – 200nM, 0.02ml of per millilitre of blood). These inhibitors were added to preserve the hormones GLP-1 and acylated ghrelin. A separate blood sample was obtained for the measure of plasma glucose and transferred to a disodium EDTA-treated tube that did not contain the protease inhibitors. Blood was centrifuged at 3500 RPM and a temperature of 4°C for 15 minutes to isolate plasma. Plasma was separated, transferred to sample cups and stored at a temperature of -70°C until analyses were conducted. In Chapters 5 and 6, separate aliquots were transferred to two sample cups that had been pre-treated with hydrochloric acid (1N, 100microlitres per millilitre of plasma), to further protect acylated ghrelin from degradation.

2.2.3.5.2 Analyses

2.2.3.5.2.1 Glucose

Plasma glucose concentration was measured (glucose oxidase reagent, Instrumentation Laboratories Company, Monza 338, Milan, Italy) using the ILab 650 Clinical Chemistry System (Instrumentation Laboratories Company, Lexington, MA, USA).

2.2.3.5.2.2 Appetite-associated hormones

Acylated ghrelin, PP, total PYY and total GLP-1 were measured in duplicate using ELISA (Human Ghrelin(active) ELISA kit, Millipore, MA, USA; Human PP ELISA kits, Millipore, MA, USA; Human PYY(total) ELISA kit, Millipore, MA, USA; Mulit Species GLP-1(total) ELISA kit, MA, USA). The sensitivity of these ELISA kits were 8 pg•ml⁻¹, 12.3pg•ml⁻¹, 1.4 pg•ml⁻¹ and 1.5 pg•ml⁻¹ respectively. The coefficient of variation values were 5.80% for PP and 5.94% for the PYY in Chapter 4; 2.36%, 5.26% and 3.28% for acylated ghrelin, PYY

and GLP-1, respectively, for Chapter 5 and 3.83%, 3.15% and 3.02% for acylated ghrelin, PYY and GLP-1, respectively, for Chapter 6.

No measure or markers of plasma volume were obtained. However, given the nature of the exercise undertaken and findings of previous studies utilising similar exercise bouts (Burns et al., 2007; Martins et al., 2007; Ueda et al., 2009), changes in plasma volume would be expected to be negligible and hence haemoconcentration is unlikely to have affected the observed plasma concentrations of metabolites and hormones.

2.2.4 Statistical Analysis

Specific statistical tests and analyses are described within the individual chapters. Throughout, data are presented as means \pm standard deviation in text and tables and as mean \pm SEM in figures. Any missing values were calculated using the multiple imputations method, with the mean value of five imputations used. A statistical significance level of $p < 0.05$ was used for all analyses of variance. All statistical analysis was carried out using the SPSS software programme (SPSS inc., Chicago, Illinois, USA).

2.3 REFERENCES

- Allison, D.B., Kalinsky, L.B., and Gorman, B.S. (1992). A comparison of the psychometric properties of three measures of dietary restraint. *Psychological Assessment* 4, 391-398.
- Arvaniti, K., Richard, D., and Tremblay, A. (2000). Reproducibility of energy and macronutrient intake and related substrate oxidation rates in a buffet-type meal. *The British journal of nutrition* 83, 489-495.
- Bellisle, F. (1999). Food choice, appetite and physical activity. *Public Health Nutrition* 2, 357-361.
- Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., Van Der Knaap, H., *et al.* (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity Reviews* 11, 251-270.
- Borg, G.A.V. (1973). "Perceived exertion: a note on history and methods". *Medicine and Science in Sports & Exercise* 5, 90-93.
- Carter, J., and Jeukendrup, A.E. (2002). Validity and reliability of three commercially available breath-by-breath respiratory systems. *European Journal of Applied Physiology* 86, 435-441.
- Farah, N.M.F., Brunstrom, J.M., and Gill, J.M.R. (2012). Using a novel computer-based approach to assess the acute effects of exercise on appetite-related measures. *Appetite* 58, 196-204.
- Health and Social Care Information Centre (2013). Health Survey for England. Statistics on Obesity, Physical Activity and Diet – England, 2013. <http://www.hscic.gov.uk/searchcatalogue?productid=11194&q=obesity&sort=Relevance&size=10&page=1#top>
- Hill, A.J., and Blundell, J.E. (1982). Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research* 17, 203-212.
- King, N.A., Caudwell, P.P., Hopkins, M., Stubbs, J.R., Naslund, E., and Blundell, J.E. (2009). Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. *The American Journal of Clinical Nutrition* 90, 921-927.
- King, N.A., Tremblay, A., and Blundell, J.E. (1997). Effects of exercise on appetite control: implications for energy balance. *Medicine and science in sports and exercise* 29, 1076-1089.
- Larson, D.E., Rising, R., Ferraro, R.T., and Ravussin, E. (1995a). Spontaneous overfeeding with a "cafeteria diet" in men: effects on 24-hour energy expenditure and substrate oxidation. *International Journal of Obesity* 19, 331-337.
- Larson, D.E., Tataranni, P.A., Ferraro, R.T., and Ravussin, E. (1995b). *Ad libitum* food intake on a "cafeteria diet" in Native American women: relations with body composition and 24-hour energy expenditure. *American Journal of Clinical Nutrition* 62, 911-917.
- Martins, C., Morgan, L., and Truby, H. (2008). A review of the effects of exercise on appetite regulation: an obesity perspective. *Int J Obes* 32, 1337-1347.

Parker, B.A., Sturm, K., MacIntosh, C.G., Feinle, C., Horowitz, M., and Chapman, I.M. (2004). Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr* 58, 212-218.

Raben, A., Tagliabue, A., and Astrup, A. (1995). The reproducibility of subjective appetite scores. *The British journal of nutrition* 73, 517-530.

Reid, C.H., CG. Blundell, JE. Stubbs, RJ (1998). What are psychometric assessments of appetite asking: a preliminary multivariate analysis. *International Journal of Obesity* 22, 151.

Rietjens, G.J.W.M., Kuipers, H., Kester, A.D.M., and Keizer, H.A. (2001). Validation of a computerized metabolic measurement system (Oxycon-Pro) during low and high intensity exercise. *International Journal of Sports Medicine* 22, 291-294.

Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., and Blundell, J.E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* 84, 405-415.

van Strien, T., Frijters, J.E.R., Bergers, G.P.A., and Defares, P.B. (1986a). The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders* 5, 295-315.

van Strien, T., Frijters, J.E.R., van Staveren, W.A., Defares, P.B., and Deurenburg, P. (1986b). The predictive validity of the Dutch Restrained Eating Scale. *International Journal of Eating Disorders* 5, 747-755.

Venter, C.S., MacIntyre, U.E., and Vorster, H.H. (2000). The development and testing of a food portion photograph book for use in an African population. *Journal of Human Nutrition and Dietetics* 13, 205-218.

Weber, J.L., Cunningham-Sabo, L., Skipper, B., Lytle, L., Stevens, J., Gittelsohn, J., Anliker, J., Heller, K., and Pablo, J.L. (1999). Portion-size estimation training in second- and third-grade American Indian children. *The American Journal of Clinical Nutrition* 69, 782S-787S.

**The development of a novel tool for the measurement of subjective
appetite. Part B: The Visual Meal Creator**

3.1 ABSTRACT

Background: We previously attempted to address the limitations of commonly-used methods for obtaining measures of appetite, with the development of the Birmingham Photographic Appetite Rating (BPAR). Results of initial validation testing proved encouraging (see Chapter 2, section 2.1, p50). However, a number of limitations of the BPAR became apparent. These included the abstract nature of asking the participant to imagine they are to consume a meal of only a single food item (i.e., a meal of chocolate raisins) and the inability to combine different food items to replicate a more habitual eating episode, i.e., create a meal.

Purpose: To further develop and validate the BPAR, creating a tool that would allow for the creation of “computerised visual meal” to measure subjective appetite – the Visual Meal Creator (VIMEC).

Method: Participants experienced dietary control over a 5-hour period to manipulate hunger state on three occasions (small breakfast (SB) vs. large breakfast (LB) vs. large breakfast + snacks (LB+S)). Appetite measures were obtained every 60 minutes using the VIMEC and VAS. At 4.5 hours, participants were presented with an *ad libitum* test meal, from which energy intake (EI) was measured. The efficacy of the VIMEC was assessed by its ability to detect expected patterns of appetite and its strength as a predictor of energy intake. Day-to-day and test-retest reproducibility were assessed.

Results: There was a significant time x condition interaction effect for appetite, using both VAS and VIMEC methods, indicating that appetite was successfully manipulated and expected changes in appetite were detected. Between- and within-condition differences in VAS and VIMEC scores were significantly correlated with one another throughout. Between- and within-condition changes in appetite scores obtained with the VIMEC exhibited a stronger correlation with EI at the test meal than those obtained with VAS. Pearson correlation coefficients for within-condition comparisons were 0.951, 0.914 and 0.875 (all $p < 0.001$) for SB, LB and LB+S respectively. Correlation coefficients for between-condition

differences in VIMEC and EI were 0.273, 0.904 ($p < 0.001$) and 0.575 ($p < 0.05$) for SB – LB+S, SB – LB and LB – LB+S respectively. The VIMEC exhibited a similar degree of reproducibility to VAS.

Conclusion: The VIMEC appears to be a stronger predictor of energy intake and may prove to be a preferable measure of subjective appetite than VAS.

3.2 INTRODUCTION

With the most recent figures showing that 65% of adult males and 58% of adult females in England are now overweight or obese (Health and Social Care Information Centre, 2013), the need for a greater understanding of energy balance and weight management is necessary. In an attempt to address the “energy in” side of the energy balance equation, the regulation of appetite has been extensively investigated (the reader is directed to a number of comprehensive review articles (Bellisle, 1999; King *et al.*, 2009; King *et al.*, 1997; Martins *et al.*, 2008). In conducting such research, a wide range of techniques have been utilised to obtain both subjective and objective measures of appetite and measures of eating behaviour.

These techniques have been discussed previously, in the General introduction (Section 1.5.3, p35). Briefly, self-report questionnaires and scales are commonly used for the measure of subjective appetite, with the visual analogue scale method (VAS) being the most prevalent within appetite research (Blundell *et al.*, 2010). These subjective appetite measures are usually used in conjunction with more objective measures, such as measures of appetite-associated hormones, surrogates of eating behaviour, typically in the form of a food intake or energy intake measure. Commonly, *ad libitum* energy intake is measured from buffet-style or constant composition test meals.

The benefits and limitations of both subjective VAS appetite measure and *ad libitum* food intake measures have also been documented previously. The VAS method is inexpensive and both quick and simple to administer and is generally considered a valid measure of subjective appetite (Blundell *et al.*, 2010; Stratton *et al.*, 1998; Stubbs *et al.*, 2000), especially when used to address within-subject comparisons (Flint *et al.*, 2000). VAS's ability to predict eating behaviour, however, is less clear. While some studies have demonstrated a significant correlation between VAS scores and subsequent aspects of eating behaviour (Flint *et al.*, 2000; Parker *et al.*, 2004), others have shown a lack of a relationship (Parker *et al.*, 2004; Raben *et al.*, 1995). Test-retest reproducibility has been

shown to be good (Flint *et al.*, 2000; Stratton *et al.*, 1998), but day-to-day reproducibility is considerably weaker (Flint *et al.*, 2000; Raben *et al.*, 1995). There are limitations associated with the use of the VAS method, notably the abstract nature of the question and line format and the difficulty in conceptualising the constructs of “hunger” and “fullness,” and the lack of any indication of desired portion size or food choice. Hence, important aspects of eating behaviour are neither assessed nor predicted when using this method of measurement. The *ad libitum* food intake method has been shown to exhibit a high degree of day-to-day reproducibility, both when presented as a buffet (Arvaniti *et al.*, 2000) and when the meal is of a constant composition (Gregersen *et al.*, 2008). While allowing for a valid quantitative proxy of eating behaviour, food intake in test meals can be influenced by a number of external factors, such as the amount of food presented (Rolls *et al.*, 2002; Wansink *et al.*, 2005), the variety of foods available (Hetherington *et al.*, 2006) and the perceived palatability of the food (Yeomans *et al.*, 2001). In addition, buffet-style presentation and a laboratory setting are not habitual eating environments for the majority of people and may influence intake (Blundell *et al.*, 2010; George and Morganstein, 2003; Hetherington *et al.*, 2006). Such external cues are potent stimuli for appetite regulation and can override physiological determinants of hunger. A key limitation of the *ad libitum* intake method is that it does not allow for independent, repeated measures within a short space of time, in contrast to VAS, which can be repeated frequently to track acute changes in appetite. Any food intake measure will have a large impact upon subsequent measures and, while total or mean intake values can be calculated over a study period, each separate intake or eating episode will not be independent from previous measures. This means that *ad libitum* intake measures do not allow the time course of changes in appetite to be monitored, which is often of interest to researchers. From a practical viewpoint, the *ad libitum* intake method can be expensive, time-consuming and labour-intensive to administer and can result in large amounts of food wastage.

Partly in an attempt to reduce food wastage and the time and money investment required to prepare food, techniques employing the use of photographic images of food have

been increasingly utilised within weight management and food intake research (Brunstrom *et al.*, 2008a; Brunstrom *et al.*, 2008b; Frobisher and Maxwell, 2003; Nelson and Haraldsdóttir, 1998; Riley *et al.*, 2007; Subar *et al.*, 2010; Turconi *et al.*, 2005; Venter *et al.*, 2000; Williamson *et al.*, 2003). Most commonly, this technique has been developed and used to assist with retrospective food recall (Frobisher and Maxwell, 2003; Subar *et al.*, 2010; Turconi *et al.*, 2005) or as a reference for the estimation of habitual food intake (Riley *et al.*, 2007; Venter *et al.*, 2000). While these investigations assessing the efficacy and validity of such tools have produced mixed results, it would appear that photographic images of food do show potential for use in measures of habitual and retrospective food intake.

More recently, Brunstrom and colleagues have used photographic images of food for measures of everyday portion size estimates (Brunstrom *et al.*, 2008a) and expected satiety (Brunstrom and Rogers, 2009; Brunstrom and Shakeshaft, 2009; Brunstrom *et al.*, 2008b), with the “constant stimuli” technique. While the preliminary version of the technique consisted of just 8 images, representing 8 different portion sizes of each food item, the later version consisted of 17 food items, with 40-60 images of each. Expected satiety is measured by using a “standard” – a known portion size of a commonly-consumed food item – and using all other items as “comparison” foods. The participant is asked to select the portion size, or image, of the comparison food that they would expect to satiate them to the same extent as the standard.

This work was extended further, with the studies of Farah *et al.* (2012) and Sadoul *et al.* (2012). Photographic images of foods were used to assess liking for certain foods and desired portion size during exercise (Farah *et al.*, 2012) and ileal infusion (Sadoul *et al.*, 2012) interventions. The techniques used allowed for sensitive measures, detecting expected changes in appetite as a consequence of the intervention, although desired portion size did not correlate with VAS scores for hunger in every population of participant and in every study condition (Farah *et al.*, 2012), and neither did it correlate with *ad libitum* food intake measures (Sadoul *et al.*, 2012). However, despite some short-comings, the effectiveness of photographic image techniques at measuring expected satiety and desired

portion size suggests that a subtle adaptation to this approach may result in a tool capable of measuring subjective appetite through the selection of desired portion size.

With an appreciation of the aforementioned limitations of the commonly used methods of measuring subjective appetite and food intake, and in light of recent data on the potential efficacy of food photography for the selection of portion size, an investigation into the possibility of developing a new tool and technique for the measure of appetite was undertaken. The early development of this project, along with pilot experimentation was discussed in Chapter 2, (section 2.1, p50). This work led to the conception of the Birmingham Photographic Appetite Rating (BPAR) – a computer-based tool utilising food photography which enabled participants to select desired portions sizes of a number of food items. Initial pilot validation testing of the BPAR proved encouraging. However, it was clear that the tool harboured some limitations. Primarily, the BPAR asks the participant to consider each food item in isolation, imagining that they are about to eat a meal consisting of only that item. This is not a normal way in which individuals consume food. Therefore, it was considered desirable to attempt to further develop the BPAR, allowing for the combination of a number of food items to create a meal. Secondly, the pilot validation testing of the BPAR did not include any measures of reliability or reproducibility.

Therefore, the aim of the current study was two-fold. Firstly, to further develop the previously-created BPAR tool for the measure of subjective appetite, improving on the current format and allowing for the creation of a “meal.” The new model must adhere to the remit of the BPAR: to provide a less abstract subjective measure than VAS, using a portion selection method, while also allowing for indicative measures of food choice and enabling independent, repeated measures in a cost-effective and time-efficient manner. Secondly, we aimed to address the validity and reproducibility of the new tool – the Visual Meal Creator (VIMEC) – relative to both the VAS and *ad libitum* intake methods. This was achieved by attempting to manipulate appetite state through acutely controlling diet from breakfast until an *ad libitum* lunch meal.

3.3 METHOD

3.3.1 Participants

Twelve recreationally active participants (8 female, 4 male; mean age 23 ± 2 years; mean body mass 70.4 ± 17.3 kg; mean BMI 22.8 ± 3.6 kg•m⁻²) were recruited from the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. Those suffering from illness such as cold or flu, those taking medication that was likely to affect appetite or that needed to be taken with food more frequently than once a day, those with food allergies and those suffering from diabetes were excluded from taking part. Ethical approval was obtained from the Ethics Committee of the University of Birmingham.

3.3.2 Study design

The validity of any form of rating scale is not easily addressed. To attempt this, we used the three assessments as highlighted by Stubbs *et al* (Stubbs *et al.*, 2000). These are a) the apparent validity of the measure in terms of its ability to predict the behaviour which is being assessed, which was assessed by comparing the VIMEC score with an *ad libitum* test meal energy intake; b) the change in rating score under conditions where it should change if sensitive, with changes compared with those seen with a valid, commonly-used technique for the measure of subjective appetite – the visual analogue scale (VAS) test and c) the reproducibility of the measures, which was assessed by comparing day-to-day measures and short-term test-retest measures.

A within-subject, randomised crossover study design was utilised. Participants were randomly assigned to each of the three experimental conditions; small breakfast (SB), large breakfast (LB) and large breakfast with snacks (LB+S). These feeding conditions were used to manipulate hunger state. This study design differs from that of Chapter 2 in so much that the “fasting” condition was replaced with a “small breakfast” condition. This alteration was made, as it became clear that remaining fasted for up to 15 hours could be quite distressing. Some participants complained of feeling light-headed and nauseous, while some claimed to

have “gone past being hungry.” A “small breakfast” condition was considered more ethical and a better reflection of normal eating habits.

3.3.3 Procedure & protocol

Participants arrived at the Exercise Metabolism Laboratory within the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham between 07.00 and 09.00, after a ten-hour overnight fast. Upon arrival at the laboratory for the first time, participants were provided with further verbal information regarding the nature of the study and given the opportunity to ask any questions regarding their participation. A written consent form was then signed. Health questionnaires were completed and breakfast food selections were made. Participants were then randomly allocated to one of the three trial conditions. The study protocol for each condition is shown in diagrammatical form in **figure 3.1**. Participants remained sedentary throughout the trial period. Trials were separated by a minimum wash out period of 3 days, but typically, this period between trials was 7 days.

Prior to the first trial, participants were provided with a food diary and instructed on how to complete it. They were asked to complete the diary on the day before their first trial and instructed to replicate this diet on the day prior to the following two trials.

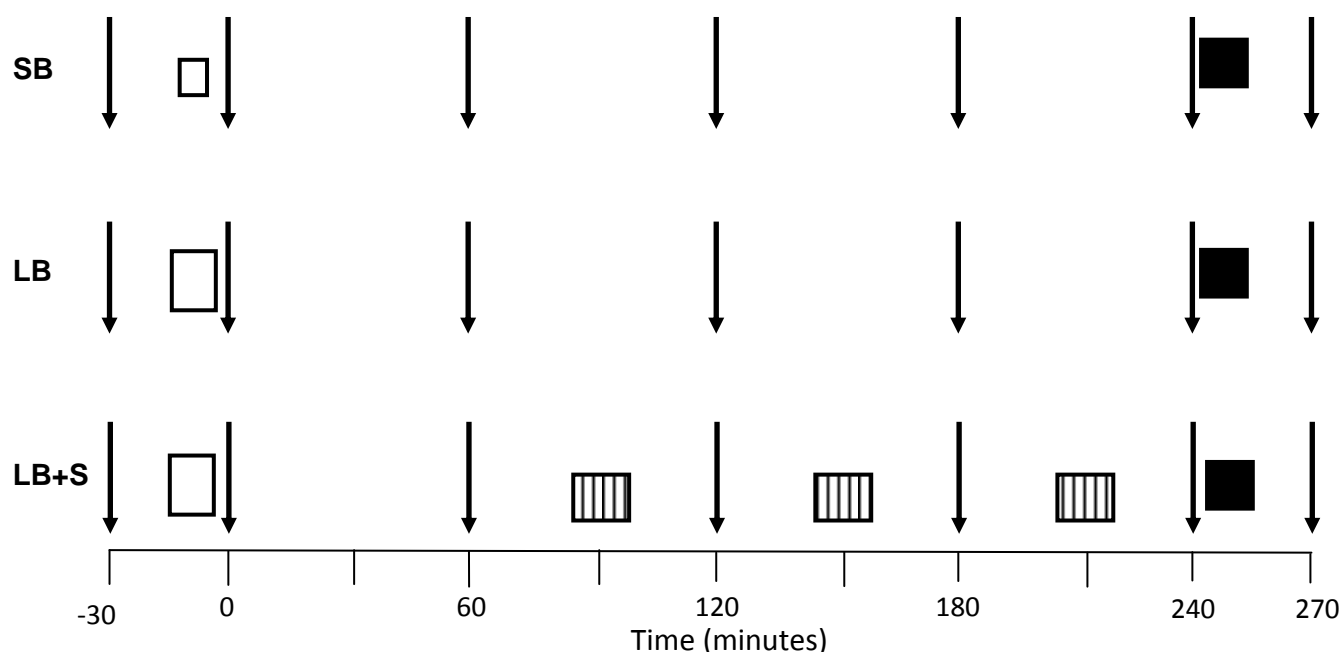


Figure 3.1. Study protocol. Arrow = appetite measure (VAS and VIMEC). Small clear rectangle = small breakfast. Large clear rectangle = large breakfast. Striped rectangle = snack. Black square = *ad libitum* lunch meal

3.3.4 Breakfast meals and snacks

The small breakfast meal consisted of a 25g cereal bar (oat and raisin, Sainsbury's) with 200ml of pure orange or apple juice (Sainsbury's), exhibiting the following characteristics: ~140 kcal, 27g carbohydrate, 2.3g fat, 1.8g protein, 1.1g fibre. The large breakfast consisted of cereal (80g of Original Swiss-style Alpen, or 55g of Kellogg's Bran Flakes); 125-150ml of semi-skimmed milk (Sainsbury's); 2 slices of toast (Kingsmill 50/50 thick slice, ~88g); 16 g of margarine (Flora light) and 30g of jam (strawberry, Sainsbury's) with 200ml of pure orange or apple juice (Sainsbury's). A choice of two cereals, with similar energy density and macronutrient content was provided to allow for individual preferences and dislikes. However, when Bran Flakes were selected, a banana was added to the meal in order for energy content to be similar between the two options, accounting for the smaller portion of Bran Flakes. The same cereal was consumed for both large breakfast conditions. The large breakfast meal (Alpen cereal chosen) typically exhibited the following

characteristics: ~763 kcal, 133.4g carbohydrate, 15.2g fat, 22.5g protein, 10.6g fibre. The meals were consumed within 15 minutes.

Three snack items were administered at 1.5, 2.5 and 3.5 hours in the LB+S condition. These were, in order, a 50g flapjack bar (Sainsbury's, 223 kcal); a 25g cereal bar (oat and raisin, Sainsbury's, 98 kcal) and a ~152g, medium sized banana (Sainsbury's, ~98 kcal). This provided an additional 419 kcal, 67.1g carbohydrate, 13.9g fat, 5.4g protein, 6.6g fibre, resulting in a total intake in the LB+S of approximately 1182 kcal, 200.6g carbohydrate, 29.2g fat, 27.9g protein, 17.2g fibre. This compared with a total energy intake of ~763 kcal in the LB condition and ~140 kcal in the SB condition.

3.3.5 Measures

Subjective appetite was measured using the VIMEC and the widely used visual analogue scale technique (VAS). The VIMEC is a computer programme test, designed and developed in the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, in which the participant is asked to construct a computerised visual meal from an extensive menu, represented by a library of food images. The participant is asked to select the foods that they would opt to consume, should they eat a meal or snack at this moment in time. Selecting no food is an option available. The participant is presented with a screen exhibiting the food items available (see **figure 3.2a**). The participant is free to select up to a maximum of four "main meal" items (from a selection of 17, see Appendix 7), which can be displayed on the meal plate, along with any number of "snack or dessert" items, which are selected individually and displayed separately. Once selections are made, the participant is then presented with a screen consisting of a meal plate on which their selected food items appear. The portion size of each item can then be manipulated individually using sliding bar scales (see **figure 3.2b**). The number of images for each food item varies, depending on the nature of the item, typical portion sizes and the number of food items selected. Typically, however, this number ranges from 10-40 images per food item, allowing for a high resolution. This process is then repeated separately for any "snack or dessert" items selected. Typically, this task took between 30 seconds and 2 minutes to complete.

Once the computerised meal was fully constructed, the meal was saved. The results were analysed and the investigator was able to see which portion size was selected and what number photograph this selection corresponded to. All food images were of a known weight and the food characteristics of each food item were recorded (energy density, macronutrient content). Hence, it was then possible to calculate the nutritional content of the meal.

Subjective appetite was also assessed using the 4-question, 150mm-line VAS test for subjective appetite, addressing “hunger”, “fullness”, “desire to eat” and “expected food intake” (Hill and Blundell, 1982). A composite VAS test score was calculated (hunger score + desire score + expected intake score + (150-fullness score)). This single score was used for the ease of data analysis and presentation. With the original 6 question VAS technique of Hill & Blundell (Hill and Blundell, 1982), the scores for each question co-vary to a large extent (Stubbs *et al.*, 2000) and that the first principal component of the questions is the mean value of the scores (Reid *et al.*, 1998). The two appetite measures, VIMEC and VAS, were completed in a counterbalanced order to negate any order effect.

Energy intake was measured with the use of an *ad libitum* test meal. The content of this test meal was dependant on the food choices made by the participant when using the VIMEC. The food items selected during the measure obtained 60 minutes before the test meal (t=180) were presented for the test meal. At the appetite measure obtained immediately prior to the test meal, the participant was asked not to select any new, additional food items. Participants were accompanied to the Research Kitchen within the School of Sport, Exercise and Rehabilitation Sciences, where they were provided with a dinner plate and a bowl at a table. The food items of the test meal were presented buffet-style on a separate work surface, and of portion-size similar to that of the largest portion available on the VIMEC tool. Participants were instructed to serve the food that they desired to eat from the buffet on to the plate or into the bowl and return to the table to eat. They were informed that they could return for further servings and that more of each food item was available. They were instructed to eat until they felt satisfyingly full. Covertly, each food item

presented was weighed prior to the meal commencing and again at the cessation of eating, with the difference between the two indicating the amount consumed. Subtracted from this was food left remaining on the plate or in the bowl, which was also weighed after the meal. Energy density of all food was known, allowing for the calculation of energy intake.

The reproducibility of VIMEC and VAS was assessed by comparing day-to-day and test-retest reproducibility. Day-to-day comparisons were made between the first measure obtained, prior to the breakfast meal, for each condition. A second comparison was made between the second, post-breakfast measures obtained in the LB and LB+S conditions, as the same breakfast was consumed in each condition. One appetite measure was randomly selected for each participant for a retest measure. In this instance, participants were asked to repeat the measure within 2-3 minutes of the initial measure. These comparisons were made for both the VAS and VIMEC techniques, hence allowing for between-measure comparisons, as well as within-measure comparisons.

3.3.6 Statistical analysis

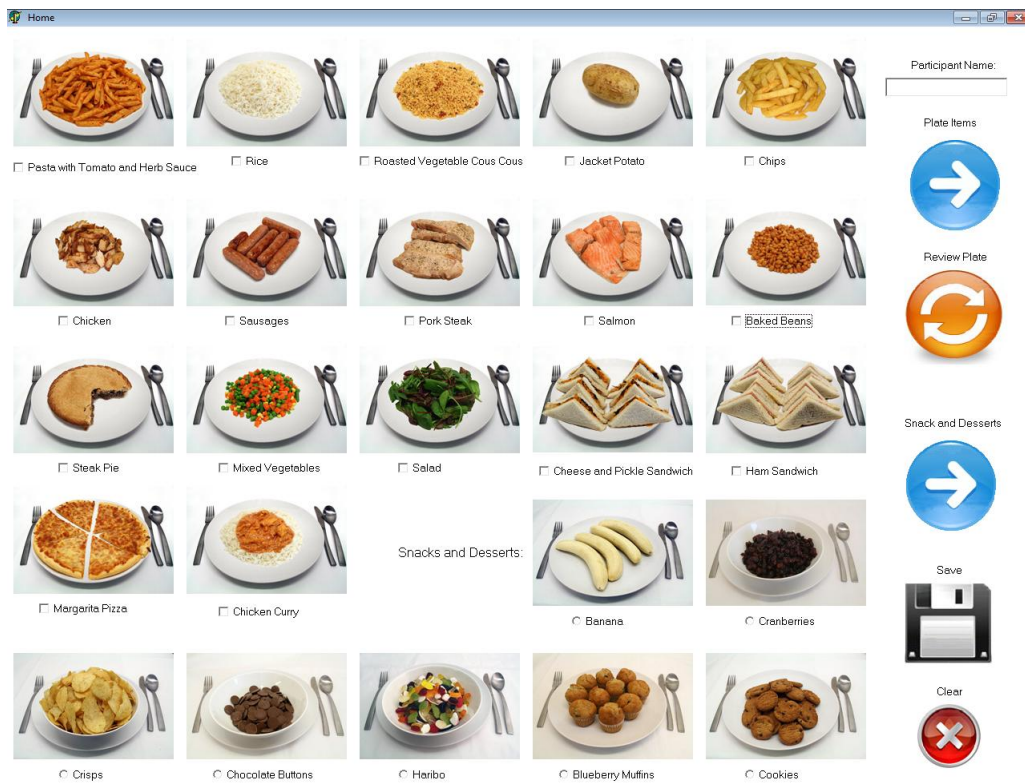
Data are presented as means \pm standard deviation in text and tables and as mean \pm SEM in figures. The mean energy intake values of the test meal for each condition were compared using a one-way, repeated measures ANOVA. To test for sensitivity to change in appetite, appetite scores from the VIMEC and the VAS were both assessed using a 3 (condition: SB, LB, LB+S) \times 7 (time: -30, 0, 60, 120, 180, 240, 300) factorial, repeated-measures ANOVA. Significant main effects and interactions from ANOVA were further assessed by pairwise comparisons using Bonferroni post-hoc analysis. VIMEC appetite scores were also compared with VAS test scores, using Pearson product moment correlation analysis, for all measures obtained within each condition, separately. This was also conducted for between-condition, within-subject comparison, by assessing percentage difference between the conditions (SB – LB+S, SB – LB and LB – LB+S). This approach allows for comparisons of the ability to detect inter-subject changes in appetite.

To assess the ability of the VIMEC to predict between-subject differences in energy intake, appetite scores obtained immediately prior to the test meal were compared with

energy intake at the test meal .To assess the ability of the VIMEC to predict within-subject differences in energy intake, between-condition percentage difference ($SB - LB+S$, $SB - LB$ and $LB - LB+S$) for energy intake, VAS score and VIMEC score was calculated and these differences were compared using correlation analysis. Differences in correlation coefficients were assessed using t-tests for non-independent correlation coefficients.

Day-to-day measures were compared using a one-way, repeated measures ANOVA (pre-breakfast measures, SB vs. LB vs. $LB+S$) and a paired samples t-test (post-breakfast measures, LB vs. $LB+S$). Test-retest measures were compared using a paired samples t-test. The coefficient of variation was calculated for all reproducibility measures, with these coefficient of variation values for the VIMEC and VAS methods compared using paired samples t-tests. A statistical significance level of $p < 0.05$ was used throughout. All statistical analysis was carried out using the SPSS software programme (SPSS inc. Chicago, Illinois, USA).

(a)



(b)

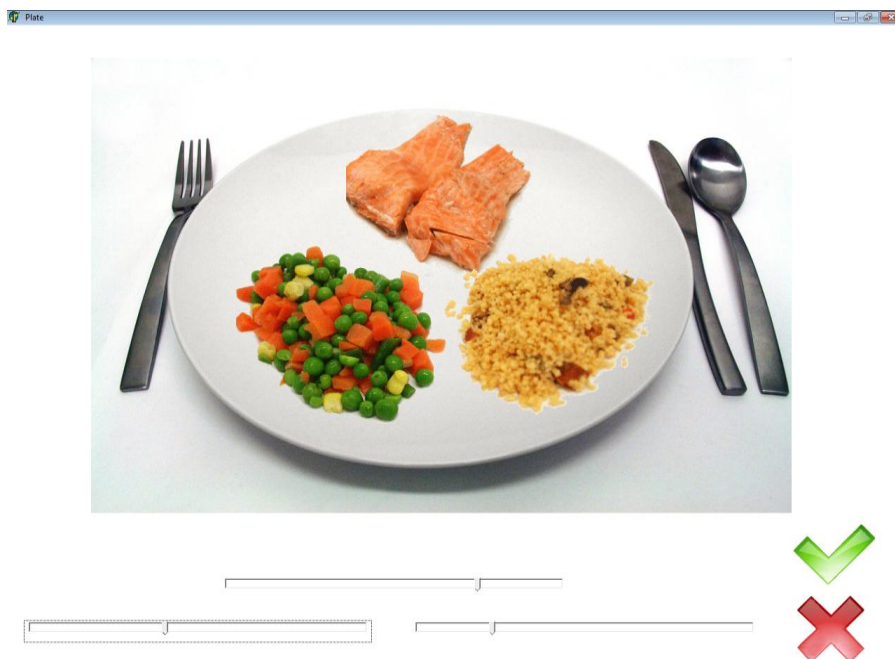


Figure 3.2. The Visual Meal Creator. The menu screen (a) and an example meal (b). Portion size of each item in the meal can be manipulated using the sliding bar scales.

3.4 RESULTS

3.4.1 Energy intake at the test meal

Mean energy intake values at the test meal, for each of the three trial conditions are shown in **figure 3.3**. A significant condition effect was observed for mean energy intake ($F(2) = 8.253$, $p = 0.002$). Pairwise comparisons demonstrated that the mean intake in the LB+S (404 ± 255 kcal) was significantly lower than both mean LB intake (675 ± 313 kcal, $p = 0.003$) and SB intake (786 ± 519 kcal, $p = 0.02$), which did not differ.

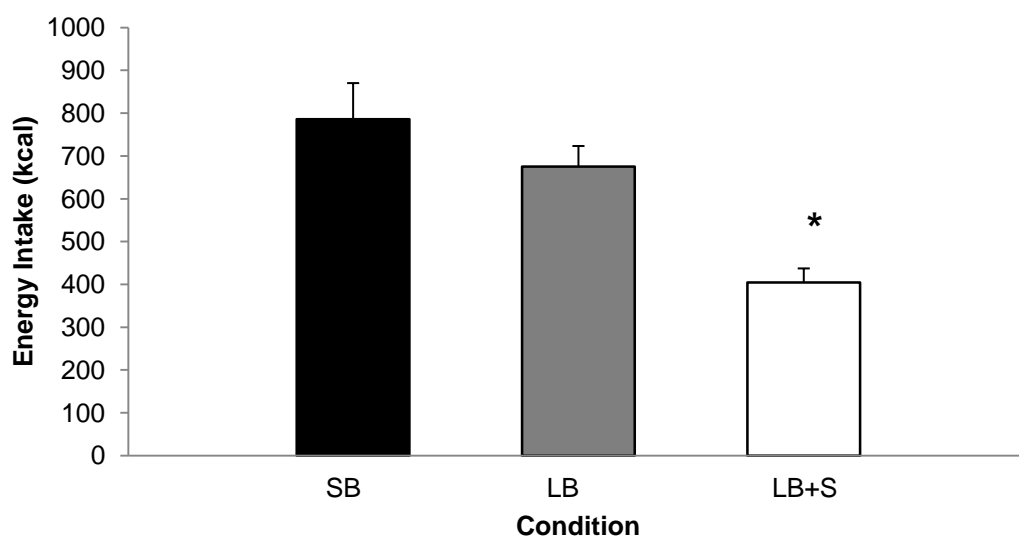


Figure 3.3. Mean energy intake values (\pm SEM) for the SB, LB and LB+S conditions.
* = significantly different to LB and SB.

3.4.2 Subjective appetite scores

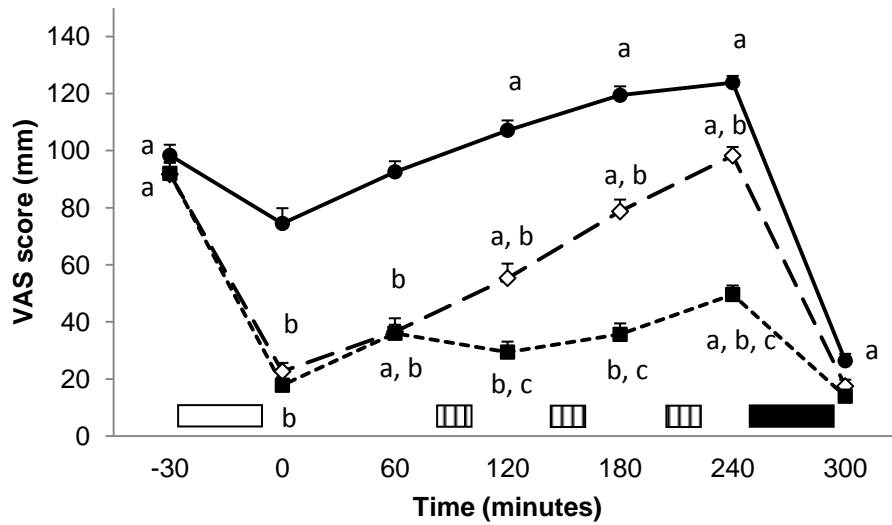
3.4.2.1 VAS

Changes in appetite scores, obtained with the VAS measure, over the trial periods, for each of the three trial conditions are shown in **figure 3.4a**. There were no significant differences at baseline. A factorial, repeated measures ANOVA demonstrated a significant condition \times time interaction effect ($F(12) = 21.039$, $p < 0.001$). Post-hoc pairwise comparisons showed significant within- and between-subject differences, as illustrated in **figure 3.4a**.

3.4.2.2 VIMEC

Changes in appetite scores, obtained with the VIMEC, over the trial periods, for each of the three trial conditions are shown in **figure 3.4b**. VIMEC score was significantly lower in the LB+S condition, compared with SB and LB, at baseline. A factorial, repeated measures ANOVA demonstrated a significant condition x time interaction effect ($F(12) = 6.973$, $p < 0.001$). Pairwise comparisons highlighted significant within- and between condition differences. These are shown in **figure 3.4b**.

(a)



(b)

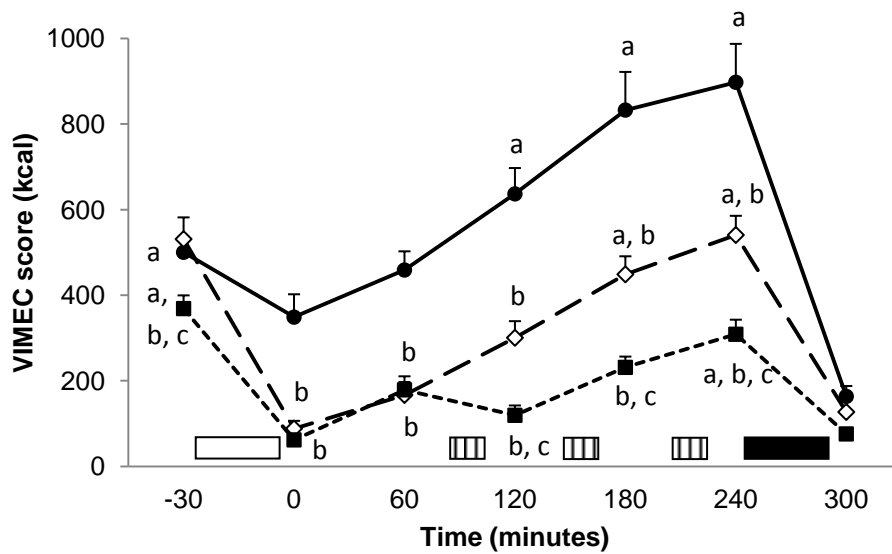


Figure 3.4. Appetite profiles for the SB, LB and LB+S conditions for (a) VAS and (b) VIMEC methods. Values are means \pm SEM. SB (•), LB (◊) and LB+S (■) conditions. Solid line indicates SB, dashed line indicates LB, dotted line indicates LB+S. Hollow rectangle = breakfast meal. Vertical lined rectangles = snacks. Solid black rectangle = *ad libitum* lunch meal. a = within-condition effect, significantly different to t=0. b = between-condition effect, significantly different to SB. c = between-condition effect, significantly different to LB.

3.4.3 Correlation between subjective appetite scores obtained with VAS and VIMEC

Between-subject, within-condition correlations for VAS scores and VIMEC scores were of moderate-strength to strong and statistically significant in each condition (SB, $r = 0.656$, $p < 0.001$; LB, $r = 0.813$, $p < 0.001$; LB+S, $r = 0.673$, $p < 0.001$).

Within-subject, between-condition correlations for percentage difference in VAS and VIMEC scores were also statistically significant, demonstrating moderate-strength correlation (SB – LB+S, $r = 0.570$; SB – LB, $r = 0.526$; LB – LB+S, $r = 0.503$, all $p < 0.001$).

3.4.4 Ability to predict between-subject differences in energy intake

3.4.4.1 VAS

Correlation between VAS scores immediately prior to the lunch test meal and EI at the test meal for each of the three trial conditions are shown in **table 3.1**. Pearson correlation coefficients were significant for LB but not for SB or LB+S.

3.4.4.2 VIMEC

Correlation between VIMEC scores immediately prior to the lunch test meal and EI at the test meal for each of the three trial conditions revealed significant correlation coefficients for all conditions (**table 3.1**).

T-tests for non-independent correlation coefficients revealed that, for each condition, the correlation coefficient for VIMEC vs. EI was significantly greater than for VAS vs. EI (all p values < 0.01).

	VAS vs. EI	VIMEC vs. EI
SB	0.548 ($p=0.065$)	0.951 ($p<0.001$) *
LB	0.632 ($p=0.027$)	0.914 ($p<0.001$) *
LB+S	0.401 ($p=0.196$)	0.875 ($p<0.001$) *

Table 3.1. Pearson product moment correlation coefficients for VAS scores vs. EI and VIMEC scores vs. EI. * = significantly greater than VAS vs. EI, $p < 0.01$. ** = significantly greater than VAS vs. EI, $p < 0.001$.

3.4.5 Ability to predict within-subject differences in energy intake

3.4.5.1 VAS

After the percentage differences in EI and VAS scores between each of the three conditions were calculated, the correlation between EI and VAS scores proved to be weak (table 3.2).

3.4.5.2 VIMEC

Correlation between percentage differences in EI and percentage differences in VIMEC score immediately prior to the test meal across the three conditions proved strong (table 3.2). The correlation coefficients for two of the three comparisons (SB – LB and LB – LB+S) were statistically significant, exhibiting moderate-strength positive correlation ($r = 0.525$, $p = 0.04$) and very strong positive correlation ($r = 0.940$, $p < 0.001$) respectively.

Comparisons of EI vs. VAS correlation with EI vs. VIMEC correlations showed that the correlation between EI and VIMEC was significantly stronger for the SB – LB difference.

	SB - LB+S	SB - LB	LB - LB+S
EI vs. VAS	0.063 ($p = 0.423$)	-0.016 ($p = 0.480$)	0.011 ($p = 0.193$)
EI vs. VIMEC	0.273 ($p = 0.195$)	0.940 ($p < 0.001$)**	0.525 ($p = 0.04$)

Table 3.2. Product moment correlation coefficients for comparison of differences in EI with differences in VAS score and differences in VIMEC score between the three trial conditions. * = EI vs. VIMEC correlation significantly greater than EI vs. VAS correlation, $p < 0.05$, ** = EI vs. VIMEC correlation significantly greater than EI vs. VAS correlation, $p < 0.01$

3.4.6 Reliability and reproducibility of subjective appetite measures

3.4.6.1 Day-to-day measures

Comparisons of measures at $t=-30$ (baseline) showed that there were no significant differences between measures for VAS. There was a significant condition effect for VIMEC scores at baseline ($F(1) = 11.63$, $p = 0.006$), with post hoc analysis demonstrating that VIMEC scores were lower in the LB+S condition (369 ± 214 kcal), compared with both the SB (500 ± 251 kcal, $p = 0.017$) and LB (531 ± 351 kcal, $p = 0.047$) conditions. There were no differences between measures obtained at $t=0$ for the LB and LB+S conditions for either

subjective appetite method. Between-measure comparisons of the coefficient of variation (CV) for t=-30 measures (SB vs. LB vs. LB+S) and CV for mean appetite scores for measures obtained at t=-30, t=0 and t=60 (LB vs. LB+S) were conducted. The mean CV value for VAS measures at t=-30 was significantly lower than that for VIMEC measures ($19.1 \pm 11.7\%$ vs. $32.2 \pm 15.4\%$, $p = 0.033$). There was no significant difference in mean CV values for meaned VAS and VIMEC measures obtained at t=-30, t=0 and t=60 ($23.3 \pm 12.1\%$ for VAS and $25.6 \pm 21.4\%$ for VIMEC, $p = 0.754$).

3.4.6.2 Test-retest measures

Paired sample T-tests comparing the test-retest scores showed that retest measures were similar to initial measures for both VAS and VIMEC methods. Mean CV values were small and did not differ between the two methods ($6.0 \pm 6.1\%$ vs. $5.7 \pm 6.2\%$ for VAS and VIMEC respectively).

3.5 DISCUSSION

The aim of the current study was to assess the validity and reliability of the Visual Meal Creator (VIMEC) as a method for measuring subjective appetite. The VIMEC demonstrated the ability to detect expected changes in subjective appetite, as shown by the appetite profiles. By time point $t=240$, immediately prior to the lunch test meal, the appetite scores were significantly different between each trial condition. This was reflected by a significant trial condition effect for energy intake at the lunch test meal, although it should be noted that intakes in the SB and LB conditions, while differing by 14%, were not significantly different. The appetite profile for VIMEC measures was almost identical to the profile obtained from using the VAS method – a valid, reliable and highly-used method for the measure of subjective appetite. Between-subject, within-condition comparisons of VIMEC and VAS scores demonstrated significant, moderate-strength to strong correlation. Further, between-condition percentage difference for VIMEC and VAS scores demonstrated a moderate-strength relationship. While proving validity for such measures is difficult, this comparison suggests that the VIMEC was performing as intended: providing a quantitative measure of subjective appetite and detecting changes in subjective appetite after dietary manipulation.

The VIMEC showed potential as a predictor of eating behaviour, of which the lunch test meal energy intake acted as a proxy. Correlations between VIMEC scores immediately prior to the test meal and the energy intake values were very strong for each of the three conditions and compared favourably with those for VAS and energy intake, indicating that the VIMEC is a stronger predictor of between-subject differences in energy intake. The correlation for within-subject, between-condition differences in EI and differences in VIMEC scores immediately prior to the test meal was significant and of moderate-strength to strong in two of the three comparisons. This relationship was stronger than that of differences in VAS score and differences in EI for all three comparisons, proving significantly so in one of these cases.

Within appetite research, when the effect of an intervention upon appetite is under investigation, VAS is commonly used in conjunction with objective appetite measures, such as circulating levels of appetite-associated hormones or a measure of eating behaviour, such as *ad libitum* energy intake. In these instances, the correlation between VAS scores and these objective or behavioural measures are rarely assessed, so direct evidence of VAS's strength as a predictor of eating behaviour in such circumstances is not abundant. Nevertheless, it is generally considered that VAS exhibits good predictive strength when more severe interventions are implemented (pharmacological), but when more subtle interventions are in place, such as exercise, the reliability of VAS to predict eating behaviour is poor (Martins *et al.*, 2007; Parker *et al.*, 2004; Thompson *et al.*, 1988). The intervention in the current study was achieved by controlling food intake at breakfast and for the following four hours until lunch in an attempt to manipulate appetite. Under these circumstances, the VIMEC proved a strong predictor of eating behaviour. It remains to be seen whether the VIMEC will prove a strong predictor of eating behaviour within exercise intervention studies.

The correlation coefficients for between-subject, within-condition comparisons of VIMEC score and EI in the present study were extremely high. It is possible that the study design contributed. The food items selected at time point $t=180$ were the items that were presented at the buffet meal. This measure was obtained 60 minutes prior to the lunch test meal, allowing sufficient time for food to be prepared. At $t=240$, immediately prior to the meal, food item selection for the VIMEC was restricted to those items selected at $t=180$. This ensured that the items selected here were those that the participant would be presented with at the lunch test meal, allowing a strong comparison of the amount of each item selected. It was possible that the number of food items selected (and hence made available at the lunch test meal) could have constrained the subsequent energy intake. As a result, the magnitude of correlation could have been artificially inflated, as food variety has been shown to influence energy intake at a meal (Hetherington *et al.*, 2006). Therefore, partial correlations were calculated to remove the influence of the number of food items on the energy intake of the test meal. These partial correlations differed minimally from the original correlation

coefficients (SB: 0.930 vs. 0.951; LB: 0.934 vs. 0.914; LB+S: 0.870 vs. 0.875). Hence, it would appear that the number of food items selected was not a strong predictor of energy intake in this study and did not contribute to the very strong correlation observed between VIMEC score and energy intake.

Stubbs (2000) highlighted the large between-subject variability in subjective appetite measures when using VAS and recommended that the method was therefore more appropriate for within-subject comparisons. Large between-subject variability is not uncommon with appetite measures, including *ad libitum* test meal intakes (Stubbs *et al.*, 1998) due to large biological variation in appetite, food preference and eating behaviour. The between-subject variability of the VIMEC scores immediately prior to the lunch test meal, was large (coefficient of variation (CV) values for 69%, 60% and 76% for SB, LB and LB+S respectively), although not vastly larger than the variability in the energy intake measures (CV values of 66%, 46% and 60% for SB, LB and LB+S respectively). Therefore, as with a number of other subjective appetite and eating behaviour measures, it could be argued from this data that the VIMEC is likely to be best suited to within-subject comparisons and repeated-measure study designs. However, the stronger correlation between VIMEC score and energy intake for between-subject differences, compared with within-subject changes, may suggest that the VIMEC is better suited to assessing between-subject comparisons of subjective appetite and predicted food intake.

The VAS has previously been shown to exhibit good test-retest reproducibility (Flint *et al.*, 2000; Stratton *et al.*, 1998), but considerably poorer day-to-day reproducibility (Flint *et al.*, 2000; Raben *et al.*, 1995; Stratton *et al.*, 1998). The results of the current study would suggest that the VIMEC exhibits a similar degree of test-retest reproducibility, with CV values very comparable to those observed with VAS. While the CV for day-to-day repeated measures at $t=-30$ was significantly higher than VAS, suggesting poorer day-to-day reproducibility when using the VIMEC, the mean VIMEC scores for measure $t=-30$, $t=0$ and $t=60$ for the LB and LB+S conditions were similar, suggesting a similar degree of reproducibility. One would perhaps have expected a greater degree of variation with the

VIMEC, due to the option of choosing different food items of different energy densities. This large degree of choice, allowing for the selection of vastly different meal creations would lend itself to large variations in the measure. It should also be noted that, with any variation in day-to-day measure, it is difficult to disentangle the contribution of biological and methodological variation, especially when obtaining subjective measures, prone to variation (Flint *et al.*, 2000).

There were, however, significant differences in baseline values for VIMEC, between the LB+S condition and LB condition and between LB+S and SB. This questions the day-to-day reproducibility. Inspection of the data would suggest that this was not driven by a single or small number of outliers. Other than the previously mentioned large degree of choice and consequent increased likelihood of variability, and the biological variation in appetite sensations from day-to-day, it is difficult to explain this observation. Food intake during the 24 hours prior to each trial was controlled by asking participants to record their dietary intake on the day prior to their first trial, then asking them to repeat this intake on the day before subsequent trials. It is possible that this was not well adhered to and that differences in dietary intake on the day prior to trials may have influenced baseline appetite measures.

When obtaining a subjective appetite measure using the VIMEC, there is an upper limit to the portion size available. This maximum portion is dependent on the food item and, for the main meal items, the number of food items selected. To alleviate this limitation, participants were informed that, should they desire more than the upper limit, they could save the current measure, clear the screen and complete a second measure for any additional food desired. While this option is not ideal, with the participant unable to visualise their entire meal creation, it does allow for unlimited portion size selection. In the present study, no participant chose to complete a second test for any measure. In addition, the 252 measures obtained in total resulted in 564 different food item selections. Only 31 times (5.5%) were maximum portions selected (15 x salad). In addition, 26 of these 31 maximum portion selections occurred during instances where the participant selected 4 or more food items in the measure, when space on the plate for individual food item portions was limited.

We are therefore confident that the VIMEC does not substantially restrict the upper limit of a subjective appetite measure.

While the use of photographic images of food is not a new concept within the area of appetite research, the VIMEC is, to our knowledge, the first subjective appetite tool that allows for the user to create a whole meal. Similar tools have asked users to select a desired portion size of a range of individual food items (Sadoul *et al.*, 2012) or a mixture of individual items and ready-made meals (Farah *et al.*, 2012), showing potential as useful appetite measures. However, in neither of these studies did the technique demonstrate a relationship between desired portion size and *ad libitum* food intake. The progressive step evident with the VIMEC, allowing for the creation of a meal from an extensive menu of food items allows a stronger measure of food choice and preference that is limited with the aforementioned format of other tools. It is also possible that the more sophisticated nature of the VIMEC allows for a stronger prediction of feeding behaviour, as is supported by the findings of the current study.

One limitation that persists with the VIMEC, is that, when investigating the effects of exercise on appetite, it would prove very difficult to obtain an appetite measure using the VIMEC while exercising. While the validity and relevance of during-exercise measures could be considered questionable in circumstances other than very prolonged exercise bouts, such measures are often obtained and can be informative. These measures can be obtained with the VAS method but due to the time, equipment and dexterity required to obtain a VIMEC measure, using the VIMEC is unlikely to be feasible during exercise, particularly exercise involving movement of the trunk and upper body.

In conclusion, the Visual Meal Creator demonstrates the potential to be a strong predictor of between-subject difference in energy intake and a moderate strength predictor of within- subject differences in energy intake. Test-retest reproducibility was good. Day-to-day reproducibility was quite large, but this may be due to the large degree of food choice allowable with the VIMEC. In comparison with the VAS technique, the VIMEC proved equally as proficient at detecting expected changes in subjective appetite, while exhibiting a similar

degree of reproducibility. There was evidence that the VIMEC was a significantly stronger predictor of energy intake – a fundamental aspect of eating behaviour – than VAS. Therefore, the VIMEC demonstrates potential as a preferable tool for the measurement of subjective appetite.

3.6 REFERENCES

- Arvaniti, K., Richard, D., and Tremblay, A. (2000). Reproducibility of energy and macronutrient intake and related substrate oxidation rates in a buffet-type meal. *The British journal of nutrition* 83, 489-495.
- Bellisle, F. (1999). Food choice, appetite and physical activity. *Public Health Nutrition* 2, 357-361.
- Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., Van Der Knaap, H., *et al.* (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity Reviews* 11, 251-270.
- Brunstrom, J.M., and Rogers, P.J. (2009). How many calories are on our plate? Expected fullness, not liking, determines meal-size selection. *Obesity* 17, 1884-1890.
- Brunstrom, J.M., Rogers, P.J., Pothos, E.M., Calitri, R., and Tapper, K. (2008a). Estimating everyday portion size using a "method of constant stimuli" in a student sample, portion size is predicted by gender, dietary behaviour, and hunger, but not BMI. *Appetite* 51, 296-301.
- Brunstrom, J.M., and Shakeshaft, N.G. (2009). Measuring affective (liking) and non-affective (expected satiety) determinants of portion size and food reward. *Appetite* 52, 108-114.
- Brunstrom, J.M., Shakeshaft, N.G., and Scott-Samuel, N.E. (2008b). Measuring "expected satiety" in a range of common foods using a method of constant stimuli. *Appetite* 51, 604-614.
- Farah, N.M.F., Brunstrom, J.M., and Gill, J.M.R. (2012). Using a novel computer-based approach to assess the acute effects of exercise on appetite-related measures. *Appetite* 58, 196-204.
- Flint, A., Raben, A., Blundell, J.E., and Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* 24, 38-48.
- Frobisher, C., and Maxwell, S.M. (2003). The estimation of food portion sizes: a comparison between using descriptions of portion sizes and a photographic food atlas by children and adults. *Journal of Human Nutrition and Dietetics* 16, 181-188.
- George, V.A., and Morganstein, A. (2003). Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite* 40, 43-46.
- Gregersen, N.T., Flint, A., Bitz, C., Blundell, J.E., Raben, A., and Astrup, A. (2008). Reproducibility and power of *ad libitum* energy intake assessed by repeated single meals. *The American Journal of Clinical Nutrition* 87, 1277-1281.
- Hetherington, M.M., Foster, R., Newman, T., Anderson, A.S., and Norton, G. (2006). Understanding variety: Tasting different foods delays satiation. *Physiology & Behavior* 87, 263-271.
- Hill, A.J., and Blundell, J.E. (1982). Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research* 17, 203-212.

- King, N.A., Caudwell, P.P., Hopkins, M., Stubbs, J.R., Naslund, E., and Blundell, J.E. (2009). Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. *The American Journal of Clinical Nutrition* 90, 921-927.
- King, N.A., Tremblay, A., and Blundell, J.E. (1997). Effects of exercise on appetite control: implications for energy balance. *Medicine and science in sports and exercise* 29, 1076-1089.
- Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 193, 251-258.
- Martins, C., Morgan, L., and Truby, H. (2008). A review of the effects of exercise on appetite regulation: an obesity perspective. *Int J Obes* 32, 1337-1347.
- Nelson, M., and Haraldsdóttir, J. (1998). Food photographs: practical guidelines I. Design and analysis of studies to validate portion size estimates. *Public Health Nutrition* 1, 219-230.
- Parker, B.A., Sturm, K., MacIntosh, C.G., Feinle, C., Horowitz, M., and Chapman, I.M. (2004). Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr* 58, 212-218.
- Raben, A., Tagliabue, A., and Astrup, A. (1995). The reproducibility of subjective appetite scores. *The British journal of nutrition* 73, 517-530.
- Reid, C.H., CG. Blundell, JE. Stubbs, RJ (1998). What are psychometric assessments of appetite asking: a preliminary multivariate analysis. *International Journal of Obesity* 22, 151.
- Riley, W.T., Beasley, J., Sowell, A., and Behar, A. (2007). Effects of a web-based food portion training program on food portion estimation. *Journal of Nutrition Education and Behavior* 39, 70-76.
- Rolls, B.J., Morris, E.L., and Roe, L.S. (2002). Portion size of food affects energy intake in normal-weight and overweight men and women. *American Journal of Clinical Nutrition* 76, 1207-1213.
- Sadoul, B.C., Schuring, E.A.H., Symersky, T., Mela, D.J., Masclee, A.A.M., and Peters, H.P.F. (2012). Measuring satiety with pictures compared to visual analogue scales. An exploratory study. *Appetite* 58, 414-417.
- Stratton, R.J., Stubbs, R.J., Hughes, D., King, N., Blundell, J.E., and Elia, M. (1998). Comparison of the traditional paper visual analogue scale questionnaire with an Apple Newton electronic appetite rating system (EARS) in free living subjects feeding *ad libitum*. *European Journal of Clinical Nutrition* 52, 737-741.
- Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., and Blundell, J.E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* 84, 405-415.
- Stubbs, R.J., Johnstone, A.M., O'Reilly, L.M., and Poppitt, S.D. (1998). Methodological issues relating to the measurement of food, energy and nutrient intake in human laboratory-based studies. *Proc Nutr Soc* 57, 357-372.

Subar, A.F., Crafts, J., Zimmerman, T.P., Wilson, M., Mittl, B., Islam, N.G., McNutt, S., Potischman, N., Buday, R., Hull, S.G., *et al.* (2010). Assessment of the accuracy of portion size reports using computer-based food photographs aids in the development of an automated self-administered 24-hour recall. *Journal of the American Dietetic Association* 110, 55-64.

Thompson, D.A., Wolfe, L.A., and Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medicine & Science in Sports & Exercise* 20, 222-227.

Turconi, G., Guarcello, M., Berzolari, F.G., Carolei, A., Bazzano, R., and Roggi, C. (2005). An evaluation of a colour food photography atlas as a tool for quantifying food portion size in epidemiological dietary surveys. *Eur J Clin Nutr* 59, 923-931.

Venter, C.S., MacIntyre, U.E., and Vorster, H.H. (2000). The development and testing of a food portion photograph book for use in an African population. *Journal of Human Nutrition and Dietetics* 13, 205-218.

Wansink, B., Painter, J.E., and North, J. (2005). Bottomless Bowls: Why visual cues of portion size may influence intake[ast][ast]. *Obesity* 13, 93-100.

Williamson, D.A., Allen, H.R., Martin, P.D., Alfonso, A.J., Gerald, B., and Hunt, A. (2003). Comparison of digital photography to weighed and visual estimation of portion sizes. *Journal of the American Dietetic Association* 103, 1139-1145.

Yeomans, M.R., Lee, M.D., Gray, R.W., and French, S.J. (2001). Effects of test-meal palatability on compensatory eating following disguised fat and carbohydrate preloads. *International Journal of Obesity* 25, 1215-1224.

The effect of exercise intensity on subjective appetite, food intake and appetite associated hormones in highly-trained male endurance athletes.

4.1 ABSTRACT

Background: Aerobic exercise of a high intensity ($\geq 60\%$ VO_{2max}) has been shown to elicit a transient suppression of appetite. This is commonly coupled with anorexigenic alterations in the profile of appetite-associated hormones, but little effect on subsequent food intake is usually observed. As research has primarily been undertaken in low- to moderately-active populations, the appetite response to exercise of differing intensities in highly-trained individuals is unknown. In addition, sustained, continuous bouts of aerobic exercise of an intensity $>70\%$ VO_{2max} have rarely been utilised, due to the limited exercise capacity of non-athletic populations.

Purpose: To investigate the effect of exercise of different intensities on subjective appetite, satiety peptides and food intake, studying a highly-trained population and therefore allowing for the utilisation of a strenuous, high-intensity exercise condition.

Method: Twelve highly-trained (≥ 6 hours of training per week) male athletes (age = 25 ± 7 years; BMI = 22.4 ± 1.7 $kg \cdot m^{-2}$; VO_{2max} = 60.8 ± 4.2 $ml \cdot min^{-1} \cdot kg^{-1}$) completed three exercise trials on a cycle ergometer in a counterbalanced, within-subject design: low-intensity ($47 \pm 5\%$ VO_{2max} – LOW); moderate-intensity ($63 \pm 5\%$ VO_{2max} – MOD) and high-intensity ($80 \pm 4\%$ VO_{2max}). Exercise was matched for an individualised energy expenditure target, meaning that the LOW, MOD and HIGH bouts lasted for a duration of approximately 60, 40 and 30 minutes respectively. Upon completion of exercise, participants rested for 60 minutes, with appetite measures and blood sampling every 20 minutes. At 60 minutes post-exercise, an *ad libitum* breakfast meal was consumed, with energy intake covertly recorded. Post-breakfast appetite was recorded and a final blood sample obtained. Plasma was analysed for pancreatic polypeptide (PP) and polypeptide YY (PYY) concentrations.

Results: VAS score for appetite was significantly lower after HIGH ($74.8 \pm 26.7mm$) compared with LOW ($102.3 \pm 26.3mm$, $p < 0.001$) and MOD ($97.8 \pm 30.7mm$, $p = 0.009$) immediately post-exercise and also significantly lower after HIGH compared with LOW ($100.7 \pm 23.4mm$ vs. $113.4 \pm 20.4mm$, $p = 0.006$) 20 minutes post-exercise. There were no

significant differences in PP or PYY concentration between conditions, neither was there a difference pre- versus post-exercise. Energy intake at the *ad libitum* breakfast meal did not differ between conditions.

Conclusion: Subjective appetite was transiently suppressed after high-intensity exercise in highly-trained male endurance athletes. However, no such suppression was observed after exercise at 60% $\text{VO}_{2\text{max}}$ – an intensity commonly seen to elicit a transient appetite suppression in non-athletic populations. The observed suppression was independent of significant changes in satiety peptide concentration and the transient nature resulted in no effect on food intake. This would suggest that athletes may exhibit some resistance to an exercise-induced suppression of appetite at an intensity lower than 80% $\text{VO}_{2\text{max}}$, while also questioning the regulatory role of PP and PYY for subjective appetite in the post-exercise period.

4.2 INTRODUCTION

The short-term coupling between exercise-induced energy expenditure and intake is weak, advocating the absence of a fast-acting compensatory mechanism by which energy intake is upregulated after physical activity (King *et al.*, 1997a; King *et al.*, 1996; Lluch *et al.*, 2000). In fact, it would appear that an acute bout of aerobic exercise of a sufficiently high intensity ($\geq 60\%$ $\text{VO}_{2\text{max}}$), can transiently suppress appetite (Broom *et al.*, 2009; Broom *et al.*, 2007; Burns *et al.*, 2007; Kawano *et al.*, 2013; King *et al.*, 2010; King *et al.*, 1994; Kissileff *et al.*, 1990; Thompson *et al.*, 1988; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b; Westerterp-Plantenga *et al.*, 1997). This is often accompanied by increases in anorexigenic hormones such as pancreatic polypeptide (PP), glucagon-like peptide-1 (GLP-1) and polypeptide YY (PYY) (Broom *et al.*, 2009; King *et al.*, 2011a; Martins *et al.*, 2007; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b), which has led to suggestions that this may be a mechanism which underpins an exercise-induced suppression of appetite. The relative time-courses for changes in hormonal profiles, subjective appetite and food intake remain unknown; hence, the influence of exercise-induced changes in circulating concentrations of appetite-associated hormones on appetite and food intake remains relatively unclear.

As this exercise-induced suppression of appetite, termed “anorexia of exercise” (King *et al.*, 1994) is short-lived, a subsequent reduction in post-exercise food intake has often been reported only when food is available within close proximity (~15-30 minutes) to the cessation of exercise (Kissileff *et al.*, 1990; Westerterp-Plantenga *et al.*, 1997). Any such suppression of appetite has rarely been seen to reduce food intake when test meals are administered 60 minutes post-exercise (Thompson *et al.*, 1988), although the exact duration of this transient suppression, and whether it can vary depending upon the nature of the exercise and the population, is unclear.

To date, the effect of exercise on appetite has rarely been assessed using athletic populations. Highly trained athletes regularly complete very strenuous, high energy cost bouts of exercise. After completing these sessions, post-exercise nutrition is often

considered of vital importance to optimise recovery and adaptation to training (Burke, 1997). Further, many endurance athletes value weight management highly (Filaire *et al.*, 2007), as an increase in body weight can result in an increase in the energy cost of performing. The majority of investigations into the effect of exercise on appetite have been conducted with overweight and obese, healthy-weight sedentary or moderately-active individuals. Further, partly due to the difficulty of non-athletic populations exercising at a high intensity for prolonged periods, few studies have used “high intensity” exercise bouts of greater than 60-70% $\text{VO}_{2\text{max}}$ (Imbeault *et al.*, 1997; Kissileff *et al.*, 1990; Pomerleau *et al.*, 2004; Ueda *et al.*, 2009b; Westerterp-Plantenga *et al.*, 1997) and exercise bouts used have often yielded relatively low energy expenditure values (e.g., ~85 kcal (350 kJ) (Pomerleau *et al.*, 2004); ~145- 200 kcal (George and Morganstein, 2003); ~240 kcal (Kissileff *et al.*, 1990)). Studies conducted within active individuals have failed to observe a suppression of appetite after 60 minutes of treadmill running at 70% $\text{VO}_{2\text{max}}$ (King *et al.*, 2010; King *et al.*, 2011a), while in the few investigations that have addressed the effect of exercise on measures of appetite in athletic populations, the focus has primarily been on hormone measures and no measures of subjective appetite or food intake have been obtained (Jurimae *et al.*, 2006; Jurimae and Jurimae, 2005; Jurimae *et al.*, 2007; Jürimäe *et al.*, 2003; Jurimae *et al.*, 2009; O'Connor *et al.*, 1995; O'Connor *et al.*, 2006; Russel *et al.*, 2009).

The purpose of the current study was to address the effect of exercise intensity on subjective appetite, food intake and circulating concentrations of appetite-associated hormones in highly trained endurance athletes, utilising a strenuous exercise protocol. It was hypothesised that a transient exercise-induced suppression of appetite would be observed after moderate- and high-intensity exercise, with a greater, longer lasting suppression evident after high-intensity exercise leading to a lower post-exercise energy intake in this condition.

4.3 METHOD

4.3.1 Participants

Twelve healthy-weight, highly trained male endurance athletes were recruited principally from the University of Birmingham cycling, triathlon and athletics clubs. The participant characteristics are shown in **Table 4.1**. Inclusion criteria were: a minimum total training duration of 6 hours per week and aged between 18 and 40 years. Exclusion criteria were: a score of 3.5 or greater for restricted eating on the Dutch Eating Behaviour Questionnaire (DEBQ, van Strien *et al.*, 1986, Appendix 4); illness such as upper respiratory tract infections; smoking and the taking of medication likely to affect appetite or induce weight-loss. Ethical approval was obtained from the Ethics Subcommittee of the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. One participant suffered from a phobia of needles, so blood samples were not obtained. As a result, hormone analysis was conducted for eleven participants.

Age (years)	25 ± 7
BMI (kg•m⁻²)	22.4 ± 1.7
VO_{2max} (mL•kg⁻¹•min⁻¹)	60.8 ± 4.1
W_{max} (Watts)	322 ± 55
DEBQ score for restraint	1.4 ± 0.1

Table 4.1. Participant characteristics. Values are mean ± SD.

4.3.2 Experimental trial conditions

Participants were randomly assigned to each exercise intensity condition: high- (HIGH, 80% VO_{2max}), moderate- (MOD, 60% VO_{2max}) and low- (LOW, 40% VO_{2max}) intensity exercise, with measures of subjective appetite and circulating concentrations of peptide tyrosine tyrosine (PYY) and pancreatic polypeptide (PP) recorded throughout each trial.

4.3.3 Procedure & protocol

After a minimum period of 3 days after pre-testing (see General methods, section 2.2.2.1, p70), participants returned to the laboratory at approximately 08:00 after an overnight fast for the first of three exercise trials. Upon arrival, the participants were weighed (weight was recorded at each visit to ensure participants were weight-stable throughout). A resting blood sample was obtained following the insertion of a venous cannula into the antecubital vein of the arm by a qualified member of staff. The participant then completed the pre-exercise tests of subjective appetite. Once the tests were completed, the exercise trial commenced. This consisted of a bout of exercise on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) at an intensity of 40%, 60% or 80% $\text{VO}_{2\text{max}}$, for a duration that elicited an individualised, standardised energy expenditure. This individual energy expenditure target equated to approximately 60, 40 and 30 minutes at each of the respective exercise intensities. This allowed for intra-subject standardisation of energy expenditure, while avoiding large inter-subject variation in exercise duration that would have been unavoidable had a generic, standardised energy target been used. During each exercise bout, exhaled gas samples were obtained intermittently to monitor VO_2 and to retrospectively calculate energy expenditure. A blood sample was obtained at the half way point of the exercise trial. Upon reaching the energy expenditure target, the exercise ceased. Measures of subjective appetite were completed and a blood sample was obtained immediately, as the participant sat resting. This resting period continued, with the participant sat reading or watching television, for a duration of 60 minutes to allow for subsequent repetitions of appetite measures and repeated blood sampling at 20, 40 and 60 minutes post-exercise. At 60 minutes post-exercise, the participant consumed an *ad libitum* intake test meal, as described in General methods, section 2.2.3.2, (p73).

4.3.4 Blood sampling and analysis

The blood sampling and plasma analysis techniques are described in General methods, section 2.2.3.5, (p76). Briefly, blood samples were obtained and immediately transferred to disodium EDTA-treated tubes for analysis of hormones. Blood was centrifuged

at 3500 RPM and a temperature of 4°C for 15 minutes to isolate plasma. Plasma was stored at a temperature of -70°C until hormone assays for PYY and PP were conducted.

4.3.5 Statistical analysis

For the determination of differences in energy intake from the test meal between each exercise condition, a one-way analysis of variance (ANOVA) with repeated measures was carried out. To compare differences in both subjective appetite and plasma concentration of appetite-associated hormones with time and between trial conditions, a 2-way factorial ANOVA with repeated measures was conducted. Post-hoc pairwise comparisons were conducted using the Bonferroni correction for multiple comparisons. In the event of a significant difference between conditions at baseline in any outcome measure, percentage change from baseline (%Δ) was calculated and analysis repeated with these calculated values. Area under the curve (AUC) was calculated for all profiles plotted, using the trapezoidal method. AUC values were then compared using a one-way ANOVA with repeated measures.

In an attempt to further elucidate the relationship between changes in appetite hormones and subjective appetite with exercise, correlation analysis was conducted using Pearson product moment correlation. This was conducted for comparisons of within-subject, between-condition changes. Between-condition differences (HIGH – LOW, HIGH – MOD, MOD – LOW) for VAS score, BPAR score, PP concentration and PYY concentration were calculated for percentage change from baseline score (%Δ, calculated for values at baseline and at $t = 0$). Changes in PP and PYY concentration were correlated with both VAS and BPAR values. Between condition percentage differences were calculated for measures obtained immediately prior to the *ad libitum* test meal and for energy intake values, with percentage change in PP and PYY and percentage changes in VAS and BPAR correlated with percentage changes in energy intake.

4.4 RESULTS

4.4.1 Exercise trials

Physiological measures of each exercise trial condition are shown in **table 4.2**. As intended, the exercise intensity (VO_2) was significantly different across trials ($F(2) = 112.689$, $p < 0.001$), with pairwise comparisons showing that each trial was different to the other two ($p < 0.001$). Percentage $\text{VO}_{2\text{max}}$, rating of perceived exertion, heart rate and percentage of maximum heart rate were also significantly different across all three conditions (all $p < 0.001$). There was also a condition main effect for energy expenditure ($F(2) = 14.114$, $p < 0.001$). Pairwise comparisons demonstrated that the energy expenditure of LOW was significantly greater than that of MOD ($p = 0.008$) and HIGH ($p = 0.007$).

	LOW	MOD	HIGH
Energy Expenditure (kcal)	599 ± 67 *	531 ± 62	509 ± 64
$\text{VO}_2 - (\text{L} \cdot \text{min}^{-1})$	2.03 ± 0.23 *	2.71 ± 0.32 *	3.47 ± 0.44 *
% $\text{VO}_{2\text{max}}$	47 ± 5 *	63 ± 5 *	80 ± 4 *
Perceived Exertion	9 ± 1 *	12 ± 1 *	16 ± 1 *
Heart rate – (bpm)	104 ± 6 *	130 ± 8 *	158 ± 7 *
% HR_{max}	58 ± 3 *	73 ± 3 *	89 ± 3 *

Table 4.2. Characteristics of the exercise bouts. Values are mean ± SD. * = significantly different to the other two conditions, $p < 0.001$.

4.4.2 Subjective appetite

4.4.2.1 VAS

The mean subjective appetite profile for each exercise intensity condition is shown in **figure 4.1**. There was no difference in baseline measures of appetite between the three exercise intensity conditions. The results of a factorial repeated measures ANOVA showed that there was a significant exercise intensity x time interaction effect ($F(10) = 3.653$, $p < 0.001$). Post hoc pairwise comparisons highlighted that appetite scores were significantly lower after HIGH ($75 \pm 27\text{mm}$) compared with LOW ($102 \pm 26\text{mm}$, $p < 0.001$) and MOD ($98 \pm 31\text{mm}$, $p = 0.009$) at time $t=0$ and also significantly lower after HIGH compared with LOW ($101 \pm 23\text{mm}$ vs. $113 \pm 21\text{mm}$, $p = 0.006$) at time $t=20$. Appetite scores were similar across

all three conditions by 40 minutes post-exercise and remained so for the rest of the test period.

Within-condition analysis showed that there were no significant differences in VAS scores immediately post-exercise, compared with baseline in any of the three trials. Appetite was significantly lower after exercise in the HIGH condition ($p = 0.035$). However, after the implementation of the Bonferroni adjustment, this difference was not significant. While in LOW and MOD, appetite scores rose throughout the trial period, until the test meal, with scores significantly greater than baseline by $t=20$ in MOD ($111 \pm 26\text{mm}$ vs. $101 \pm 26\text{mm}$, $p = 0.015$) and by $t=40$ in LOW ($120 \pm 15\text{mm}$ vs. $97 \pm 22\text{mm}$, $p = 0.04$), a non-significant decrease in appetite score with exercise in HIGH ($100 \pm 21\text{mm}$ vs. $75 \pm 27\text{mm}$) attenuated this increase throughout the trial, meaning that VAS score was not significantly greater than baseline until $t=60$ ($124 \pm 17\text{mm}$ vs. $100 \pm 21\text{mm}$, $p = 0.025$).

Area under the curve (AUC) analysis (data not presented) was conducted for the appetite profiles. There was a significant condition main effect for AUC for the entire testing period ($F(2) = 4.264$, $p = 0.027$). Post hoc pairwise comparisons showed that AUC was significantly lower in HIGH compared with LOW ($p = 0.007$).

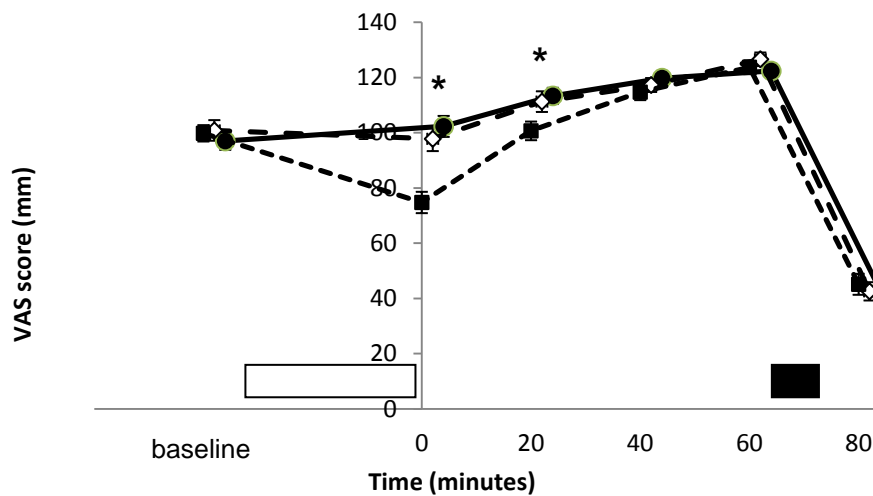


Figure 4.1 - Mean appetite scores (\pm SEM) with the VAS method for LOW (●), MOD (◇) and HIGH (■) conditions. Solid line indicates LOW, dashed line indicates MOD, dotted line indicates HIGH. Hollow rectangle indicates exercise period, solid shaded rectangle indicates *ad libitum* test meal. * = significantly condition effect, $p < 0.05$.

4.4.2.2 BPAR

The mean subjective appetite profile for each exercise intensity condition is shown in **figure 4.2**. There was no difference in baseline measures of appetite between the three exercise intensity conditions. The results of a factorial repeat measures ANOVA showed that there was a significant exercise intensity \times time interaction ($F(4.741) = 4.264$, $p = 0.003$). Appetite was significantly lower for HIGH (336 ± 211 kcal) compared with LOW (455 ± 231 kcal, $p = 0.001$) and MOD (427 ± 215 kcal, $p = 0.004$) at time $t=0$ and remained significantly lower for HIGH vs. LOW (419 ± 213 kcal vs. 545 ± 213 kcal, $p = 0.010$) at 20 minutes post-exercise. Within-condition analysis showed no significant difference in BPAR scores immediately post-exercise, compared with baseline in any of the three trials. In all trials, appetite scores rose throughout the trial period, until the test meal, with scores significantly greater than baseline by $t=20$ (545 ± 213 kcal vs. 362 ± 203 kcal, $p = 0.003$) in LOW. This rise was attenuated in both MOD and HIGH, with BPAR score only significantly higher than baseline at $t=60$ in HIGH (529 ± 228 kcal vs. 431 ± 190 kcal, $p = 0.003$).

AUC analysis (data not presented) demonstrated that there was a significant main effect for exercise intensity condition, ($F(2) = 4.146$, $p = 0.030$). Pairwise comparisons demonstrated that the AUC was lower after HIGH compared with LOW, with this difference approaching significance ($p = 0.077$).

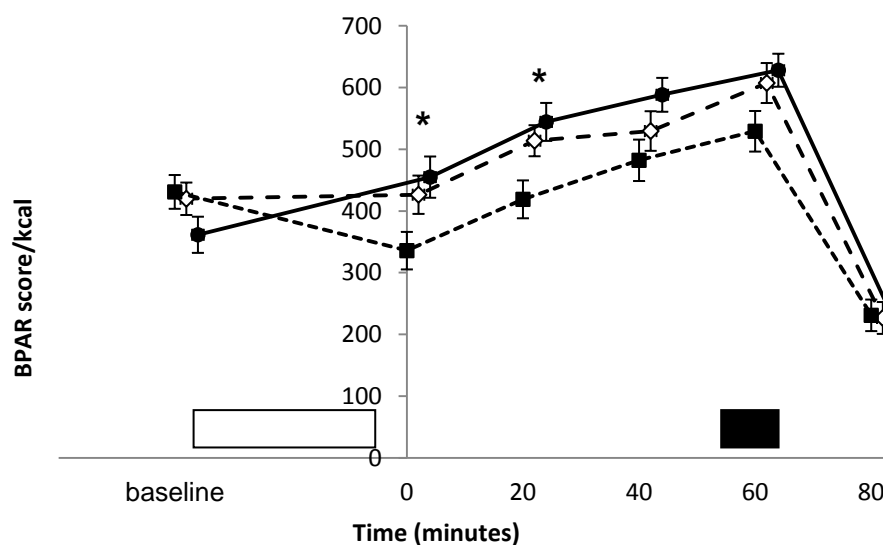
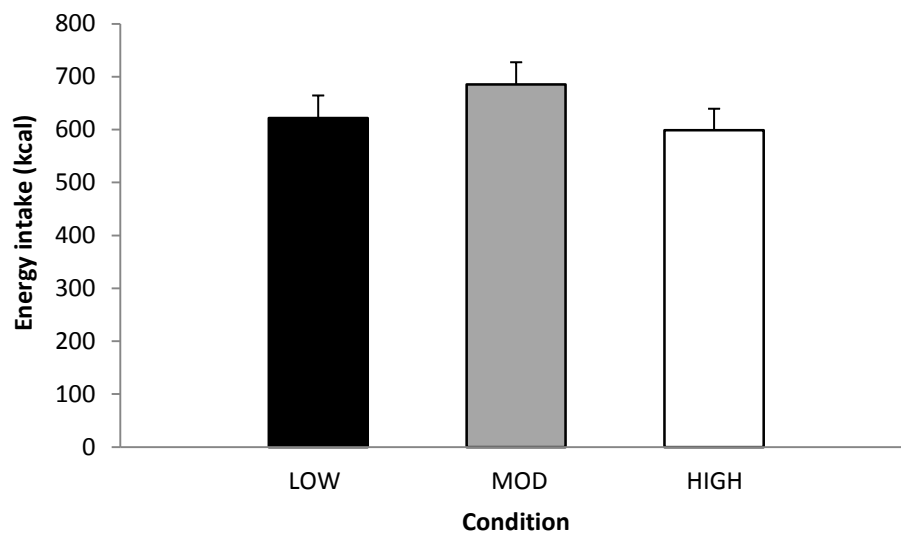


Figure 4.2 - Mean appetite scores (\pm SEM) with the BPAP for LOW (\bullet), MOD (\diamond) and HIGH (\blacksquare) conditions. Solid line indicates LOW, dashed line indicates MOD, dotted line indicates HIGH. Hollow rectangle indicates exercise period, solid shaded rectangle indicates *ad libitum* test meal. * = significant condition effect.

4.4.3 Energy intake at *ad libitum* test meal

The mean energy intake values for each exercise intensity condition are shown in **figure 4.3a**. There was no significant difference between the three trial conditions ($F(2) = 0.596$, $p = 0.560$). When accounting for the energy expenditure of the exercise bout, relative energy intake (energy intake – energy expenditure) was also not significantly different between conditions ($F(2) = 1.196$, $p = 0.323$; **figure 4.3b**).

(a)



(b)

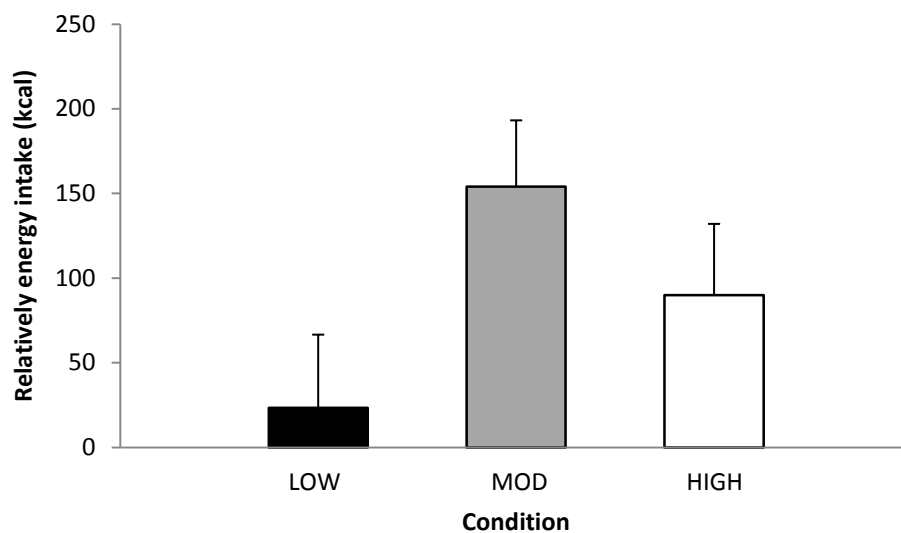


Figure 4.3 - Mean energy intake (a) and relative energy intake (b) values (\pm SEM) for LOW, MOD and HIGH conditions.

4.4.4 Satiety peptide concentration

4.4.4.1 PP

The mean plasma PP concentrations for each exercise trial condition are shown in **figure 4.4**. There were no significant differences in PP concentrations at baseline. A factorial repeated measures ANOVA showed no significant time x condition interaction effect. The

condition main effect showed a trend for higher PP concentrations in the HIGH condition, particularly compared with the LOW condition ($p = 0.106$). Further inspection of a significant ($p < 0.001$) time main effect highlighted that PP concentration was significantly higher during exercise and immediately post-exercise, compared with baseline ($p = 0.005$ and $p = 0.007$ respectively). PP concentration was also significantly higher post-test meal, compared with all pre-meal measures. AUC analysis for the entire trial period failed to show any difference between conditions ($F(2) = 2.562$, $p = 0.102$).

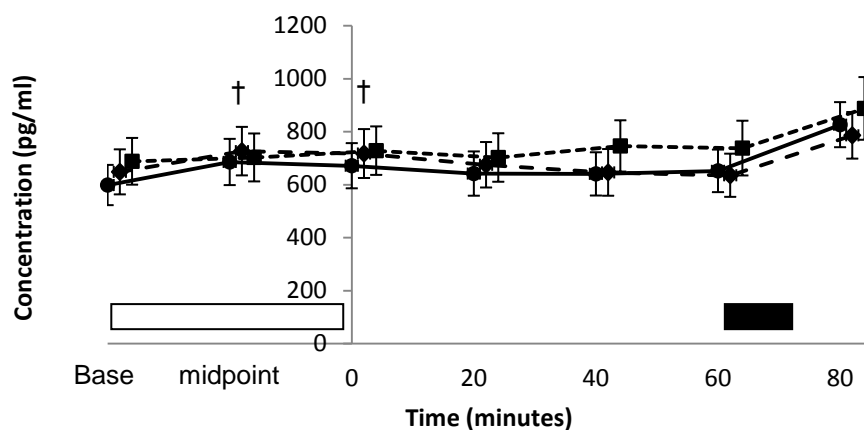


Figure 4.4 – Mean plasma PP concentration (\pm SEM) for LOW (●), MOD (◇) and HIGH (■) conditions. Solid line indicates LOW, dashed line indicates MOD, dotted line indicates HIGH. Hollow rectangle indicates exercise period, solid shaded rectangle indicates *ad libitum* test meal. † = significant time main effect, significantly higher than baseline.

4.4.4.2 PYY

PYY concentrations were similar across all three conditions at baseline. There was no significant time \times condition interaction ($p = 0.125$, **figure 4.5**). The time main effect was significant ($p < 0.001$), with pairwise comparisons showing that PYY concentration were lower at 60 minutes post-exercise, compared with during exercise and in the immediate post-exercise ($t=0$ and $t=20$) period. AUC analysis for the entire trial period failed to show any difference between conditions ($F(2) = 1.729$, $p = 0.203$).

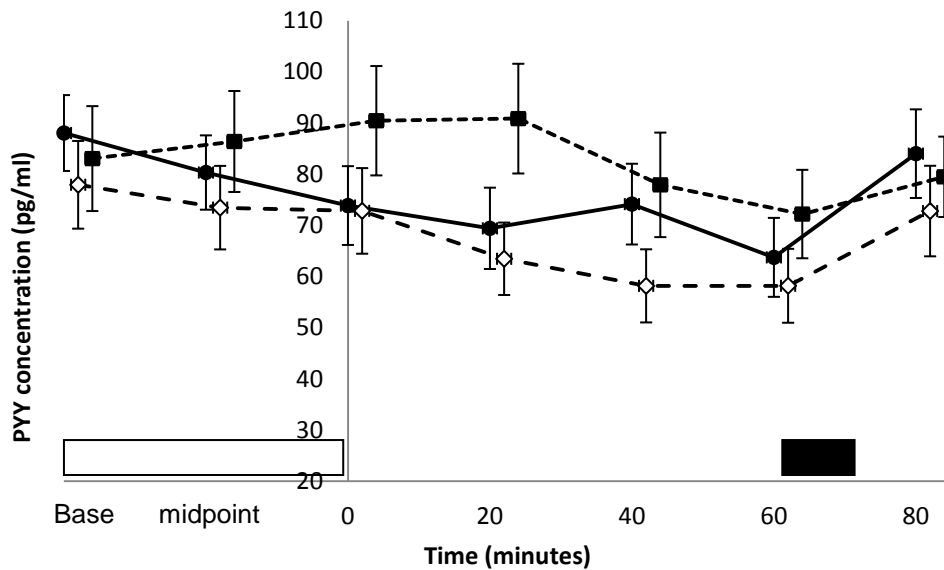


Figure 4.5 – Mean plasma PYY concentration (\pm SEM) for LOW (\bullet), MOD (\diamond) and HIGH (\blacksquare) conditions. Solid line indicates LOW, dashed line indicates MOD, dotted line indicates HIGH. Hollow rectangle indicates exercise period, solid shaded rectangle indicates *ad libitum* test meal.

4.4.5 Relationship between hormones, subjective appetite and food intake.

4.4.5.1 Between-condition correlations of change in hormone concentration with exercise and change in appetite with exercise

In order to investigate if a change in hormone concentration from one trial to another influenced subsequent change in subjective appetite, within-subject, between-condition correlations were conducted. Change in $\% \Delta$ PYY concentration was not significantly associated with change in $\% \Delta$ VAS score or change in $\% \Delta$ BPAR score for any trial condition. For HIGH – MOD differences, the correlation between change in $\% \Delta$ PYY and change in $\% \Delta$ VAS was moderate-strength and showed a trend towards significance ($r = 0.479$, $p = 0.068$) Change in $\% \Delta$ PP was positively correlated with change in $\% \Delta$ VAS for differences between MOD and LOW, ($r = 0.587$, $p = 0.029$).

4.4.5.2 Between-condition correlations of percentage change in hormone concentration with percentage change in energy intake, and of percentage change in appetite score with percentage change in energy intake

In order to investigate if a change in hormone concentration or subjective appetite from one trial to another influenced subsequent change in energy intake, within-subject, between-condition correlations were conducted. There was little relationship between percentage changes in energy intake and percentage changes in either appetite scores or satiety peptide concentration; for MOD – LOW differences, the relationship between percentage change in BPAR score and percentage change in energy intake showed a trend towards significance ($r = 0.469$, $p = 0.073$).

4.5 DISCUSSION

The primary purpose of this study was to address the effect of exercise intensity on subjective appetite, food intake and circulating concentrations of appetite-associated hormones, utilising three aerobic exercise intensity conditions (high, moderate and low intensity), in highly trained male endurance athletes. Subjective appetite scores were transiently suppressed with high intensity exercise, compared with exercise at a moderate or low intensity. VAS scores were 27% lower in the high-intensity condition immediately post-exercise compared with moderate-intensity exercise and 31% lower than in the low-intensity condition. While this provides some evidence for the notion of exercise-induced suppression of appetite, and its occurrence after high-intensity aerobic exercise, no such effect was observed after a bout of exercise at 60% VO_{2max} , an intensity that has commonly been shown to suppress appetite in overweight and obese, (Ueda *et al.*, 2009b; Westerterp-Plantenga *et al.*, 1997), and healthy-weight, non-athletic populations (King *et al.*, 1994; Martins *et al.*, 2007; Ueda *et al.*, 2009a; Westerterp-Plantenga *et al.*, 1997). It is possible that individuals accustomed to moderate-high intensity exercise are more resistant to an exercise-induced suppression of appetite, with a possible threshold for such an effect (should the phenomenon be purely intensity dependent) increased with exercise training. Imbeault and co-workers (Imbeault *et al.*, 1997) failed to observe any suppression of subjective hunger or a decrease in food intake with just 15 minutes of exercise at a high intensity (72% VO_{2max}) in moderately-trained individuals ($VO_{2max} = 56.7 \pm 5.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). While this provides no evidence of a “higher threshold” for exercise-induced appetite suppression, it does suggest that exercise at an intensity of 60% VO_{2max} is insufficient to cause a suppression of appetite in moderately-trained athletes.

The observed suppression of appetite with high intensity aerobic exercise was rather short-lived, with VAS test score returning to baseline levels 20 minutes post-exercise. While appetite scores remained significantly lower after HIGH, compared with after LOW at the 20 minute post-exercise time point, scores at 40 minutes post-exercise were similar across all

conditions. However, AUC analysis for this post-exercise period showed a significant main effect for condition, with AUC for HIGH significantly lower than AUC for LOW when using both the VAS and BPAR measures. These results are similar to those of previous investigations that have demonstrated the transient nature of an exercise-induced suppression of appetite, even with lower intensities of exercise than in the current study (Blundell *et al.*, 2003; Broom *et al.*, 2009; Broom *et al.*, 2007; King *et al.*, 1994; Martins *et al.*, 2007). Broom and colleagues (Broom *et al.*, 2009; Broom *et al.*, 2007) investigated the effect of aerobic exercise on hunger, food intake and circulating concentrations of acylated ghrelin in two separate studies. Common to both studies was a suppression of subjective hunger (measured using the VAS technique) during and immediately after aerobic exercise (60 minutes of treadmill running at 72% $\text{VO}_{2\text{max}}$ (Broom *et al.*, 2007) and 69% $\text{VO}_{2\text{max}}$ (Broom *et al.*, 2009), that was maintained at 30 minutes post-exercise. At 60 minutes post-exercise, there was no difference in subjective hunger between the exercise condition and a control condition. In keeping with the current study, although changes in subjective appetite were short-lived, AUC analysis demonstrated lower hunger scores over more prolonged post-exercise periods of up to 3 hours.

Unsurprising in light of transient changes in subjective appetite, analysis of the energy intake values at the test meal demonstrated that intake did not differ significantly across the exercise intensity conditions. Intake was 11.7% lower after high intensity exercise, compared with moderate intensity exercise. This equated to an absolute difference of 78 kcal. It is difficult to speculate about the impact of such an acute effect upon energy balance (78 kcal shift in favour of deficit) on likely changes in body mass in the long term. The failure of transient appetite suppression to induce a reduction in post-exercise energy intake is in agreement with the findings of studies that administered the test meal at a similar time point post-exercise (60 minutes (King *et al.*, 2010; King *et al.*, 2011b; King *et al.*, 1997b; Schubert *et al.*, 2013; Thompson *et al.*, 1988)) and contrasts the findings of Kissileff *et al.* (Kissileff *et al.*, 1990) who demonstrated a reduced energy intake after strenuous exercise, but not moderate exercise in non-obese females, when food was presented 15 minutes post-

exercise. These findings support the concept of the any exercise-induced suppression of appetite being a short-lived, transient phenomenon. However, what has yet to be concluded is whether an even higher exercise load can elicit a greater anorexigenic effect. It is possible that exercise bouts such as supramaximal exercise that is often undertaken by highly trained athletes in the form of high-intensity interval training or high-intensity aerobic exercise sustained for longer periods could lead to perhaps a greater suppression of appetite or a suppression that lasts longer than that which has been observed with exercise bouts such as those used in the present study.

To investigate the effect of exercise intensity on satiety peptides, the plasma concentration of PP and PYY were measured. Plasma PP concentration increased with exercise, with no difference between exercise intensity conditions. This would suggest that any PP response to exercise is independent of exercise intensity. However, while the time main effect showed a significant increase from baseline during and immediately after exercise, the magnitude of these increases was small (~9%), indicating a muted response. There is little in the current literature regarding the effect of exercise and exercise intensity on plasma PP concentration. Martins and colleagues (Martins *et al.*, 2007) showed an increase in PP during a 60 minute bout of intermittent cycling at ~65% of estimated maximum heart rate in healthy, normal-weight individuals. The magnitude of the increase was considerably greater than that found in the current study (~170% vs. ~9%) but was equally as transient. The large discrepancy in the magnitude of the increase may be due to the difference in training status of the participants; while the current study population consisted of highly trained athletes, Martins and colleagues excluded those exhibiting greater than moderate activity levels.

PYY concentration exhibited a small (~9%), non-significant increase from baseline in the HIGH trial. It could be said that HIGH appeared to attenuate the gradual fall in PYY concentration observed with LOW and MOD, to the extent that, at t=20, the PYY concentration was ~24% higher than LOW and ~30% higher than MOD, in the HIGH condition. It is possible that some degree of exercise-intensity-dependent PYY response

could exist in this population, but that this study lacked the statistical power to observe a significant difference and it may be that a greater duration of high-intensity exercise, or exercise of a higher intensity would see a greater response. However, from the current data, it would appear that PYY was not very responsive to exercise, even exercise of a high intensity, in this athletic population.

A decline in plasma PYY concentration during and after moderate- and low-intensity exercise observed in the current study is not a familiar finding. Ueda and colleagues (Ueda *et al.*, 2009a) observed that moderate intensity exercise (50% $\text{VO}_{2\text{max}}$) resulted in a transient increase in PYY concentration. Other studies have also shown transient increases in PYY during moderate intensity exercise (Martins *et al.*, 2007; Ueda *et al.*, 2009b) and greater increases with higher intensity aerobic exercise (Broom *et al.*, 2009; King *et al.*, 2011a; Ueda *et al.*, 2009a). This data could also suggest some degree of blunting of the exercise-induced changes in PYY concentration in trained individuals. Previous studies used study populations of obese individuals (Ueda *et al.*, 2009b), those of low (Ueda *et al.*, 2009b) or moderate activity levels (Martins *et al.*, 2007; Ueda *et al.*, 2009a) or those described as being “physically active” (King *et al.*, 2011a). In comparison, the participants in the current study all completed a minimum of 6 hours of aerobic exercise training per week, resulting in high fitness levels, as demonstrated by a mean $\text{VO}_{2\text{max}}$ value of $60.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. It is possible that habitual exercisers have developed an adaptive response whereby the exercise-induced increase in satiety peptides is downregulated. This may be a response to promote post-exercise feeding in order to maintain energy balance and body weight and hence oppose weight-loss, or it may be an adaptive response in order to restore depleted fuel stores, such as muscle and liver glycogen, after exercise. Trained individuals experience reduced physiological and metabolic perturbations during exercise. As redistribution of blood flow away from the gastrointestinal tract (Blundell *et al.*, 2003; Burns *et al.*, 2007) and increases in body temperature (Shorten *et al.*, 2009) have been postulated as mediators of the exercise-induced alterations in hormone concentration (as well as alterations in

subjective appetite), a reduction in these responses to exercise in trained athletes may explain the blunted PP and PYY responses.

Significant elevations in plasma PYY were observed in highly trained runners, after a 10km time trial, preceded by a 90 minute preload (Russel *et al.*, 2009). However, such a large stimulus still only elicited an 11% increase in PYY concentration, which is in line with the increase observed in the present study and is a slightly smaller increase than is commonly seen in non-athletic populations, after smaller exercise stimuli (~15% - (Ueda *et al.*, 2009b); ~20% - (Martins *et al.*, 2007); ~20% (Broom *et al.*, 2009)) Conversely, the commencement of regular physical activity by previously sedentary individuals has been shown to result in an increase in fasting plasma PYY concentrations (Jones *et al.*, 2009; Roth *et al.*, 2005). However, these studies did not directly compare acute exercise-induced perturbations in plasma PYY, pre- and post-training.

The influence of within-subject, between-condition changes in satiety peptide concentration on subjective appetite and food intake were assessed using correlation analysis. Within-subject comparisons were conducted, as opposed to the more commonly-used between-subject analysis. It was felt that such an approach was preferable, as within-subject changes are of greater interest and relevance than between-subject differences, especially when considering the likely degree of variability in between-subject responses to exercise. There was no association between within-subject changes in appetite and within-subject changes in either PP or PYY. Neither was there any relationship between changes in PP or PYY and energy intake.

These data question the role of the satiety peptides PP and PYY as regulators of post-exercise subjective appetite. It has recently been shown that PYY concentrations were strongly, positively correlated with VAS scores for satiety over a 12 hour period after continuous exercise, but there was no correlation between PYY concentration and suppressed appetite scores after intermittent exercise (Holmstrup *et al.*, 2013). Further, in a study assessing changes in appetite-associated hormones with strenuous endurance running in trained runners, total ghrelin and PYY₃₋₃₆ concentrations both increased

significantly, meaning an increase in both orexigenic and anorexigenic drive (Larson-Meyer, Palm *et al.* 2012). No measures of subjective appetite, nor energy intake were obtained, so the influence of this perplexing dual elevation was not known. The association between changes in subjective appetite and changes in satiety peptides with exercise requires further investigation.

It could be argued that the differing duration of exercise is a confounding variable in this study. However, it was decided to control for energy expenditure. It was felt that this was important to avoid differences in energy expenditure and short-term energy balance in the post-exercise period and prior to the test meal, as certain appetite-regulating hormones may be sensitive to energy status (Black *et al.*, 2005; King *et al.*, 2011a). Therefore, it is not possible to have exercise bouts of varying intensity but yet be equal in both energy expenditure and duration. Effort was made to eliminate large variations in inter-subject durations of exercise within each trial condition by calculating standardised energy expenditure targets that were individualised for each participant, thus equating to the exercise durations of 30 minutes, 40 minutes and 60 minutes in the high-, moderate- and low-intensity conditions respectively. It may be of interest to investigate the effect of exercise duration on appetite directly by controlling for exercise intensity but varying the duration of the bout of exercise. Again however, it will be impossible to distinguish between duration and energy expenditure using such an approach.

A limitation with this study is that the energy expenditure of the three exercise bouts were poorly controlled and not accurately matched, as intended. The energy expenditure of the low-intensity bout was significantly greater than that of the moderate- and high-intensity bouts, by 67 kcal (12%) and 89 kcal (16%) respectively. This error occurred due to an overestimation of the power output to elicit an intensity of 40% $\text{VO}_{2\text{max}}$ in the low-intensity condition. The linear regression equation, calculated from the $\text{VO}_{2\text{max}}$ test of the pre-testing session turned out to be inaccurate at lower intensities. This is evidenced by the mean relative intensities for the low-, moderate- and high-intensity conditions being 47%, 63% and 80% $\text{VO}_{2\text{max}}$, as opposed to the targeted 40%, 60% and 80% $\text{VO}_{2\text{max}}$. It is felt that the error in

relative intensities is unlikely to have impacted upon the study findings, as the intensities remained significantly different from one another and could still be classified as low-, moderate- and high-intensities. It is currently thought that any acute post-exercise suppression of appetite is exercise-intensity dependent, with the duration or energy cost of exercise not postulated as a regulatory factor. Hence, it would seem unlikely that the energy expenditure differences of the current study influenced post-exercise subjective appetite scores. Nonetheless, it was decided to calculate relative energy intake (energy intake – energy expenditure of exercise), as well as absolute intake. Relative energy intake mirrored absolute intake, in not being significantly different between conditions.

There are further limitations of this study. As the primary interest was the effect on appetite parameters of exercise intensity, not exercise per se, it was decided not to include a control condition. It is accepted that the inclusion of a control group would have been optimal. However, as the appetite response to rest, in similar situations (overnight fast) has previously been established within the literature (Erdmann *et al.*, 2007; King *et al.*, 2010; King *et al.*, 2011b), it was felt that a control condition would add little and compromise other aspects of the study due to imposing a greater participant time commitment. It was also decided to use a very simple form of constant composition *ad libitum* test meal, in the shape of a cornflakes and milk breakfast meal. Such a homogeneous meal offers no information to the researcher with regard to food selection and preference and a limited food choice may have led to under eating. However, as the researchers of this current study were not directly concerned with energy balance, issues of under eating were not a primary concern and opted for a test meal that would be representative of a more habitual eating episode of preparing a single meal consisting of breakfast-type food at that time of day, as opposed to the less habitual buffet-style *ad libitum* test meal.

In conclusion, appetite was transiently suppressed with high intensity exercise (~80% $\text{VO}_{2\text{max}}$), compared with low- and moderate-intensity exercise. Although appetite scores were similar across all conditions at 40 minutes post-exercise, total appetite scores (obtained by AUC analysis) remained lower for HIGH over the 60min post-exercise period, compared with

lower intensity exercise. Post-exercise energy intake was similar across all conditions. There were no significant changes in PYY plasma concentrations with exercise, while exercise induced a modest increase in PP concentration. Changes in neither hormone correlated with changes in subjective appetite or energy intake, which questions the role of PYY and PP in regulating post-exercise appetite.

4.6 REFERENCES

- Black, S.E., Mitchell, E., Freedson, P.S., Chipkin, S.R., and Braun, B. (2005). Improved insulin action following short-term exercise training: role of energy and carbohydrate balance. *Journal of Applied Physiology* 99, 2285-2293.
- Blundell, J.E., Stubbs, R.J., Hughes, D.A., Whybrow, S., and King, N.A. (2003). Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proc Nutr Soc* 62, 651-661.
- Borg, G.A.V. (1973). "Perceived exertion: a note on history and methods". *Medicine and Science in Sports & Exercise* 5, 90-93.
- Broom, D.R., Batterham, R.L., King, J.A., and Stensel, D.J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 296, R29-R35.
- Broom, D.R., Stensel, D.J., Bishop, N.C., Burns, S.F., and Miyashita, M. (2007). Exercise-induced suppression of acylated ghrelin in humans. *J Appl Physiol* 102, 2165-2171.
- Burke, L.M. (1997). Nutrition for post-exercise recovery. *Australian journal of science and medicine in sport* 29, 3-10.
- Burns, S.F., Broom, D.R., Miyashita, M., Mundy, C., and Stensel, D.J. (2007). A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of Sports Sciences* 25, 635-642.
- Erdmann, J., Tahbaz, R., Lippl, F., Wagenpfeil, S., and Schusdzarra, V. (2007). Plasma ghrelin levels during exercise -- Effects of intensity and duration. *Regulatory Peptides* 143, 127-135.
- Filaire, E., Rouveix, M., Pannafieux, C., and Ferrand, C. (2007). Eating attitudes, perfectionism and body-esteem of elite male judoists and cyclists. *Journal of Sports Science and Medicine* 5, 50-57.
- George, V.A., and Morganstein, A. (2003). Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite* 40, 43-46.
- Hill, A.J., and Blundell, J.E. (1982). Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research* 17, 203-212.
- Holmstrup, M.E., Fairchild, T.J., Keslacy, S., Weinstock, R.S., and Kanaley, J.A. (2013). Satiety, but not total PYY, is increased with continuous and intermittent exercise. *Obesity*, n/a-n/a.
- Imbeault, P., Saint-Pierre, S., Alm-Ras, N., and Tremblay, A. (1997). Acute effects of exercise on energy intake and feeding behaviour. *British Journal of Nutrition* 77, 511-521.
- Jones, T.E., Basilio, J.L., Brophy, P.M., McCammon, M.R., and Hickner, R.C. (2009). Long-term exercise training in overweight adolescents improves plasma peptide YY and resistin. *Obesity* 17, 1189-1195.

Jurimae, J., Hofmann, P., Jurimae, T., Maestu, J., Purge, P., Wonisch, M., Pokan, R., and von Duvillard, S.P. (2006). Plasma adiponectin response to sculling exercise at individual anaerobic threshold in college level male rowers. *Int J Sports Med* 27, 272-277.

Jurimae, J., and Jurimae, T. (2005). Leptin responses to short term exercise in college level male rowers. *Br J Sports Med* 39, 6-9.

Jurimae, J., Jurimae, T., and Purge, P. (2007). Plasma ghrelin is altered after maximal exercise in elite male rowers. *Experimental Biology and Medicine* 232, 904-909.

Jürimäe, J., Mäestu, J., and Jürimäe, T. (2003). Leptin as a marker of training stress in highly trained male rowers? *Eur J Appl Physiol* 90, 533-538.

Jurimae, J., Ramson, R., Maestu, J., Purge, P., Jurimae, T., Arciero, P.J., and Von Duvillard, S.P. (2009). Plasma visfatin and ghrelin response to prolonged sculling in competitive male rowers. *Medicine & Science in Sports & Exercise* 41, 137-143
110.1249/MSS.1240b1013e31818313e31818316.

Kawano, H., Mineta, M., Asaka, M., Miyashita, M., Numao, S., Gando, Y., Ando, T., Sakamoto, S., and Higuchi, M. (2013). Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite* 66, 26-33.

King, J.A., Miyashita, M., Wasse, L.K., and Stensel, D.J. (2010). Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* 54, 492-498.

King, J.A., Wasse, L.K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R.L., and Stensel, D.J. (2011a). Differential acylated ghrelin, peptide YY₃₋₃₆, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology & Metabolism* 96, 1114-1121.

King, J.A., Wasse, L.K., and Stensel, D.J. (2011b). The acute effects of swimming on appetite, food intake, and plasma acylated ghrelin. *Journal of Obesity* 2011.

King, N.A., Burley, V.J., and Blundell, J.E. (1994). Exercise induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition* 48, 715-724

King, N.A., Lluch, A., Stubbs, R.J., and Blundell, J.E. (1997a). High dose exercise does not increase hunger or energy intake in free living males. *Eur J Clin Nutr* 51, 478-483.

King, N.A., Snell, L., Smith, R.D., and Blundell, J.E. (1996). Effects of short-term exercise on appetite responses in unrestrained females. *European Journal of Clinical Nutrition* 50, 663-667.

King, N.A., Tremblay, A., and Blundell, J.E. (1997b). Effects of exercise on appetite control: implications for energy balance. *Medicine & Science in Sports & Exercise* 29, 1076-1089.

Kissileff, H.R., Pi-Sunyer, F.X., Segal, K., Meltzer, S., and Foelsch, P.A. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition* 52, 240-245.

Lluch, A., King, N.A., and Blundell, J.E. (2000). No energy compensation at the meal following exercise in dietary restrained and unrestrained women. *British Journal of Nutrition* 84, 219-225.

Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 193, 251-258.

O'Connor, A.M., Johnston, C.F., Buchanan, K.D., Boreham, C., Trinick, T.R., and Riddoch, C.J. (1995). Circulating gastrointestinal hormone changes in marathon running. *Int J Sports Med* 16, 283-287.

O'Connor, A.M., Pola, S., Ward, B.M., Fillmore, D., Buchanan, K.D., and Kirwan, J.P. (2006). The gastroenteroinsular response to glucose ingestion during postexercise recovery. *Am J Physiol Endocrinol Metab* 290, E1155-1161.

Pomerleau, M., Imbeault, P., Parker, T., and Doucet, E. (2004). Effects of exercise intensity on food intake and appetite in women. *Am J Clin Nutr* 80, 1230-1236.

Reid, C.H., CG. Blundell, JE. Stubbs, RJ (1998). What are psychometric assessments of appetite asking: a preliminary multivariate analysis. *International Journal of Obesity* 22, 151.

Roth, C.L., Enriori, P.J., Harz, K., Woelfle, J., Cowley, M.A., and Reinehr, T. (2005). Peptide YY is a regulator of energy homeostasis in obese children before and after weight loss. *J Clin Endocrinol Metab* 90, 6386-6391.

Russel, R.R., Willis, K.S., Ravussin, E., and Larson-Meyer, E.D. (2009). Effects of endurance running and dietary fat on circulating ghrelin and peptide YY. *Journal of sports science & medicine* 8, 574-583.

Schubert, M.M., Desbrow, B., Sabapathy, S., and Leveritt, M. (2013). Acute exercise and subsequent energy intake. A meta-analysis. *Appetite* 63, 92-104.

Shorten, A.L., Wallman, K.E., and Guelfi, K.J. (2009). Acute effect of environmental temperature during exercise on subsequent energy intake in active men. *The American Journal of Clinical Nutrition* 90, 1215-1221.

Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., and Blundell, J.E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* 84, 405-415.

Thompson, D.A., Wolfe, L.A., and Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medicine & Science in Sports & Exercise* 20, 222-227.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., and Fujimoto, S. (2009a). Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J Endocrinol* 203, 357-364.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., and Fujimoto, S. (2009b). Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J Endocrinol*, JOE-08-0500.

Venter, C.S., MacIntyre, U.E., and Vorster, H.H. (2000). The development and testing of a food portion photograph book for use in an African population. *Journal of Human Nutrition and Dietetics* 13, 205-218.

Weber, J.L., Cunningham-Sabo, L., Skipper, B., Lytle, L., Stevens, J., Gittelsohn, J., Anliker, J., Heller, K., and Pablo, J.L. (1999). Portion-size estimation training in second- and third-grade American Indian children. *The American Journal of Clinical Nutrition* 69, 782S-787S.

Westerterp-Plantenga, M.S., Verwegen, C.R., Ijeda, M.J., Wijckmans, N.E., and Saris, W.H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* 62, 1345-1354.

**The effect of exercise duration on appetite, food intake and
appetite-associated hormones.**

5.1 ABSTRACT

Background: The importance of the intensity of exercise for appetite responses post-exercise is well established, with an intensity of $\geq 60\%$ $\text{VO}_{2\text{max}}$ commonly observed to elicit a transient suppression of appetite. What is much less clear, is the role of the duration of exercise in regulating responses in subjective appetite and plasma concentrations of appetite-associated hormones to exercise.

Purpose: To investigate the effect of high-intensity aerobic exercise of differing durations on subjective appetite, food intake and appetite-associated hormones in highly-trained male endurance athletes.

Method: Twelve highly-trained (≥ 6 hours of training per week) male endurance runners, cyclists and triathletes (age = 21 ± 2 years; BMI = $21.0 \pm 1.6 \text{ kg}\cdot\text{m}^{-2}$; $\text{VO}_{2\text{max}} = 61.6 \pm 6.0 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) completed four trials in a counterbalance, within-subject study design: resting (REST); 15 minutes exercise bout (15 min); 30 minute exercise bout (30 min) and 45 minute exercise bout (45 min). All exercise was completed on a cycle ergometer and at an intensity of $\sim 80\%$ $\text{VO}_{2\text{max}}$ ($76 \pm 8\%$, $77 \pm 8\%$ and $76 \pm 8\%$ for 15 min, 30 min and 45 min respectively). Upon cessation of exercise, a 60 minute resting period followed, with measures of appetite and blood samples obtained every 20 minutes. After 60 minutes, participants consumed an *ad libitum* breakfast meal, with food intake covertly recorded. Appetite measures and blood samples were obtained throughout the trial, with plasma analysed for acylated ghrelin, polypeptide YY (PYY) and glucagon-like peptide 1 (GLP-1) concentrations.

Results: A composite VAS score for appetite did not differ between trial conditions, or with time. Food intake at the breakfast meal also did not differ between conditions. When considering the energy expenditure of exercise, relative energy intake (intake – expenditure) was significantly lower after 30 min and 45 min, compared with REST. Relative energy intake was also lower for 45 min, compared with 15 min. GLP-1 concentration was increased with exercise in 30 min and 45 min, with concentration remaining elevated during the post-

exercise period. Acylated ghrelin was transiently suppressed with exercise in all three exercise trials, with the greatest suppression observed in 45 min. PYY concentration did not change with exercise and did not differ between conditions. However, correlation analysis failed to show strong relationships between changes in hormone concentration and either changes in subjective appetite or changes in *ad libitum* energy intake.

Conclusion: High intensity aerobic exercise did not elicit a suppression of subjective appetite, irrespective of the duration of exercise. Consequently, absolute energy intake was largely unaffected by exercise. This was despite changes in acylated ghrelin and GLP-1 towards a more anorexigenic state. This would suggest a dissociation between hormonal signals of appetite and subjective appetite in the post-exercise period. Both acylated ghrelin and GLP-1 appeared to exhibit some degree of dose response to increasing exercise duration.

5.2 INTRODUCTION

High-intensity aerobic exercise commonly elicits a transient suppression of appetite in lean, recreationally active individuals (Broom *et al.*, 2009; Broom *et al.*, 2007; Burns *et al.*, 2007; Kawano *et al.*, 2013; King *et al.*, 2010; Martins *et al.*, 2007; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b). Typically, this phenomenon, termed the “anorexia of exercise,” (King *et al.*, 1994) is observed with exercise of an intensity $\geq 60\%$ $\text{VO}_{2\text{max}}$. This response in subjective appetite is often coupled with anorexigenic changes in appetite-associated hormones. Plasma concentrations of the acylated form of the orexigenic hormone ghrelin have been shown to decrease with exercise (Broom *et al.*, 2009; Broom *et al.*, 2007; King *et al.*, 2010; King *et al.*, 2011a; Marzullo *et al.*, 2008; Shiya *et al.*, 2011), while concentrations of the satiety peptides peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) have been shown to increase (Broom *et al.*, 2009; King *et al.*, 2011a; Larson-Meyer *et al.*, 2012; Martins *et al.*, 2007; Russel *et al.*, 2009; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b). However, the ghrelin response to exercise is somewhat equivocal, because no change (Burns *et al.*, 2007; Kraemer *et al.*, 2004; Martins *et al.*, 2007; Schmidt *et al.*, 2004) and even increases in ghrelin post-exercise have been observed (Larson-Meyer *et al.*, 2012; Russel *et al.*, 2009), with differences likely due to the form of ghrelin measured.

While the intensity dependency of post-exercise appetite suppression appears well established, the existence of a dependency with regard to duration of exercise or energy expenditure of the bout of exercise has yet to be comprehensively investigated. Suppressions in appetite, accompanied by increases in the satiety peptides PYY and GLP-1 have been observed with continuous, high-intensity aerobic bouts lasting as little as 30 minutes (Ueda *et al.*, 2009a), and with intermittent exercise bouts yielding energy expenditure values of as little as 142 kcal (Deighton *et al.*, 2012). Conversely, bouts of very low energy cost have elicited increases in subjective appetite (Bellissimo *et al.*, 2007), or failed to influence appetite, despite a response in plasma concentration of the hunger hormone ghrelin (Erdmann *et al.*, 2007). However in this instance, ghrelin was seen to

increase during exercise; a change in the opposite direction to that which is more usually observed with higher intensity exercise. Furthermore, the role of the duration of an exercise bout on plasma concentrations has not been directly assessed. It remains unknown whether any of the appetite-associated hormones are released in a dose-response manner to exercise duration or energy cost, or whether there is a duration or energy cost threshold for a hormonal response.

The transient nature of both a suppression of subjective appetite and changes in plasma hormone concentration means that food intake can be reduced when administered in close proximity to the cessation of exercise (~10 minutes (Westerterp-Plantenga *et al.*, 1997); ~15 minutes (Kissileff *et al.*, 1990); ~30 minutes (Ueda *et al.*, 2009a); ~60 minutes (Ueda *et al.*, 2009b), but is largely unaffected when a meal is consumed ≥ 60 minutes after exercise (King *et al.*, 2010; King *et al.*, 2011b; King *et al.*, 1997; Martins *et al.*, 2007; Schubert *et al.*, 2013; Thompson *et al.*, 1988). Although, exercise bouts that yield a considerable energy cost can elicit a decrease in relative energy intake (energy intake – energy expenditure) compared with non-exercise control conditions (Imbeault *et al.*, 1997; King *et al.*, 2010; Lluch *et al.*, 2000; Martins *et al.*, 2007). Due to the commonly used study population of recreationally active individuals, large exercise loads of both a high intensity and long duration have rarely been utilised. It may be possible that a larger exercise stimulus could lead to a greater, sustained appetite suppression that could impact upon food intake.

Highly trained athletes regularly complete very strenuous, high energy cost bouts of exercise. After completing these sessions, post-exercise nutrition is often considered of crucial importance to optimise recovery and maximise adaptations to training (Burke, 1997). In addition, many endurance athletes value weight management highly (Filaire *et al.*, 2007), as an increase in body weight can result in an increase in the energy cost of performing. Therefore, the appetite response to exercise could be doubly important for athletes. Nevertheless, there is a dearth of literature regarding the effect of exercise on appetite and food intake in athletic populations. Of the few investigations that have addressed the effect of exercise on any appetite-related measures in athletic populations, the focus has been

primarily on hormone measures and no measures of subjective appetite or food intake have been obtained (Jurimae *et al.*, 2006; Jurimae and Jurimae, 2005; Jurimae *et al.*, 2007; Jürimäe *et al.*, 2003; Jurimae *et al.*, 2009; O'Connor *et al.*, 1995; O'Connor *et al.*, 2006). These studies have shown both increases (Jurimae *et al.*, 2006; O'Connor *et al.*, 2006) and decreases (Jurimae and Jurimae, 2005; Jürimäe *et al.*, 2003) in anorexigenic gut hormones with strenuous exercise, while increases in the orexigenic hormone ghrelin have also been reported (Jurimae *et al.*, 2007; Jurimae *et al.*, 2009). These data suggest that it may be possible that highly-trained athletes respond differently to exercise than non-athletic populations, with regard to changes in appetite-associated hormones. It has yet to be investigated whether this translates to altered appetite and food intake responses.

The purpose of the current study was to address the effect of exercise duration on subjective appetite, food intake and circulating concentrations of acylated ghrelin, PYY and GLP-1 in highly trained endurance athletes, utilising high-intensity exercise bouts lasting 15, 30 and 45 minutes. It was hypothesised that a transient exercise-induced suppression of appetite would be observed after all exercise durations, but in a dose response fashion, with greater, longer lasting suppression evident after greater duration bouts. It is surmised that due to a large exercise load, this may lead to a lower post-exercise energy intake in the 45 minute condition.

5.3 METHOD

5.3.1 Participants

Twelve healthy-weight, highly trained male endurance athletes were recruited principally from the University of Birmingham cycling, triathlon and athletics clubs. Participant characteristics are shown in **table 5.1**. Inclusion criteria were: a minimum total training duration of 6 hours per week and aged between 18 and 40 years. Exclusion criteria were: a score of 3.5 or greater for restricted eating on the Dutch Eating Behaviour Questionnaire (DEBQ, van Strien *et al*, 1986); illness such as upper respiratory tract infections; smoking and the taking of medication likely to affect appetite or induce weight-loss. Ethical approval was obtained from the Ethics Subcommittee of the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. One of the twelve participants that completed the study suffered from a phobia of needles. Hence, blood samples were not obtained from this participant. Hormone analysis was therefore conducted for eleven participants.

Age (years)	21 ± 2
BMI (kg•m⁻²)	21.0 ± 1.6
VO_{2max} (mL•kg⁻¹•min⁻¹)	61.6 ± 6.0
W_{max} (Watts)	309 ± 44.5
DEBQ score for restraint	1.9 ± 0.4

Table 5.1. Participant characteristics. Values are mean ± SD.

5.3.2 Experimental trial conditions

Participants were randomly assigned to each trial condition: resting (REST), 15 minutes of cycling exercise (15min), 30 minutes of cycling exercise (30min), and 45 minutes of cycling exercise (45min). For each condition, exercise was completed at an intensity of

80% $\text{VO}_{2\text{max}}$. Subjective appetite measures and measures of circulating concentrations of acylated ghrelin, PYY and GLP-1 were obtained throughout.

5.3.3 Procedure & protocol

Participants arrived at the laboratory at approximately 08:00, after a minimum 10-hour overnight fast. On arriving, participants were weighed (weight was recorded at each visit to ensure participants were weight-stable throughout) and a resting blood sample was obtained following the insertion of a venous cannula into the antecubital vein of the arm by a qualified member of staff. Pre-exercise tests of subjective appetite were then completed, before the exercise bout commenced. This consisted of a bout of exercise on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) at an intensity of 80% $\text{VO}_{2\text{max}}$, lasting a duration of 15, 30 or 45 minutes. During each exercise bout, exhaled gas samples were obtained intermittently to monitor VO_2 and to retrospectively calculate energy expenditure. This also allowed the ergometer resistance to be adjusted to maintain the correct VO_2 output. A blood sample was obtained at the half way point of the exercise trial. At 5 minute intervals throughout the exercise bout, measures of heart rate and perceived exertion were obtained. These values were averaged for the entire bout. Upon reaching the exercise duration target, the exercise ceased. A blood sample was obtained and measures of subjective appetite were completed immediately. The resting period then commenced, with the participant sat reading or watching television, for a duration of 60 minutes, allowing for subsequent repetitions of the subjective appetite measures and repeated blood sampling at; 20, 40, 60 minutes post-exercise. In the resting condition, the participant remained rested throughout, resting for an additional 30 minutes to equate to the period of time spent exercising. At 60 minutes post-exercise, the participant consumed an *ad libitum* intake breakfast test meal, as described in General methods, section 2.2.3.2 (p73).

5.3.4 Blood sampling and analysis

The blood sampling and plasma analysis techniques are described in General methods, section 2.2.3.5 (p76). Briefly, blood samples were obtained and immediately

transferred to the appropriate pre-treated blood collection tubes for analysis of hormones. Blood was centrifuged at 3500 RPM and a temperature of 4°C for 15 minutes to isolate plasma. Plasma was stored at a temperature of -70°C until hormone assays for acylated ghrelin, PYY and GLP-1 were conducted.

5.3.5 Statistical analysis

For the determination of differences in energy intake from the test meal between each exercise condition, a one-way analysis of variance (ANOVA) with repeated measures was carried out. To compare differences in both subjective appetite and plasma concentration of appetite-associated hormones with time and between trial conditions, a 2-way factorial ANOVA with repeated measures was conducted. Post-hoc pairwise comparisons were conducted using the Bonferroni correction for multiple comparisons. In the event of a significant difference between conditions at baseline in any outcome measure, percentage change from baseline (%Δ) was calculated and analysis repeated with these calculated values. Area under the curve (AUC) was calculated for all profiles plotted, using the trapezoidal method. AUC values were then compared using a one-way ANOVA with repeated measures.

To further investigate any possible exercise duration dose-response on acylated ghrelin, PYY or GLP-1, percentage changes in hormone concentration from baseline (%Δ) were correlated with time spent exercising. This was performed using the measures obtained across all three exercise trials, at t=7.5 (midpoint for 15min), t=15 (midpoint for 30min and endpoint of 15min), t=22.5 (midpoint of 45min), t=30 (endpoint of 30min) and t=45 (endpoint 45min). Correlation analysis was conducted using Pearson product moment correlation.

To further investigate the relationship between changes in appetite hormones and changes in subjective appetite with exercise, correlation analysis was conducted for within-subject, between-condition comparisons. Change from REST trial values were calculated for each of the three exercise conditions (15 min – REST; 30 min – REST; 45 min – REST) for percent change from baseline score (%Δ, calculated from value at baseline to value at t = 0)

in acylated ghrelin, PYY and GLP-1, with these values correlated with corresponding values for VAS and VIMEC. Between-condition percentage change from REST was calculated for hormone measures and appetite measures obtained immediately prior to the test meal and these values were correlated with between-condition percentage change in energy intake at the *ad libitum* test meal. Correlation coefficients were calculated using Pearson product moment correlation analysis.

5.4 RESULTS

5.4.1 Exercise trials

The characteristics of the four trial conditions are shown in **table 5.2**. With regard to the three exercise trials, relative exercise intensity (VO_2 and $\%\text{VO}_{2\text{max}}$) did not differ between trials. As expected, energy expenditure was significantly greater in each of the three exercise conditions, compared with REST, though did not differ significantly between the three exercise conditions.

	REST	15 min	30 min	45 min
$\text{VO}_2 (\text{mL} \cdot \text{min}^{-1})$	$341 \pm 33^{\text{aa}}$	3150 ± 368	3180 ± 405	3138 ± 416
$\% \text{VO}_{2\text{max}}$	$6.0 \pm 3.7^{\text{aa}}$	76.4 ± 7.5	77.0 ± 7.8	76.0 ± 8.0
Power output (W)	-	$218 \pm 30^{\text{b}}$	207 ± 30	207 ± 33
$\% \text{W}_{\text{max}}$	-	$69.8 \pm 4.0^{\text{b}}$	66.3 ± 5.9	66.4 ± 3.9
Heart rate (beats min^{-1})	-	153 ± 13	156 ± 15	157 ± 14
$\% \text{HR}_{\text{max}}$	-	83.7 ± 4.8	83.8 ± 5.3	85.8 ± 3.1
RPE	-	$13 \pm 1^{\text{c}}$	14 ± 1	15 ± 2
Energy Expenditure of bout (kcal)	$37 \pm 23^{\text{aa}}$	$236 \pm 26.5^{\text{aa}}$	$475 \pm 60.2^{\text{aa}}$	$700 \pm 91.2^{\text{aa}}$
EE of trial (bout + rec. period. Kcal)	$112 \pm 68^{\text{aa}}$	$337 \pm 40.4^{\text{aa}}$	$584 \pm 75.1^{\text{aa}}$	$787 \pm 94.9^{\text{aa}}$

Table 5.2. Characteristics of exercise. Values are mean \pm SD. aa = significantly different to all other conditions, $p < 0.001$. b = significantly different to 45 min, $p < 0.05$. c = significantly different to 30 min and 45 min, $p < 0.05$.

5.4.2 Subjective Appetite

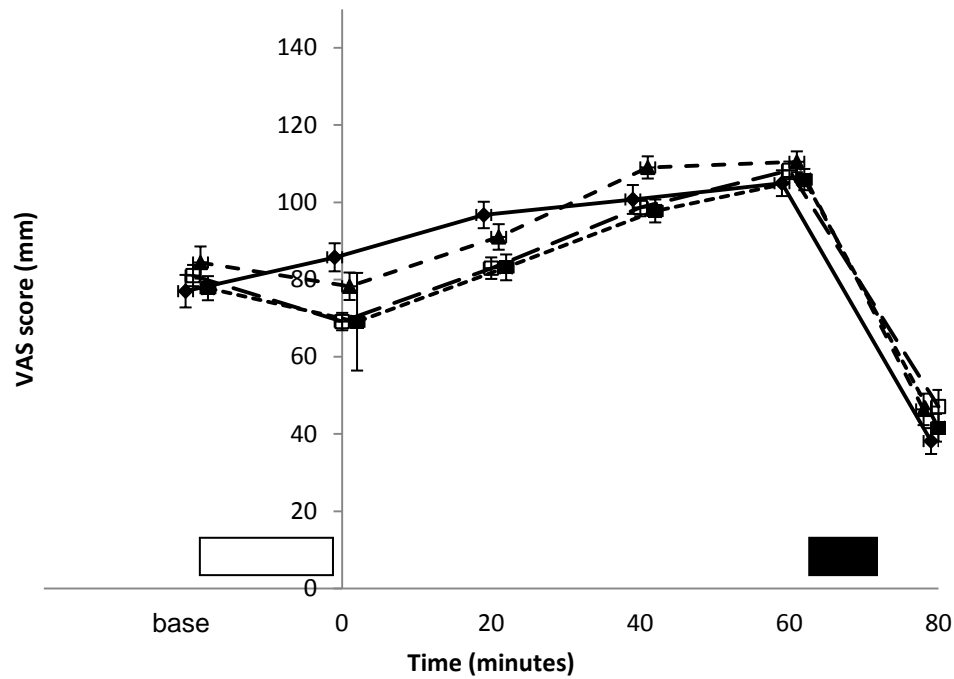
5.4.2.1 VAS

Appetite profiles for each condition, obtained using the VAS technique are shown in **figure 5.1a**. VAS scores were similar across all four conditions at baseline. There was no significant condition \times time interaction ($p = 0.083$), nor condition main effect. There was a significant main effect for time ($F(5) = 38.848$, $p < 0.001$), which showed that during all conditions, appetite rose from the cessation of exercise ($t=0$) until the test meal ($t=60$), before falling after feeding. Area under the curve (AUC) analysis for these appetite profiles also failed to demonstrate any statistically significant differences in appetite between the four conditions ($F(3) = 1.661$, $p = 0.194$).

5.4.2.2 VIMEC

Appetite profiles for each condition, obtained using the VIMEC are shown in **figure 5.1b**. There were no significant differences in VIMEC scores across the four conditions at baseline. There was no significant condition x time interaction ($F(3.592) = 2.257$, $p = 0.086$), nor condition main effect. There was a significant main effect for time ($F(1.524) = 10.572$, $p = 0.002$). Appetite scores rose from the cessation of exercise ($t=0$) until the test meal ($t=60$) and fell after feeding. AUC analysis for these appetite profiles also failed to demonstrate any statistically significant differences in appetite between the four conditions ($F(3) = 0.883$, $p = 0.416$).

(a)



(b)

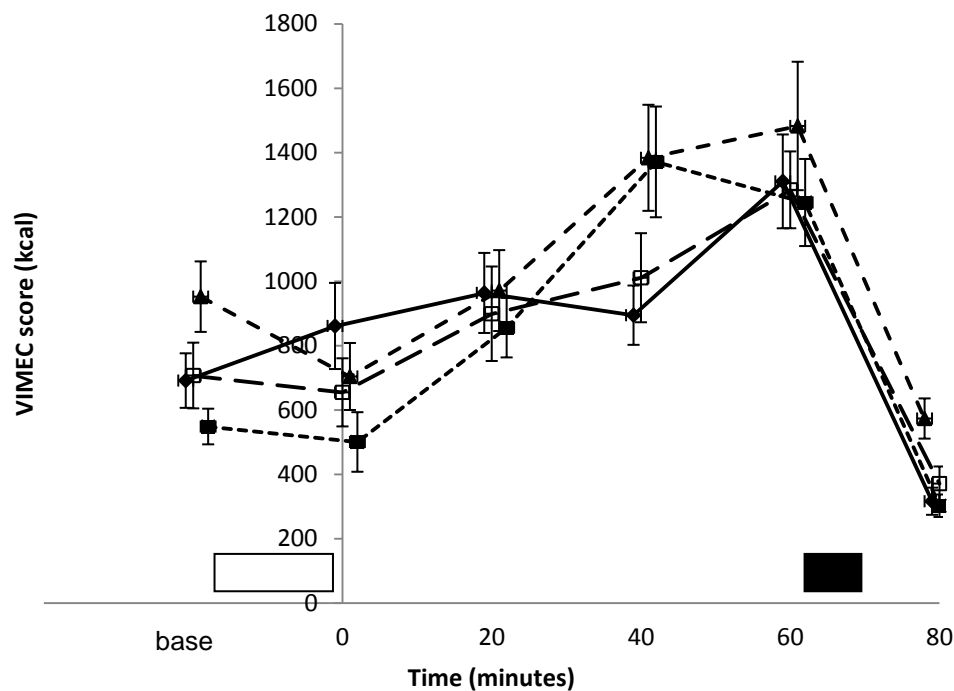
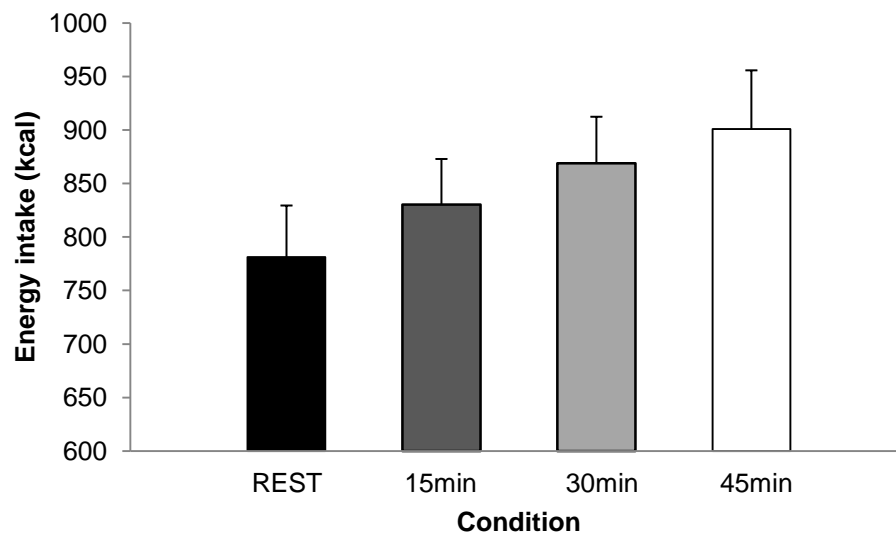


Figure 5.1 – Mean appetite scores, as measured using VAS(a) and VIMEC (b). ●, solid line = REST; ○, large dash = 15 min; ■, medium dash = 30 min; □, small dash = 45 min. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean \pm SEM.

5.4.3 Food intake at the *ad libitum* test meal

The mean energy intake values for each of the four trial conditions are shown in **figure 5.2a**. There was no significant condition effect for energy intake ($F(3) = 1.636$, $p = 0.223$), suggesting that intakes were similar (REST = 781 ± 334 kcal, 15min = 822 ± 291 kcal, 30min = 869 ± 300 , 45min = 901 ± 380 kcal). When accounting for the energy expenditure of exercise, relative energy intake (REI, intake – expenditure), there was a significant main effect for condition ($F(1.625) = 14.129$, $p < 0.001$, **figure 5.2b**). REI was significantly greater in REST (687 ± 369 kcal) versus 30min (285 ± 326 kcal) and 45min (114 ± 374 kcal) (both $p < 0.001$), while REI in 45min was also significantly lower than 15min (595 ± 280 kcal, $p = 0.02$). Total amount of food consumed, in grams, and the macronutrient content of the food consumed, as both absolute intake (grams) and percentage of total energy, are shown in **table 5.3**. There were no significant differences in any of these intake variables between the four conditions.

(a)



(b)

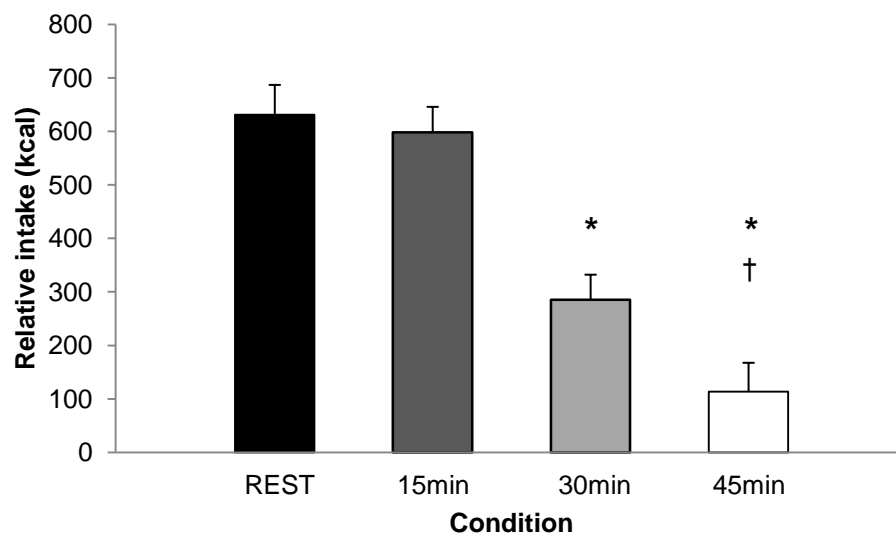


Figure 5.2. Energy intake (a) and relative energy intake (b) at the *ad libitum* breakfast test meal for REST, 15min, 30min and 45min. Values are mean \pm SEM. * = significantly lower than REST. † = significantly lower than 15min.

	REST	15 min	30 min	45 min
Weight consumed (grams)	735 ± 331	793 ± 281	836 ± 262	822 ± 264
Carbohydrate (g)	148 ± 64	157 ± 55	167 ± 55	165 ± 57
% energy CHO	76.3 ± 6.7	77.2 ± 5.7	77.5 ± 5.7	75.4 ± 9.3
Fat (g)	11.1 ± 6.6	11.3 ± 6.4	11.5 ± 6.4	17.2 ± 21.2
% energy fat	13.4 ± 5.8	12.9 ± 4.8	12.5 ± 4.7	15.3 ± 10.6
Protein (g)	20.6 ± 9.4	20.9 ± 8.2	22.3 ± 9.2	20.3 ± 8.3
% energy protein	10.3 ± 2.2	9.9 ± 2.0	10.0 ± 2.1	9.3 ± 2.3

Table 5.3. Summary of food intake at the *ad libitum* test meal for each of the four conditions. Values are mean ± SD.

5.4.4 Plasma glucose and appetite-associated hormone concentrations

5.4.4.1 Glucose

There was no significant time x condition interaction for plasma glucose concentration ($F(3) = 2.223$, $p = 0.106$), nor was there a significant main effect for condition or time (data not presented).

5.4.4.2 Acylated ghrelin

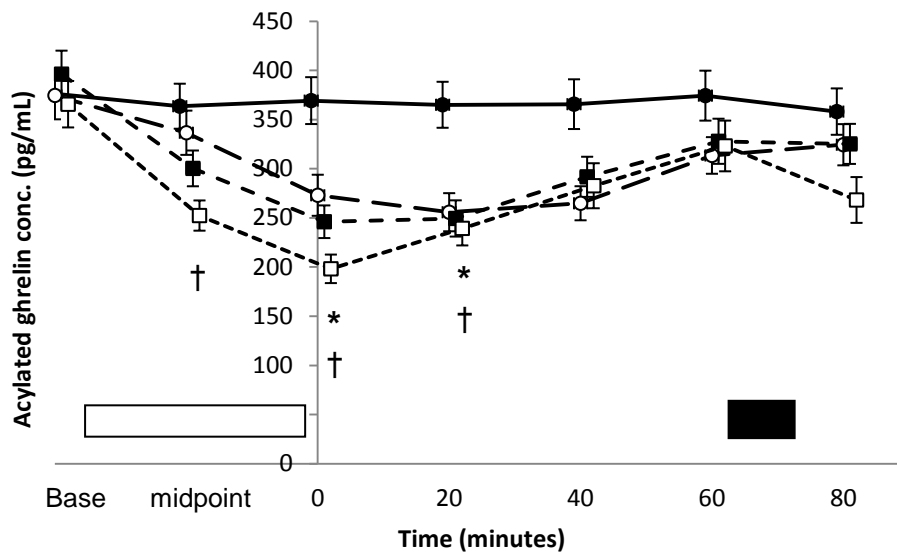
Acylated ghrelin concentrations did not differ across the four conditions at baseline. There was a significant condition x time interaction for acylated ghrelin concentration ($F(3.606) = 3.978$, $p = 0.011$, **figure 5.3a**). Post-hoc analysis of between-condition comparisons showed that, at the midpoint of exercise, acylated ghrelin concentration was significantly lower in the 45 min condition ($252 \pm 31 \text{ pg}\cdot\text{mL}^{-1}$), versus REST ($356 \pm 46 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.026$) and 15 min ($337 \pm 45 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.042$). Immediately post-exercise ($t=0$), acylated ghrelin was significantly lower in 45 min ($198 \pm 29 \text{ pg}\cdot\text{mL}^{-1}$) versus REST ($369 \pm 48 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.009$). Concentrations were also lower than REST in 15 min ($273 \pm 42 \text{ pg}\cdot\text{mL}^{-1}$) and 30 min ($246 \pm 33 \text{ pg}\cdot\text{mL}^{-1}$) at this time, with these differences approaching statistical significance ($p = 0.077$ and $p = 0.055$ respectively). The difference in acylated ghrelin concentration between 30 min and 45 min also approached significance ($p = 0.057$). The difference in plasma ghrelin concentration between 45 min and REST remained significant at

t=20 ($239 \pm 35 \text{ pg}\cdot\text{mL}^{-1}$ vs. $365 \pm 47 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.023$). There were no significant differences between conditions at t=40 onwards.

Within-condition comparisons showed that acylated ghrelin concentration did not change in the resting condition. In the 15 min condition the mean concentration fell significantly from the midpoint to immediately post-exercise ($337 \pm 45 \text{ pg}\cdot\text{mL}^{-1}$ vs. $273 \pm 42 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.001$) and remained lower than the midpoint of exercise at t=20 ($256 \pm 39 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.026$). In the 30 min condition acylated ghrelin fell during exercise, with a trend for lower concentration immediately post-exercise versus baseline ($246 \pm 33 \text{ pg}\cdot\text{mL}^{-1}$ vs. $396 \pm 48 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.098$). The difference between the acylated ghrelin concentration at t=20 and the baseline concentration almost reached statistical significance ($249 \pm 7 \text{ pg}\cdot\text{mL}^{-1}$ vs. $396 \pm 8 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.05$). Mean acylated ghrelin concentration fell to the greatest extent in the 45 min trial, with concentrations significantly lower immediately post-exercise ($198 \pm 29 \text{ pg}\cdot\text{mL}^{-1}$) and at t=20 ($239 \pm 35 \text{ pg}\cdot\text{mL}^{-1}$), versus baseline ($366 \pm 47 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.038$ and $p = 0.025$ respectively). The continued decrease in acylated ghrelin during exercise resulted in a lower immediate post-exercise concentration ($198 \pm 29 \text{ pg}\cdot\text{mL}^{-1}$), which approached significance, compared with the midpoint of exercise ($252 \pm 31 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.066$). AUC analysis showed a significant main effect for condition ($F(3,30) = 3.781$, $p = 0.021$), with Bonferroni pairwise comparisons highlighting that AUC was significantly lower in 45min, compared with REST ($p = 0.039$).

The plot of acylated ghrelin $\%\Delta$ against time spent exercising is shown in **figure 5.3b**. A significant, strong negative correlation was observed ($r = -0.945$, $p = 0.004$).

(a)



(b)

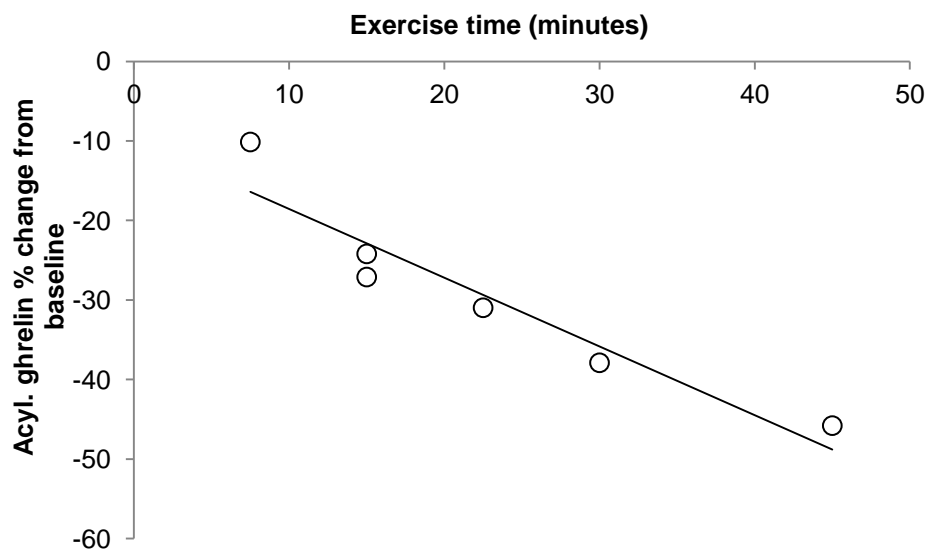


Figure 5.3 – Mean plasma concentration of acylated ghrelin (a) and mean percentage change from baseline with duration of exercise (combined data from 3 exercise trials, (b)). ●, solid line = REST; ○, large dash = 15 min; ■, medium dash = 30 min; □, small dash = 45 min. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean \pm SEM. * = significant within-condition, lower than baseline. † = significant between-condition effect, 45 min lower than REST.

5.4.4.3 PYY

Baseline PYY concentrations were similar across the four conditions. There was no significant condition x time interaction for PYY concentration, nor was there a significant main effect for condition (**figure 5.4**). A significant time main effect ($F(2.681) = 10.938$, $p < 0.001$) demonstrated, across all conditions, a trend for PYY concentration to fall during the post-exercise period, until the test meal ($t=60$), with mean concentration at $t=60$ lower than baseline, midpoint and $t=0$ ($p = 0.035$, 0.002 and 0.008 respectively) and concentration at $t=40$ lower than midpoint and $t=0$ ($p = 0.016$ and 0.02 respectively). Further, there was no correlation between PYY % Δ and time spent exercising (not shown).

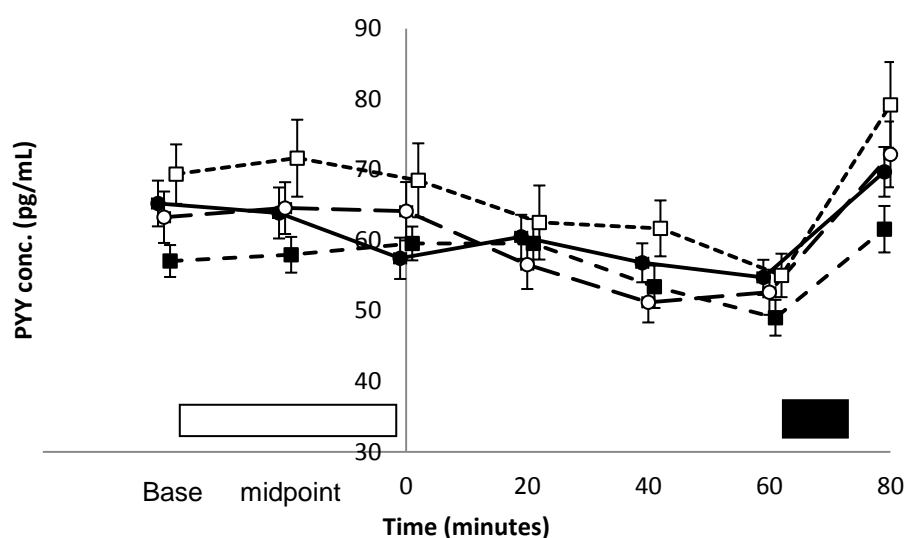


Figure 5.4. Mean plasma concentration of PYY. ●, solid line = rest; ○, large dash = 15 min; ■, medium dash = 30 min; □, small dash = 45 min. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean \pm SEM.

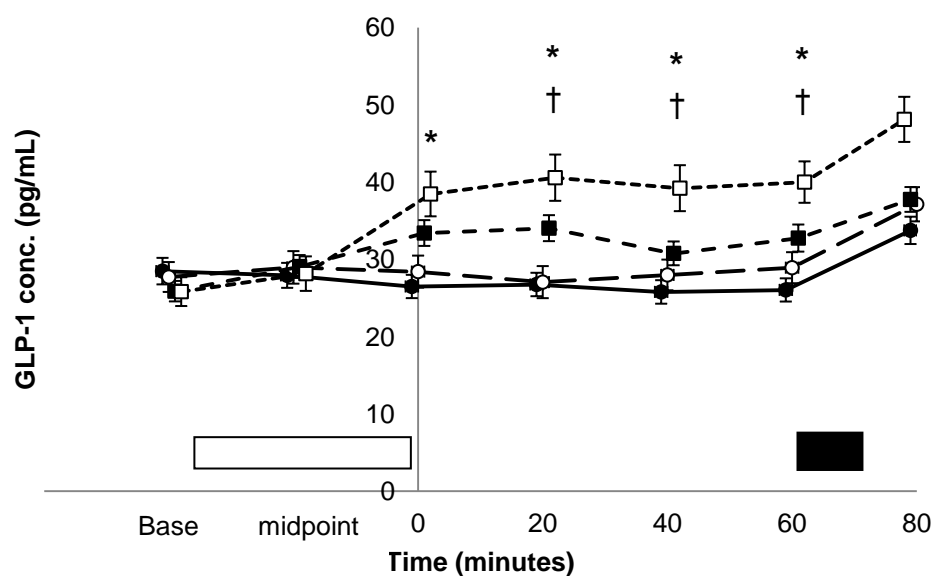
5.4.4.4 GLP-1

There were no significant differences in GLP-1 concentration at baseline. There was a significant time x condition interaction for GLP-1 plasma concentration ($F(4.74) = 7.637$, $p < 0.001$; **figure 5.5.a**). Post-hoc analysis of between-condition comparisons showed that, at $t=0$, there was a trend for higher GLP-1 concentration in the 30min trial (33.4 ± 11.1 pg•mL⁻¹) compared with REST (26.5 ± 10.0 pg•mL⁻¹, $p = 0.093$) and higher concentration in 45min (38.5 ± 19.2 pg•mL⁻¹) versus 15min (28.4 ± 13.8 pg•mL⁻¹, $p = 0.076$). At $t=20$, the trend of a

higher concentration in 30min versus REST was maintained, while plasma GLP-1 was significantly higher in 45min ($40.6 \pm 19.9 \text{ pg}\cdot\text{mL}^{-1}$) versus 15min ($27.1 \pm 13.8 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.024$) and close to significantly higher in 45min versus REST (vs. $26.7 \pm 10.1 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.080$). This elevated concentration in 45min was significantly higher than both REST and 15min at $t=40$ ($p = 0.035$ and $p = 0.047$ respectively) and $t=60$ ($p = 0.012$ and $p = 0.040$ respectively) and remained higher than rest after the test meal ($p = 0.035$). Plasma GLP-1 was higher in 30min, compared with REST at $t=60$ ($p = 0.032$). Post-hoc analysis of within-condition differences showed that, in 30min, plasma GLP-1 increased above baseline concentration by the midpoint of exercise (29.1 ± 9.8 vs. $25.9 \pm 9.0 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.022$) and remained so for the entire trial period. In 45min, GLP-1 concentration raised significantly above baseline at $t=0$ (38.5 ± 19.2 vs. $25.8 \pm 12.4 \text{ pg}\cdot\text{mL}^{-1}$, $p = 0.043$) and stayed elevated for the remainder of the trial period. AUC analysis demonstrated a main effect of condition ($F(3) = 5.036$, $p = 0.006$). Pairwise comparisons highlighted that there was a trend for a greater AUC in 45min versus REST ($p = 0.091$) and versus 15 min ($p = 0.067$).

Figure 5.5b shows the $\%\Delta$ in GLP-1 with increasing exercise time, across the three exercise trials. There was a significant, very strong, positive correlation between exercise time and GLP-1 concentration ($r = 0.946$, $p = 0.004$). Further inspection highlighted that the data appeared to fit a non-linear regression model. Curve fit analysis of the data showed the largest r^2 value when applying a quadratic polynomial function ($r^2 = 0.993$, $p = 0.017$). This would suggest a non-linear increase in GLP-1 concentration with duration of exercise.

(a)



(b)

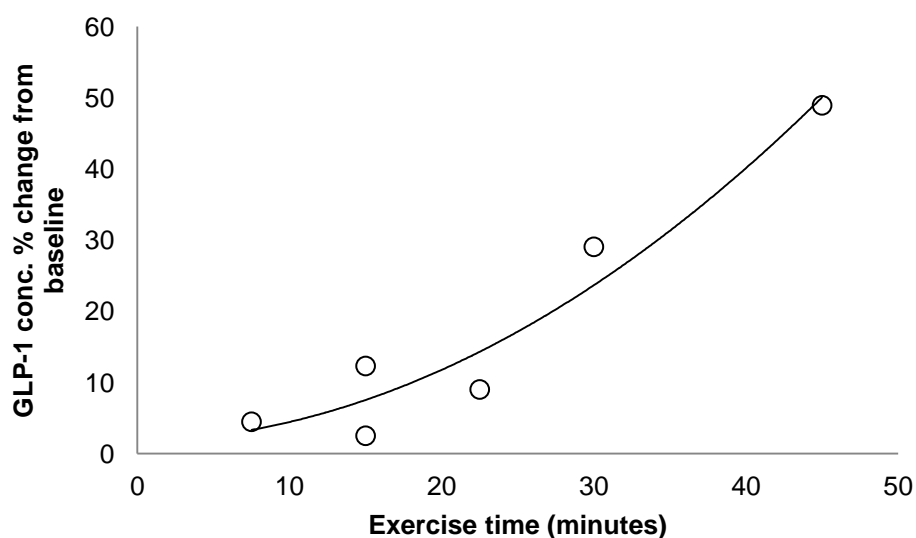


Figure 5.5. Mean plasma concentration of GLP-1 (a) and mean percentage change from baseline with duration of exercise (combined data from 3 exercise trials, (b)). ●, solid line = rest; ○, large dash = 15 min; ■, medium dash = 30 min; □, small dash = 45 min. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean \pm SEM. * = significant within-condition, lower than baseline. † = significant between-condition effect.

5.4.5 Relationship between hormones, subjective appetite and food intake.

5.4.5.1 Between-condition correlations of change in hormone concentration with exercise and change in appetite with exercise

In order to investigate if a change in hormone concentration from one trial to another influenced subsequent change in subjective appetite, within-subject, between-condition correlations were conducted. Changes in % Δ acylated ghrelin were negatively correlated with percentage changes in % Δ VAS for differences between REST and 15 min ($r = -0.570$, $p = 0.035$). Changes in % Δ GLP-1 were negatively correlated with changes in % Δ VIMEC ($r = -0.548$, $p = 0.040$). For differences between REST and 45 min, there was a positive relationship between changes in % Δ acylated ghrelin and changes in % Δ VIMEC ($r = 0.683$, $p = 0.01$). There were no significant relationships for differences between REST and 30 min.

5.4.5.2 Between-condition correlations of percentage change in hormone concentration with percentage change in energy intake, and of percentage change in appetite score with percentage change in energy intake

In order to investigate if a change in hormone concentration or subjective appetite from one trial to another influenced subsequent change in energy intake, within-subject, between-condition correlations were conducted. Percentage change in VAS score was significantly, positively correlated with percentage change in energy intake for differences between REST and 15 min ($r = 0.567$, $p = 0.034$). There was also a trend for a negative correlation between changes in PYY and changes in energy intake ($r = -0.436$, $p = 0.090$) and changes in GLP-1 and changes in energy intake ($r = -0.508$, $p = 0.055$), while a trend was evident for a positive correlation between changes in acylated ghrelin and changes in intake ($p = 0.507$, $p = 0.056$). For differences between REST and 30 min, both VAS and VIMEC scores were positively related with changes in energy intake ($r = 0.658$, $p = 0.014$ and $r = 0.936$, $p < 0.001$, respectively). The relationship between changes in PYY and changes in energy intake approached significance ($r = -0.518$, $p = 0.051$). For differences

between REST and 45 min, there was a trend for a significant negative correlation between changes in PYY and changes in energy intake ($r = -0.496$, $p = 0.060$) once again.

5.5 DISCUSSION

The aim of the current study was to assess the effect of the duration of high-intensity aerobic exercise on subjective appetite, food intake and appetite-regulating hormones in highly-trained male endurance athletes. Contrary to the hypothesis, subjective appetite was not significantly suppressed post-exercise in any of the three exercise conditions, with no significant drop in appetite scores from baseline observed with either subjective appetite measure. Similarly, neither VAS nor VIMEC scores in any of the three exercise conditions differed from resting condition scores at any point in the trial period. A lack of statistically significant difference was despite a 14% decrease in VAS score post-exercise in 15min and an 11% decrease in 45min. Further, VAS scores were 21% lower than REST in 15min and 22% lower in 45min.

It was hypothesised that completing exercise of such a high intensity would result in a suppression of appetite in the 30 minute and 45 minute conditions. Exercise at an intensity $\geq 60\%$ $\text{VO}_{2\text{max}}$ often elicits a transient suppression in recreationally active individuals (Broom *et al.*, 2009; King *et al.*, 2010; Martins *et al.*, 2007), despite being of a shorter duration and lower energy cost (Laan *et al.*, 2010) than the present study. Nonetheless, the present study is not in isolation in finding no significant suppression of appetite despite a large exercise stimulus (Imbeault *et al.*, 1997; King *et al.*, 2011a). Further, the present study was conducted in highly trained athletes – a population rarely studied with regard to post-exercise appetite. It may be possible that an appetite suppression was not observed due to a difference in responses to exercise between athletic and non-athletic populations. It may be possible that regularly exercising at an intensity of $\geq 60\%$ $\text{VO}_{2\text{max}}$ may blunt the appetite suppression response observed in those unfamiliar with such exercise. It may also be possible that ingrained behavioural habits of athletes, regarding post-exercise feeding and nutrition are more potent than physiological determinants of appetite and that this the primary regulator of not only food intake, but also perceived appetite. However, this is purely speculative. It must be acknowledged that the failure to observe a significant reduction in appetite, especially

after 30 and 45 minutes of exercise, could be due to lack of statistical power, especially when considering the substantial absolute differences. It may be that a study cohort of 12 individuals was insufficient to afford the necessary statistical power for such a difference to become statistically significant.

Unsurprisingly, in light of the absence of a post-exercise appetite suppression, there was no significant difference in food intake at the *ad libitum* test meal, administered 60 minutes after the cessation of the exercise bout. The lack of any difference in food intake post-exercise is a commonly observed finding, especially when the test meal is consumed 60 minutes post-exercise (King *et al.*, 2010; King *et al.*, 2011b; King *et al.*, 1997; Schubert *et al.*, 2013; Thompson *et al.*, 1988). Interestingly, there was a pattern for increased intake with increased exercise duration. However, the differences between conditions were small and insufficient to indicate a dose-response rebound in appetite. Relative energy intake did differ between trial conditions. This has often been observed in the absence of a lower post-exercise absolute energy intake (Imbeault *et al.*, 1997; King *et al.*, 2010; Lluch *et al.*, 2000; Unick *et al.*, 2010), or even after absolute energy intake is greater after exercise, compared with resting (Martins *et al.*, 2007; Pomerleau *et al.*, 2004). The 45 minute condition, despite yielding a mean energy cost of 787 kcal was insufficient to elicit an acute energy deficit over the course of the trial period. However, when one considers that the exercise was conducted after a 10 hour overnight fast, 114 kcal is a small energy surplus for the morning testing period, which included breakfast. Further, the REI was 573 kcal lower than in the resting condition. From a weight-management perspective, the ACSM guidelines recommend a daily energy deficit of ~170 - 300 kcal for the avoidance of weight gain and a deficit of over 500 kcal•day⁻¹ for weight-loss (Donnelly *et al.*, 2009) This would suggest that, for this study population, an exercise bout of 45 minutes of cycling at 76% VO_{2max} can elicit a reduction in energy balance that, should it be maintained for the remainder of the day could assist in weight maintenance, if not also help promote weight-loss.

While athletes may be concerned with weight management, the immediate post-exercise period is of importance with regard to nutrition for recovery and adaptation to

training. Post-exercise carbohydrate intake is valued by many endurance athletes, with exercise-induced GLUT-4 translocation leading to an increased potential for glucose uptake and glycogen resynthesis after exercise (see (Goodyear and Kahn, 1998; Ivy, 1998; Jentjens and Jeukendrup, 2003)). In addition, amino acid delivery and a positive energy balance stimulate net muscle protein synthesis after resistance (Tipton *et al.*, 1999) and endurance (Howarth *et al.*, 2009) exercise, meaning that the ingestion of protein in close proximity to exercise is often desired for optimal rates of muscle protein synthesis (Phillips, 2006; Phillips and Van Loon, 2011). Therefore, a suppression of appetite post-exercise may be detrimental for recovery and adaptation, should it impact upon nutrition. The findings of the present study would suggest that this was not the case, even after a strenuous bout of 45 minutes of cycling at 76% $\text{VO}_{2\text{max}}$.

A lack of a significant exercise-induced suppression of appetite was allied with no significant change in plasma PYY concentration. Plasma PYY fell throughout the trial period, until the test meal across all trials, and did not differ between conditions. Further, the small increase in PYY concentration observed with exercise did not appear to follow a dose-response with increasing duration of exercise. While PYY concentration has commonly been observed to be responsive to high-intensity aerobic exercise (Broom *et al.*, 2009; King *et al.*, 2011a; Larson-Meyer *et al.*, 2012; Martins *et al.*, 2007; Russel *et al.*, 2009; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b), this was not the case in the present study. It is possible that this is due to the study population; highly-trained athletes, familiar with exercising at such a high-intensity may be resistant to exercise-induced alterations in PYY secretion. Chronic exercise training has been postulated to sensitise satiety peptides to food intake, with greater late post-prandial period concentrations of PYY and GLP-1 with food intake after training (Martins *et al.*, 2010). In addition exercise training has been shown to lower fasting PYY concentrations (Jones *et al.*, 2009; Roth *et al.*, 2005). These may be mechanisms by which regular physical activity assists with tighter regulation of energy balance, through limiting over-eating. Similarly, a blunting of an exercise-induced increase in PYY through regular exercise may be a means of regulating energy balance through the avoidance of appetite

suppression and an energy deficit after the energy cost of exercise. However, no study has yet assessed the PYY response to acute exercise, pre- and post-training.

Despite no significant suppression of appetite post-exercise, there was a clear response to exercise in GLP-1. Concentrations rose with exercise in the 30 minute and 45 minute conditions, by 29% and 49% respectively, with levels remaining elevated during the 60 minute recovery period. This finding is in agreement with previous studies in obese (Ueda *et al.*, 2009b) and healthy-weight (Martins *et al.*, 2007; Ueda *et al.*, 2009a) individuals, after exercise of an intensity lower than that of the current study, lasting 30-60 minutes. As has previously been shown in these studies, the increase in GLP-1 proved lasting. In the present study, concentrations remained elevated at the time of the test meal, 60 minutes post-exercise. No such response was observed in the 15 minute condition however, with the GLP-1 profile closely resembling that of the rest condition. Moreover, there appeared a dose-response increase in GLP-1 concentration with increasing duration of exercise, as demonstrated by the very strong, positive correlation between $\% \Delta$ and time spent exercising, across the exercise conditions. The data appeared to follow a quadratic polynomial function ($y = 0.021x^2 + 0.1545x + 0.391$), which suggests a non-linear increase in GLP-1 concentration with increasing exercise duration. It must be noted at this point that this analysis should be viewed with caution, as the measures used for correlation analysis are not independent data points. Nevertheless, the exploratory investigation into this relationship still allows for an interesting observation. Together these data would suggest that just 15 minutes of high-intensity cycling at 76% $\text{VO}_{2\text{max}}$ was an insufficient stimulus to cause any exercise-induced increase in plasma GLP-1 and that GLP-1 concentration during high-intensity aerobic exercise exhibits some duration or energy expenditure dependency, possibly with a threshold duration for its secretion.

Acylated ghrelin also proved responsive to exercise. The plasma concentration fell with exercise in all three exercise conditions, with the greatest decrease seen after 45 minutes of cycling. This suppression of acylated ghrelin was transient, with concentrations not significantly different to baseline by 40 minutes post-exercise, even in 45 min; neither

was there a significant difference between any exercise condition and the resting condition at this time point. This was despite acylated ghrelin concentration being 28%, 20% and 23% lower than rest in the 15 minute, 30 minute and 45 minute conditions, respectively. There was evidence of a dose-response of acylated ghrelin suppression, to the duration of exercise. Further, when assessing changes in acylated ghrelin concentration over time during exercise, a significant, very strong, negative correlation between % Δ and time spent cycling was present (again, non-independent data points were used, so this observation must be accepted with caution). This data would suggest that, with high-intensity aerobic exercise, plasma acylated ghrelin concentration begins to fall in the very early stages of exercise and continues to fall as the bout continues. This would suggest a physiological mechanism by which the duration of exercise is an important regulatory factor in post-exercise appetite suppression. However, the absence of a significant suppression of appetite (either compared with baseline or with REST), dispels this theory somewhat and also questions the role of acylated ghrelin as a regulator of appetite in the post-exercise state.

It would appear that there are a number of inconsistencies in the hormonal response to exercise and subjective appetite in the present study. Firstly, changes in acylated ghrelin and GLP-1 in favour of an anorexigenic state were not observed with PYY. It has generally been observed that alterations in appetite-associated hormones occur concurrently, especially with regards to satiety peptides (Broom *et al.*, 2009; King *et al.*, 2011a). Larson-Meyer *et al.* (Larson-Meyer *et al.*, 2012) did see a contrasting increase in the orexigenic hormone ghrelin and in the anorexigenic hormones PYY and GLP-1, while Ueda and colleagues observed an exercise induced increase in PYY and GLP-1, with no change in total ghrelin concentration (Ueda *et al.*, 2009b). This research group did, however, demonstrate differential responses in PYY and GLP-1 following 30 minutes of cycling at 50% VO_{2max} and 75% VO_{2max} (Ueda *et al.*, 2009a). They found that PYY secretion appeared to be intensity-dependent, whereas GLP-1 concentration increased to the same extent in both exercise trials. The authors suggest that their data would advocate specific exercise responses in plasma kinetics of PYY and GLP-1. The data of the present study would

support this notion, augmenting the theory by postulating that the rise in plasma GLP-1 concentration appears to be duration and/or energy expenditure dependent. Further, if an increase in plasma PYY is intensity-dependent, then it may be the case that athletes possess a blunted response, or, due to regularly undertaking high-intensity exercise, have elevated their threshold intensity for PYY release.

Secondly, as alluded to earlier, the anorexigenic stimulus of an increase in GLP-1 concentration and a decrease in acylated ghrelin was not reflected by a suppression of subjective appetite or a reduction in food intake. Total ghrelin (Wren *et al.*, 2001), acylated ghrelin (Druce *et al.*, 2005) and GLP-1 (Verdich *et al.*, 2001) have been shown to be potent appetite regulators when administered pharmaceutically. However, in this case, exercise-induced alterations in both, that would favour an anorexigenic state, did not lead to a suppression of subjective appetite.

Assessment of the relationships for within-subject changes in appetite, hormone concentration and energy showed little consistency in the association between changes in appetite-associated hormones and subjective appetite. Changes in $\% \Delta$ GLP-1 showed a moderate-strength, negative correlation with changes in $\% \Delta$ VIMEC score in 15 min, while changes in $\% \Delta$ acylated ghrelin was moderately, positively correlated with change $\% \Delta$ VIMEC score in 45 min. However, changes in $\% \Delta$ acylated ghrelin was also negatively correlated with changes in $\% \Delta$ VIMEC for 15 min – an inverse relationship to that which was seen in 45 min, and to that which would have been expected. Within-subject percentage changes in appetite-associated hormones immediately prior to the test meal did not correlate significantly with percentage changes in energy intake, which again would suggest a weak relationship between hormonal regulators of appetite and food intake.

There is evidence that in the post-exercise period, there is blunting to hormonal regulators of appetite. In a recent study by Heden *et al.* (Heden *et al.*, 2013), acylated ghrelin and subjective appetite responded differently with exercise in healthy-weight and obese individuals; acylated ghrelin decreased post-exercise in healthy-weight participants but appetite ratings remained unchanged. Conversely, acylated ghrelin was unaltered in the

obese post-exercise, which was reflected by no alteration in subjective appetite. This data, allied with the findings of the present study, would suggest a discrepancy between changes in hormone concentration and changes in subjective appetite in the post-exercise period in highly-active, healthy-weight and obese individuals and hence questions the importance of exercise-induced changes in appetite-associated hormones for appetite regulation and acute energy balance.

In conclusion, as much as 45 minutes of cycling at 76% $\text{VO}_{2\text{max}}$ failed to suppress subjective appetite or alter acute food intake in highly-trained endurance athletes. This was despite a transient suppression of acylated ghrelin and a sustained increase in GLP-1. Both of these hormones appeared to change in an exercise-duration-dependent manner. These findings suggest that highly-trained individuals, accustomed to high-intensity aerobic exercise, may exhibit a blunted response to exercise-induced appetite suppression, as is commonly observed with recreationally active and (less commonly observed) overweight individuals. Further, it would appear that post-exercise appetite responses are not duration-dependent. The current data would also imply that changes in appetite-associated hormones, specifically acylated ghrelin and GLP-1, may not play an important regulatory role in either subjective appetite or post-exercise food intake.

5.6 REFERENCES

- Bellissimo, N., Thomas, S.G., Goode, R.C., and Anderson, G.H. (2007). Effect of short-duration physical activity and ventilation threshold on subjective appetite and short-term energy intake in boys. *Appetite* 49, 644-651.
- Borg, G.A.V. (1973). "Perceived exertion: a note on history and methods". *Medicine and Science in Sports & Exercise* 5, 90-93.
- Broom, D.R., Batterham, R.L., King, J.A., and Stensel, D.J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 296, R29-R35.
- Broom, D.R., Stensel, D.J., Bishop, N.C., Burns, S.F., and Miyashita, M. (2007). Exercise-induced suppression of acylated ghrelin in humans. *J Appl Physiol* 102, 2165-2171.
- Burke, L.M. (1997). Nutrition for post-exercise recovery. *Australian journal of science and medicine in sport* 29, 3-10.
- Burns, S.F., Broom, D.R., Miyashita, M., Mundy, C., and Stensel, D.J. (2007). A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of Sports Sciences* 25, 635-642.
- Deighton, K., Barry, R., Connon, C., and Stensel, D. (2012). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*, 1-10.
- Donnelly, J.E., Blair, S.N., Jakicic, J.M., Manore, M.M., Rankin, J.W., Smith, B.K., and null (2009). American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Medicine and science in sports and exercise* 41, 459-471.
- Druce, M.R., Neary, N.M., Small, C.J., Milton, J., Monteiro, M., Patterson, M., Ghatei, M.A., and Bloom, S.R. (2005). Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *Int J Obes Relat Metab Disord* 30, 293-296.
- Erdmann, J., Tahbaz, R., Lippl, F., Wagenpfeil, S., and Schusdziarra, V. (2007). Plasma ghrelin levels during exercise -- Effects of intensity and duration. *Regulatory Peptides* 143, 127-135.
- Filaire, E., Rouveix, M., Pannafieux, C., and Ferrand, C. (2007). Eating attitudes, perfectionism and body-esteem of elite male judoists and cyclists. *Journal of Sports Science and Medicine* 5, 50-57.
- Goodyear, P., Laurie J., and Kahn, M., Barbara B. (1998). Exercise, glucose transport, and insulin sensitivity. *Annual Review of Medicine* 49, 235-261.
- Heden, T.D., Liu, Y., Park, Y., Dellsperger, K.C., and Kanaley, J.A. (2013). Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology*.
- Hill, A.J., and Blundell, J.E. (1982). Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research* 17, 203-212.

- Howarth, K.R., Moreau, N.A., Phillips, S.M., and Gibala, M.J. (2009). Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of Applied Physiology* 106, 1394-1402.
- Imbeault, P., Saint-Pierre, S., Alm-Ras, N., and Tremblay, A. (1997). Acute effects of exercise on energy intake and feeding behaviour. *British Journal of Nutrition* 77, 511-521.
- Ivy, J.L. (1998). Glycogen resynthesis after exercise: effect of carbohydrate intake. *International journal of sports medicine* 19 Suppl 2, S142-145.
- Jentjens, R., and Jeukendrup, A. (2003). Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports medicine (Auckland, NZ)* 33, 117-144.
- Jones, T.E., Basilio, J.L., Brophy, P.M., McCammon, M.R., and Hickner, R.C. (2009). Long-term exercise training in overweight adolescents improves plasma peptide YY and resistin. *Obesity* 17, 1189-1195.
- Jurimae, J., Hofmann, P., Jurimae, T., Maestu, J., Purge, P., Wonisch, M., Pokan, R., and von Duvillard, S.P. (2006). Plasma adiponectin response to sculling exercise at individual anaerobic threshold in college level male rowers. *Int J Sports Med* 27, 272-277.
- Jurimae, J., and Jurimae, T. (2005). Leptin responses to short term exercise in college level male rowers. *Br J Sports Med* 39, 6-9.
- Jurimae, J., Jurimae, T., and Purge, P. (2007). Plasma ghrelin is altered after maximal exercise in elite male rowers. *Experimental Biology and Medicine* 232, 904-909.
- Jürimäe, J., Mäestu, J., and Jürimäe, T. (2003). Leptin as a marker of training stress in highly trained male rowers? *Eur J Appl Physiol* 90, 533-538.
- Jurimae, J., Ramson, R., Maestu, J., Purge, P., Jurimae, T., Arciero, P.J., and Von Duvillard, S.P. (2009). Plasma visfatin and ghrelin response to prolonged sculling in competitive male rowers. *Medicine & Science in Sports & Exercise* 41, 137-143
110.1249/MSS.1240b1013e31818313e31818316.
- Kawano, H., Mineta, M., Asaka, M., Miyashita, M., Numao, S., Gando, Y., Ando, T., Sakamoto, S., and Higuchi, M. (2013). Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite* 66, 26-33.
- King, J.A., Miyashita, M., Wasse, L.K., and Stensel, D.J. (2010). Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* 54, 492-498.
- King, J.A., Wasse, L.K., Ewens, J., Crystall, K., Emmanuel, J., Batterham, R.L., and Stensel, D.J. (2011a). Differential acylated ghrelin, peptide YY₃₋₃₆, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology & Metabolism* 96, 1114-1121.
- King, J.A., Wasse, L.K., and Stensel, D.J. (2011b). The acute effects of swimming on appetite, food intake, and plasma acylated ghrelin. *Journal of Obesity* 2011.
- King, N.A., Burley, V.J., and Blundell, J.E. (1994). Exercise induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition* 48, 715-724

King, N.A., Tremblay, A., and Blundell, J.E. (1997). Effects of exercise on appetite control: implications for energy balance. *Medicine & Science in Sports & Exercise* 29, 1076-1089.

Kissileff, H.R., Pi-Sunyer, F.X., Segal, K., Meltzer, S., and Foelsch, P.A. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition* 52, 240-245.

Kraemer, R.R., Durand, R.J., Acevedo, E.O., Johnson, L.G., Kraemer, G.R., Hebert, E.P., and Castracane, V.D. (2004). Rigorous running increases growth hormone and insulin-like growth factor-i without altering ghrelin. *Experimental Biology and Medicine* 229, 240-246.

Laan, D.J., Leidy, H.J., Lim, E., and Campbell, W.W. (2010). Effects and reproducibility of aerobic and resistance exercise on appetite and energy intake in young, physically active adults. *Applied Physiology, Nutrition, and Metabolism* 35, 842-847.

Larson-Meyer, D.E., Palm, S., Bansal, A., Austin, K.J., Hart, A.M., and Alexander, B.M. (2012). Influence of running and walking on hormonal regulators of appetite in women. *Journal of Obesity* 2012, 15.

Lluch, A., King, N.A., and Blundell, J.E. (2000). No energy compensation at the meal following exercise in dietary restrained and unrestrained women. *British Journal of Nutrition* 84, 219-225.

Martins, C., Kulseng, B., King, N.A., Holst, J.J., and Blundell, J.E. (2010). The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. *Journal of Clinical Endocrinology & Metabolism* 95, 1609-1616.

Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 193, 251-258.

Marzullo, P., Salvadori, A., Brunani, A., Verti, B., Walker, G.E., Fanari, P., Tovaglieri, I., Medici, C.D., Savia, G., and Liuzzi, A. (2008). Acylated ghrelin decreases during acute exercise in the lean and obese state. *Clinical Endocrinology* 69, 970-971.

O'Connor, A.M., Johnston, C.F., Buchanan, K.D., Boreham, C., Trinick, T.R., and Riddoch, C.J. (1995). Circulating gastrointestinal hormone changes in marathon running. *Int J Sports Med* 16, 283-287.

O'Connor, A.M., Pola, S., Ward, B.M., Fillmore, D., Buchanan, K.D., and Kirwan, J.P. (2006). The gastroenteroinsular response to glucose ingestion during postexercise recovery. *Am J Physiol Endocrinol Metab* 290, E1155-1161.

Phillips, S.M. (2006). Dietary protein for athletes: from requirements to metabolic advantage. *Applied Physiology, Nutrition, and Metabolism* 31, 647-654.

Phillips, S.M., and Van Loon, L.J.C. (2011). Dietary protein for athletes: From requirements to optimum adaptation. *Journal of Sports Sciences* 29, S29-S38.

Pomerleau, M., Imbeault, P., Parker, T., and Doucet, E. (2004). Effects of exercise intensity on food intake and appetite in women. *Am J Clin Nutr* 80, 1230-1236.

Reid, C.H., CG. Blundell, JE. Stubbs, RJ (1998). What are psychometric assessments of appetite asking: a preliminary multivariate analysis. *International Journal of Obesity* 22, 151.

- Rocha, J., Paxman, J., Dalton, C., Winter, E., and Broom. (2013). Effects of an acute bout of aerobic exercise on immediate and subsequent three-day food intake and energy expenditure in active and inactive men. *Appetite* 71, 369-378.
- Roth, C.L., Enriori, P.J., Harz, K., Woelfle, J., Cowley, M.A., and Reinehr, T. (2005). Peptide YY is a regulator of energy homeostasis in obese children before and after weight loss. *J Clin Endocrinol Metab* 90, 6386-6391.
- Russel, R.R., Willis, K.S., Ravussin, E., and Larson-Meyer, E.D. (2009). Effects of endurance running and dietary fat on circulating ghrelin and peptide YY. *Journal of sports science & medicine* 8, 574-583.
- Schmidt, A., Maier, C., Schaller, G., Nowotny, P., Bayerle-Eder, M., Buranyi, B., Luger, A., and Wolzt, M. (2004). Acute exercise has no effect on ghrelin plasma concentrations. *Horm Metab Res* 36, 174-177.
- Schubert, M.M., Desbrow, B., Sabapathy, S., and Leveritt, M. (2013). Acute exercise and subsequent energy intake. A meta-analysis. *Appetite* 63, 92-104.
- Shiia, T., Ueno, H., Toshinai, K., Kawagoe, T., Naito, S., Tobina, T., Nishida, Y., Shindo, M., Kangawa, K., Tanaka, H., *et al.* (2011). Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocrine journal* 58, 335-342.
- Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., and Blundell, J.E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* 84, 405-415.
- Thompson, D.A., Wolfe, L.A., and Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medicine & Science in Sports & Exercise* 20, 222-227.
- Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle, D., and Wolfe, R.R. (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *American Journal of Physiology - Endocrinology And Metabolism* 276, E628-E634.
- Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., and Fujimoto, S. (2009a). Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *Journal of Endocrinology* 203, 357-364.
- Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., and Fujimoto, S. (2009b). Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J Endocrinol*, JOE-08-0500.
- Unick, J.L., Otto, A.D., Goodpaster, B.H., Helsel, D.L., Pellegrini, C.A., and Jakicic, J.M. (2010). Acute effect of walking on energy intake in overweight/obese women. *Appetite* 55, 413-419.
- Verdich, C., Flint, A., Gutzwiller, J.-P., Naslund, E., Beglinger, C., Hellstrom, P.M., Long, S.J., Morgan, L.M., Holst, J.J., and Astrup, A. (2001). A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on *ad libitum* energy intake in humans. *J Clin Endocrinol Metab* 86, 4382-4389.

Westerterp-Plantenga, M.S., Verwegen, C.R., Ijeda, M.J., Wijckmans, N.E., and Saris, W.H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* 62, 1345-1354.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillon, W.S., Ghatei, M.A., and Bloom, S.R. (2001). Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 86, 5992-.

The effect of sprint interval cycling exercise on appetite, food intake and appetite-associated hormones in overweight and obese individuals.

6.1 ABSTRACT

Background: High-intensity, continuous, aerobic exercise ($\geq 60\%$ $\text{VO}_{2\text{max}}$) has been shown to elicit a transient suppression of appetite and create a more anorexic profile of appetite-associated hormones. It is yet to be elucidated whether such a response is observed following low-volume, intermittent exercise at supramaximal intensity and whether any effect is likely to be robust enough to impact upon post-exercise food intake.

Purpose: To investigate the effect of low-volume sprint interval cycling exercise (SICE) on subjective appetite, food intake and appetite-associated hormones in overweight and obese individuals.

Method: Eight (4 male) overweight and obese individuals ($\text{BMI } 28.8 \pm 5.7 \text{ kg}\cdot\text{m}^{-2}$) completed both a resting (REST) and an exercise (EX) trial in a counterbalanced, within-subject study design. Participants arrive at the laboratory in the fasted state and were provided with a standardised breakfast ($t = -120$ minutes), before resting. At $t = 0$, exercise commenced in the exercise trial. This consisted of 4 x 30 seconds “flat-out” cycling on a cycle ergometer (adapted Wingate test), with 4 minute recovery. After the exercise bout, or an equivalent period of time in the resting trial, a 2h recovery resting period commenced. Two hours post-exercise, the participant was presented with an *ad libitum* buffet meal. Subjective appetite measures and blood samples were obtained throughout the trial period. Plasma was analysed for the concentration of acylated ghrelin, polypeptide YY (PYY) and glucagon-like peptide 1 (GLP-1).

Results: Subjective appetite, as measured using the VAS method, was significantly lower after exercise compared with REST ($38.0 \pm 28.5\text{mm}$ vs. $75.1 \pm 26.2\text{mm}$, $p = 0.018$). This difference remained significant 30 minutes post-exercise ($53.0 \pm 32.6\text{mm}$ vs. $89.2 \pm 21.6\text{mm}$, $p = 0.034$). Acylated ghrelin concentration was suppressed in EX compared with REST immediately post-exercise ($113.4 \pm 43.0 \text{ pg}\cdot\text{mL}^{-1}$ vs. 189.2 ± 91.8

pg•mL⁻¹, $p = 0.03$) and remained lower for the entire post-exercise, pre-test-meal period. Area-under-the-curve for GLP-1 concentration was greater for EX versus REST and PYY showed a transient, non-significant increase with exercise. There was no difference in *ad libitum* intake at the test meal. Further, when the energy expenditure of exercise was accounted for, relative energy intake also did not differ between trial conditions.

Conclusion: As little as 4 x 30 seconds of “flat-out” cycle exercise was sufficient to suppress appetite. This was allied with a sustained suppression in plasma acylated ghrelin and more modest increases in PYY and GLP-1. The suppression of appetite was short-lived and elicited no difference in absolute or relative intake 2-hours post-exercise between REST and EX. Further research is required to determine whether this has implications for the undertaking of such exercise bouts as part of a fitness programme or weight-loss strategy.

6.2 INTRODUCTION

It has been repeatedly shown that high-intensity, continuous aerobic exercise elicits a transient suppression of appetite in lean, recreationally active individuals (Broom *et al.*, 2009; Broom *et al.*, 2007; Burns *et al.*, 2007; Kawano *et al.*, 2013; King *et al.*, 2010; King *et al.*, 2011b; Martins *et al.*, 2007; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b). Typically, this phenomenon, termed the “anorexia of exercise,” (King *et al.*, 1994) is observed with exercise of an intensity $\geq 60\%$ $\text{VO}_{2\text{max}}$. Post-exercise appetite suppression is a less common observation in obese and overweight individuals (Dodd *et al.*, 2008; George and Morganstein, 2003; Unick *et al.*, 2010). This may be due to a differing response in this population, or could be because exercise of an intensity of $>60\%$ $\text{VO}_{2\text{max}}$ is rarely undertaken in studies investigating acute effects of exercise on appetite in the overweight and obese (George and Morganstein, 2003; Unick *et al.*, 2010). The paucity of literature for this study population makes fully elucidating this issue difficult.

While it has been shown that a suppression of subjective appetite can lead to reductions in food intake in the immediate post-exercise period (~10 minutes (Westerterp-Plantenga *et al.*, 1997); ~15 minutes (Kissileff *et al.*, 1990); ~30 minutes (Ueda *et al.*, 2009a); ~60 minutes (Ueda *et al.*, 2009b) food intake is largely unaffected when a meal is consumed ≥ 60 minutes after exercise (King *et al.*, 2010; King *et al.*, 2011b; King *et al.*, 1997b; Martins *et al.*, 2007; Schubert *et al.*, 2013; Thompson *et al.*, 1988). However, the response in subjective appetite is often coupled with changes in appetite-associated hormones, in the favour of an anorexigenic state. While it would appear that total ghrelin plasma concentration is perhaps unresponsive to exercise (Burns *et al.*, 2007; Kraemer *et al.*, 2004; Martins *et al.*, 2007; Schmidt *et al.*, 2004), or may even be increased post-exercise (Larson-Meyer *et al.*, 2012; Russel *et al.*, 2009) concentrations of the acylated form of ghrelin, have been shown to decrease with

exercise (Broom *et al.*, 2009; Broom *et al.*, 2007; King *et al.*, 2010; Marzullo *et al.*, 2008; Shiya *et al.*, 2011). In contrast, plasma concentrations of the satiety peptides peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) have been shown to increase with exercise (Broom *et al.*, 2009; Larson-Meyer *et al.*, 2012; Martins *et al.*, 2007; Russel *et al.*, 2009; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b).

The intensity dependency of an exercise-induced suppression of appetite appears well founded. However, the findings of Chapter 5 would suggest that the exercise duration is not an important driver of exercise-induced changes in appetite.

If the exercise-induced suppression of appetite effect is exclusively driven by intensity and independent of duration and energy expenditure, it may be possible to elicit a suppression of appetite with low-volume, time efficient bouts of supramaximal exercise. High-intensity interval exercise (HIE) and high-intensity training (HIT) have recently been shown to elicit a host of physiological adaptations (Bartlett *et al.*, 2012; Burgomaster *et al.*, 2006; Burgomaster *et al.*, 2008; Burgomaster *et al.*, 2005) and health benefits (Babraj *et al.*, 2009; Little *et al.*, 2011; Whyte *et al.*, 2010), akin to that seen with more traditional, longer duration, continuous aerobic exercise. Furthermore, it has also been suggested that this form of exercise is perceived as more enjoyable than more traditional endurance exercise (Bartlett *et al.*, 2011). With such observations, allied with reduced time requirements, HIT is considered by some to be a preferable form of exercise to undertake. However, one criticism often levelled at such low-volume exercise bouts is a low energy cost. It is often argued that HIT is an ineffective form of exercise for inducing weight-loss in overweight and obese individuals, as the low energy cost is unlikely to yield an energy deficit sufficient to promote weight-loss. Typically, only modest weight-loss is observed in long-term HIT studies (Burgomaster *et al.*, 2008; Helgerud *et al.*, 2007), even in overweight and obese individuals (Whyte *et al.*, 2010), although significant reductions in body weight (Trapp *et al.*, 2008) and fat mass have been achieved (see Boutcher, 2011).

In light of a number of chronic exercise training studies, the dearth of research in to the effect of HIE on appetite and acute food intake is perhaps surprising. Despite a considerable amount of investigation into the effect of continuous aerobic exercise on appetite, there is little within the literature with respect to alternative forms of exercise. Only a small number of studies have investigated the effect of low-volume, high-intensity exercise, or sprint interval exercise (SIE) on appetite, food intake and gut hormones (Deighton *et al.*, 2012; Deighton *et al.*, 2013; Sim *et al.*, 2013). Six 30 second Wingate tests transiently suppressed appetite and circulating acylated ghrelin concentrations, as well as elevating plasma PYY₃₋₃₆ (the most abundant form of PYY) concentrations. This response was achieved with an estimated mean energy cost of exercise of just 142 kcal. However, food intake at an *ad libitum* buffet meal, provided 45 minutes after the cessation of exercise was no different to that of a control condition (Deighton *et al.*, 2012). In a follow-up study, 10 x 4 minutes of cycling at 85% VO_{2max} suppressed appetite and elevated plasma PYY₃₋₃₆ concentrations to a greater extent than isocaloric continuous exercise at 60% VO_{2max} (Deighton *et al.*, 2013). While these studies were conducted with recreationally active individuals, Sim *et al.* (Sim *et al.*, 2013) investigated the effect of high-intensity intermittent cycling on appetite in overweight males. Repetitions of 60 second intervals at an intensity of 100% VO_{2peak} (high-intensity) and of 15 second intervals at 170% VO_{2peak} (very-high-intensity) did not result in changes in subjective appetite, compared with baseline, a resting control condition or energy-matched moderate-intensity, continuous exercise. However, energy intake at a test meal provided 70 minutes post-exercise was lower after high-intensity and very-high-intensity exercise, compared with rest and rest and continuous exercise, respectively. The monitoring of *ad libitum* food intake for the remainder of the trial day and the following day showed that intake was lower after the very-high-intensity exercise trial, compared with the resting and continuous exercise trials. These data suggest that exercise of a sufficiently high intensity may provide a strong enough

stimulus to influence food intake in the hours and days post-exercise, even in the absence of changes in acute subjective appetite. However, further work is certainly required to substantiate this curious finding.

The aim of the current study was to investigate the effect of low-volume sprint interval cycling on appetite, food intake and circulating concentration of the appetite-associated hormones acylated ghrelin, PYY and GLP-1 in overweight and obese individuals. It was hypothesised that just 4 x 30 seconds maximal effort cycling would induce a suppression of subjective appetite, and that this would be associated with an anorexigenic change in hormones. However, it was expected that these changes would be transient, meaning that a difference in food intake two hours post-exercise was unlikely to be observed.

6.3 METHOD

6.3.1 Participants

Eleven overweight or obese, low- to moderate-activity level individuals were recruited principally from the University of Birmingham students and staff. Inclusion criteria were: a BMI of $\geq 25 \text{ kg}\cdot\text{m}^{-2}$, a score of ≤ 3000 MET-minutes per week on the International Physical Activity Questionnaire (IPAQ, Appendix 8), currently weight-stable and not attempting weight-loss and not currently undertaking any form of vigorous exercise. Exclusion criteria were: a score of 3.5 or greater for restricted eating on the Dutch Eating Behaviour Questionnaire (DEBQ, van Strien *et al*, 1986); resting blood pressure of $>140/90$ mm Hg, irregular cardiac activity at rest (as assessed with the use of a resting ECG), illness such as upper respiratory tract infections; smoking and the taking of medication likely to affect appetite or induce weight-loss. Ethical approval was obtained from the Ethics Subcommittee of the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. Of the eleven participants recruited, two withdrew and one was excluded from the results on the basis that their resting acylated ghrelin concentration was >2 SD from the mean. Further, we were unable to obtain blood samples from one participant who did complete the study, so hormone analysis was conducted for seven participants. The participant characteristics for the eight individuals who completed the study (4 males, 4 females) are shown in **table 6.1**.

Age (years)	38 ± 14
BMI (kg·m⁻²)	29.2 ± 5.7
DEBQ score for restraint	2.7 ± 0.8
IPAQ score for physical activity level (METS)	2063 ± 1166

Table 6.1. – Participant characteristics. Values are mean ± SD.

6.3.2 Experimental trial conditions

Participants were randomly assigned to both the exercise (EX) and resting (REST) trial conditions, with measures of subjective appetite and circulating concentrations of acylated ghrelin, PYY and GLP-1 recorded throughout.

6.3.3 Procedure & protocol

Participants arrived at the laboratory at approximately 08:00, after an overnight fast, for the first of two experimental trials. Upon arrival, the participants were weighed (weight was recorded at each visit to ensure participants were weight-stable throughout) and completed a baseline appetite measure. Participants were then provided with a standardised breakfast meal, consisting of two slices of toast (Thick slice, 50/50 bread, ~90g), margarine (~16g), jam (mixed fruit, ~30g) with a choice of orange or apple juice (~200ml). The approximate energy content of this meal was 415 kcal (71% energy from carbohydrate, 19% from fat and 10% protein), based on the addition of jam and selection of orange juice. Once the breakfast was consumed (t=0), the participant began a two-hour rest period before the exercise bout commenced. During this time, the participant remained sedentary within the laboratory, leaving them free to watch television, read or use a computer. At t=60 minutes, a second appetite measure was obtained. At t=90 minutes, a venous cannula was inserted into the antecubital vein of the arm by a qualified member of research staff. At t=120 minutes,

an appetite measure was recorded and a resting blood sample (~7ml) was obtained. An additional blood sample (~3ml) was obtained at this point for the baseline measure of blood glucose. In the exercise trial condition, the exercise bout commenced immediately.

The exercise bout consisted of 4 x 30 seconds “flat out” sprint on a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands), utilising a modified Wingate anaerobic power test (the torque factor was set at either 0.5 or 0.6, as opposed to the commonly used torque factor of 0.7, due to the fitness level of the participants and judging by their performance during the familiarisation trial). The first bout was preceded by a brief 3 minute warm-up period at a constant load of 40 watts; the first sprint was initiated immediately upon reaching the end of the 3 minute period. The 4 sprint bouts were separated by a 4 minute recovery period, during which the power of the cycle ergometer was a constant 40 watts. During this recovery period participants were encouraged to remain on the bike, being free to rest or partake in light cycling to facilitate active recovery. Heart rate was recorded throughout the bout, using a heart rate monitor (Polar S625X; Polar Electro Oy, Kempele, Finland), and noted at the end of every sprint and at the end of each recovery period. Rating of perceived exertion, measured using the Borg Scale (Borg, 1973) was also recorded at these time points. Breath-by-breath measures of exhaled gas were obtained, using the Oxycon Pro (Jaeger, Wuerzburg, Germany) apparatus, during sprint number one, recovery period number one and sprint and recovery period number three. The exercise bout ended upon completing sprint number four, with a blood sample being obtained immediately, followed by an appetite measure. An additional ~3ml blood sample was obtained for the post-exercise measure of blood glucose. Thus, the exercise bout lasted a total of 17 minutes. In the resting trial condition, the participant remained sedentary for an equal amount of time. A 10-minute resting exhaled gas

measurement was made during this time, using the Oxycon Pro (Jaeger, Wuerzburg, Germany) apparatus.

On concluding the exercise bout ($t=0^*$), a 2 hour rest period began. During this period, the participant again remained sedentary, free to watch television, read or use a computer. Blood samples and appetite measures were obtained every 30 minutes during this time. Between $t=30^*$ and $t=40^*$, a resting sample of exhaled gas was collected in order to calculate post-exercise recover period energy expenditure. At $t=120^*$, the participant was escorted to the research kitchen facility, where they were provided with a buffet lunch meal (see General methods, section 2.2.3.2, p73). The participant was instructed that they may consume as they wished and were left to do so in isolation. Upon finishing the meal, a final blood sample was obtained and final appetite measure recorded.

6.3.4 Blood sampling and analysis

The blood sampling and plasma analysis techniques are described in General methods, section 2.2.3.5 (p76). Briefly, blood samples were obtained and immediately transferred to the appropriate pre-treated blood collection tubes for analysis of hormones. Blood was centrifuged at 3500 RPM and a temperature of 4°C for 15 minutes to isolate plasma. Plasma was stored at a temperature of -70°C until hormone assays for acylated ghrelin, PYY and GLP-1 were conducted.

6.3.5 Statistical analysis

To assess changes in subjective appetite over time and between the two trial conditions, a 2 x 9 factorial ANOVA with repeated measures was conducted, using both the VAS and VIMEC scores. For the analysis of PYY, GLP-1 and acylated ghrelin concentrations, 2 x 7 factorial ANOVA with repeated measures were carried out, and for the analysis of glucose concentration, 2 x 2 factorial ANOVA with repeated measures were conducted. Any significant interactions or main effects were investigated further by carrying out pairwise comparisons, using Bonferroni post-hoc

analysis. In the event of a significant difference between conditions at baseline in any outcome measure, percentage change from baseline ($\% \Delta$) was calculated and analysis repeated with these calculated values. Area under the curve (AUC) was calculated for all profiles plotted, using the trapezoidal method. AUC values were then compared using paired-samples t-tests. Energy intake values at the *ad libitum* buffet meal, as well as total grams, grams of carbohydrate, fat and protein consumed were compared using paired-sample t-tests.

To assess the regulation of subjective appetite by appetite-associated hormones, within-subject, between-condition changes in hormone concentration were correlated with change in VAS scores and VIMEC score. For this analysis, change from the resting condition was calculated (EX – REST) for percentage change with exercise ($\% \Delta_{EX}$, from value $t = 0$ to value at $t = 0$) in VAS, VIMEC, acylated ghrelin, PYY and GLP-1. To assess the regulation of energy intake by subjective appetite and appetite-associated hormones, within-subject, between-condition percentage differences (EX – REST) for appetite and hormone concentration measures obtained immediately prior to the *ad libitum* test meal were correlated with between-condition percentage differences for energy intake at the test meal. Correlations were conducted using Pearson product moment correlation analysis.

6.4 RESULTS

6.4.1 Resting and exercise conditions.

Table 6.2 shows the characteristics of the REST and EX trials. Energy expenditure over the entire trial period was significantly higher in the exercise condition (549 ± 144 kcal vs. 420 ± 119 kcal, $p = 0.001$), as was energy expenditure during the exercise bout, compared with equivalent time point in REST (152 ± 37 kcal vs. 29.3 ± 8.3 kcal, $p = 0.001$).

	EE for trial (kcal)	EE for bout (kcal)	HR (beats min ⁻¹)	RPE (Wingate bouts)	Peak power (Watts)	Average power (Watts)	Work (kJ)
Rest	420 ± 119	29.3 ± 8.3	-	-	-	-	-
Exercise	549 ± 144*	152 ± 37*	130 ± 22	17 ± 2	774 ± 74	449 ± 151	15.0 ± 1.2

Table 6.2 – Characteristics of resting and exercise conditions. * = significantly different to REST, $p = 0.001$.

6.4.2 Subjective appetite

6.4.2.1 VAS

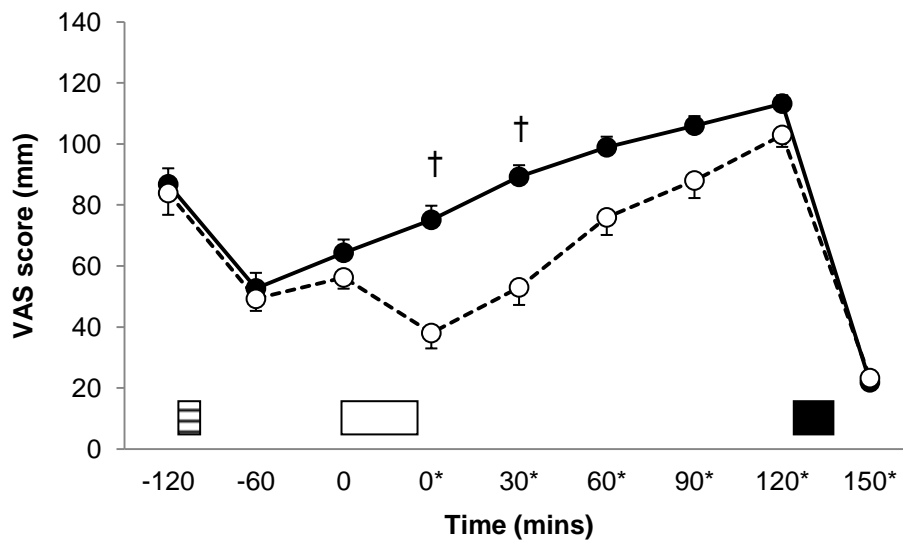
Figure 6.1 shows the appetite profiles for both VAS scores (Figure 1a) and VIMEC scores (Figure 1b). Baseline VAS scores did not differ between the two trial conditions. There was a significant condition x time effect for VAS score ($F(8) = 2.528$, $p = 0.02$). Post-hoc pairwise comparisons for between-condition differences demonstrated that appetite scores during EX were significantly lower than REST immediately post exercise ($t = 0^*$, 38 ± 29 mm vs. 75 ± 26 mm, $p = 0.018$) and $t = 30^*$ (53 ± 33 mm vs. 89 ± 22 mm, $p = 0.034$). Post-hoc pairwise comparisons for within-condition differences showed no meaningful significant differences associated with exercise; appetite rose steadily from the cessation of exercise until the test meal. Area-

under-the-curve (AUC) data for the VAS appetite profiles showed that appetite over the entire trial period was significantly lower in EX versus REST ($t(7) = 2.441$, $p = 0.045$).

6.4.2.2 VIMEC

There was no significant difference in VIMEC scores at baseline. No significant condition x time interaction was present ($F(8) = 1.517$, $p = 0.172$, **figure 6.1b**), nor was there a significant condition main effect ($F(1) = 0.718$, $p = 0.425$). A significant main effect for time ($F(8) = 7.242$, $p < 0.001$) simply indicated a general increase in appetite from breakfast to the test meal, before falling post-test-meal. Analysis of AUC data also failed to show any difference between conditions ($t(7) = 0.770$, $p = 0.466$).

(a)



(b)

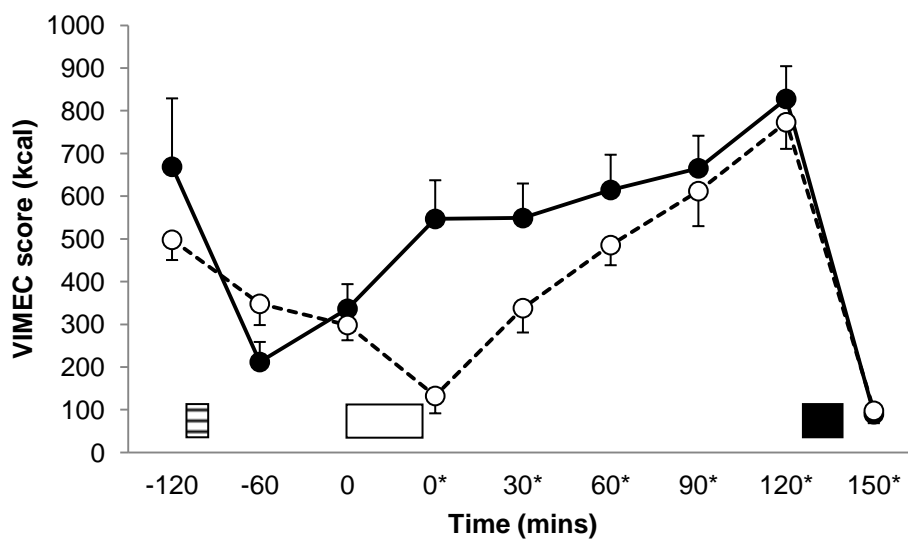


Figure 6.1 – Appetite profiles of VAS(a) and VIMEC(b) measures for REST (●, solid line) and EX (○, dashed line). Striped block represents breakfast meal. Hollow block represents exercise. Solid block represents the *ad libitum* test meal. * on x axis labels indicate minutes post-exercise. Values are mean \pm SEM. † = exercise significantly different to rest, $p < 0.05$.

6.4.3 Plasma glucose and appetite-associated hormones

6.4.3.1 Glucose

Plasma glucose concentration was similar in both conditions, at baseline and $t=0$ (4.48 ± 0.75 mmol and 4.64 ± 0.92 mmol in REST and 4.32 ± 0.89 mmol and 4.60 ± 0.61 mmol in EX, respectively (condition x time interaction, ($F(1) = 0.165$, $p = 0.697$))

6.4.3.2 Acylated ghrelin

The acylated ghrelin concentration profiles for both conditions are shown in **figure 6.2a**. Concentrations at baseline were similar between the two conditions. There was a significant condition x time interaction ($F(2.271) = 6.728$, $p = 0.008$). Post-hoc analysis for between-condition effects revealed that acylated ghrelin concentration was significantly lower at $t = 0$ in EX, compared with REST (118 ± 45 pg•mL⁻¹ vs. 197 ± 95 pg•mL⁻¹, $p = 0.03$) and remained lower at every time point post-exercise, until the test meal (all $p < 0.05$). Within-condition comparisons showed that, in the exercise condition, acylated ghrelin concentration was significantly greater immediately before and after the test meal ($t=120$ and $t=150$) versus immediately post-exercise, when the plasma concentration was at its lowest. AUC for acylated ghrelin concentration was significantly lower in the exercise trial, compared with rest ($p = 0.024$; data not shown).

6.4.3.3 PYY

There was no significant condition x time interaction for PYY concentration ($F(2.412) = 1.767$, $p = 0.203$, **figure 6.2b**). There was also no significant main effect of condition, (36.7 ± 5.7 pg•mL⁻¹ vs. 32.2 ± 8.1 pg•mL⁻¹, $F(1) = 1.485$, $p = 0.269$). A significant main effect for time ($F(1.614) = 5.765$, $p = 0.027$) simply indicated a significant increase in PYY concentration post-test meal in both conditions. Percentage change from baseline analysis was carried out in this instance, due to a significant difference at baseline (REST = 40.6 ± 11.9 pg•mL⁻¹, EX = 33.4 ± 10.1 pg•mL⁻¹, $p = 0.04$), but also failed to demonstrate a significant condition x time interaction, nor a

condition main effect (**figure 6.2c**). AUC was not significantly different between the two trials, when represented as either absolute values or % Δ values (data not presented).

6.4.3.4 GLP-1

GLP-1 concentrations did not differ between the two conditions at baseline. There was no significant condition x time interaction ($F(2.523) = 2.218$, $p = 0.135$, **figure 6.2d**). There was a significant condition main effect, with mean GLP-1 concentration across the entire trial period significantly higher in the exercise condition vs., rest ($36.2 \pm 11.0 \text{ pg}\cdot\text{mL}^{-1}$ vs. $30.2 \pm 8.9 \text{ pg}\cdot\text{mL}^{-1}$, $F(1) = 28.224$, $p = 0.002$). Comparisons of AUC for GLP-1 concentration profiles showed that the area was significantly greater in the exercise condition, compared with rest ($t(6) = 5.723$, $p = 0.001$).

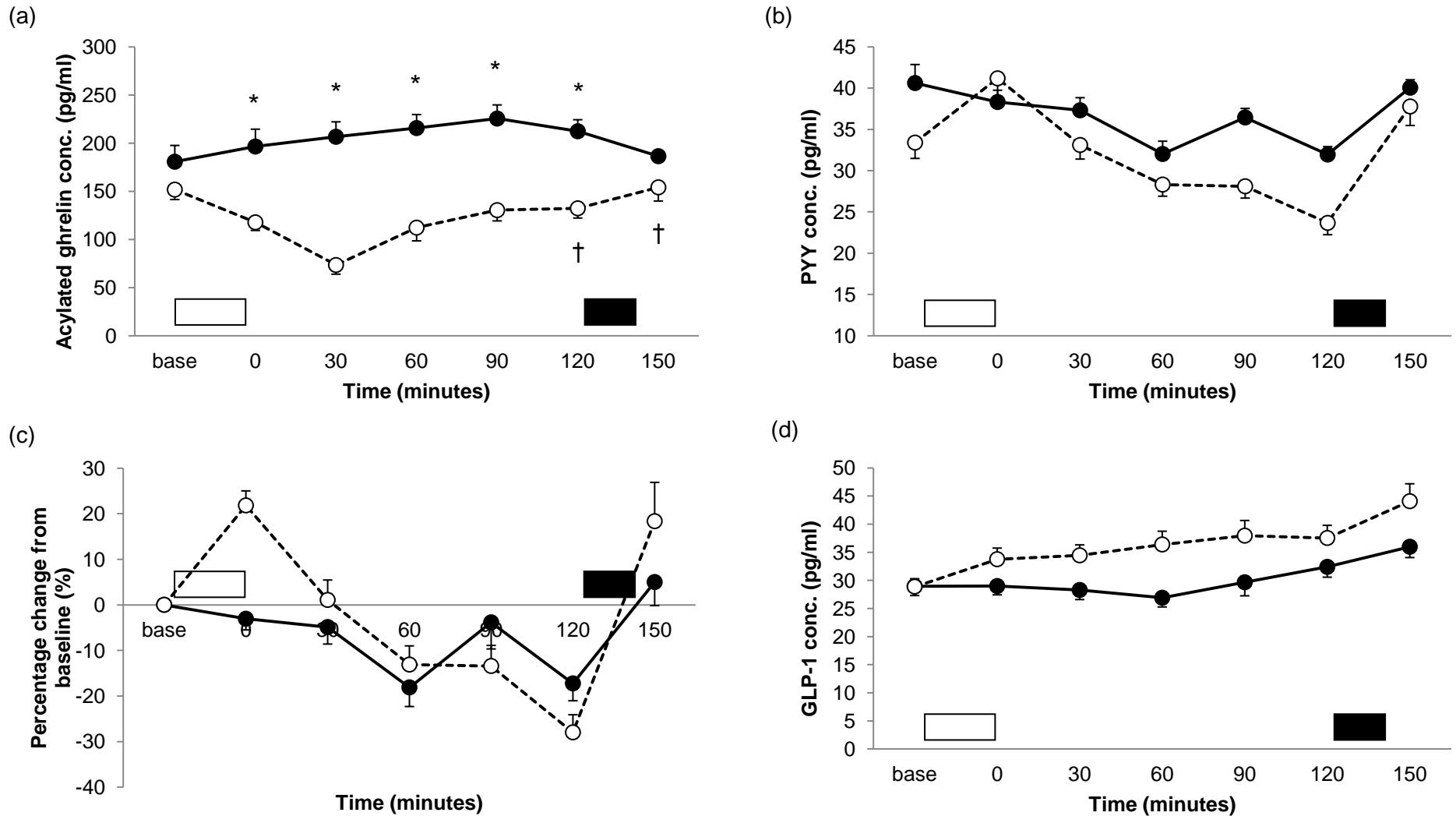
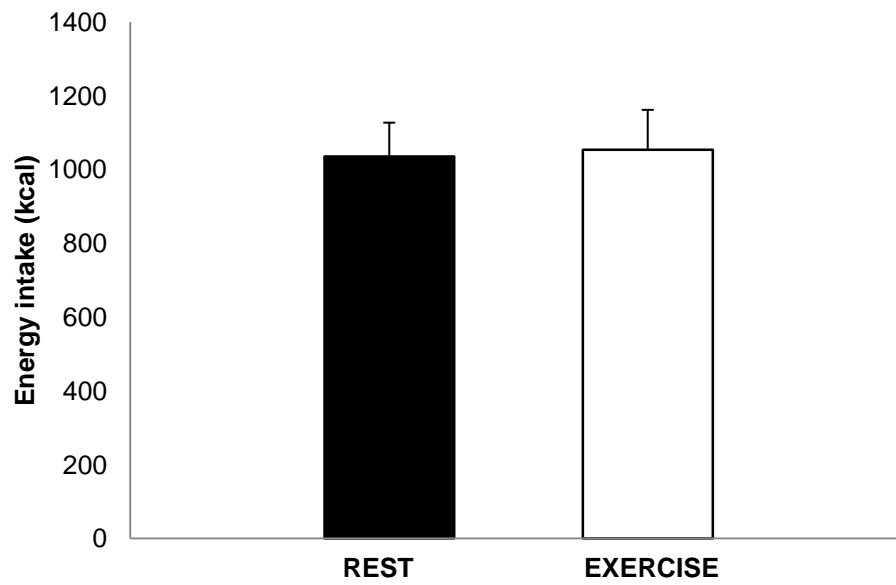


Figure 6.2 – hormone profiles of acylated ghrelin (A), PYY (B) and GLP-1 (C), for REST (●, solid line) and EX (○, dashed line). PYY data include percentage change from baseline curve (inserted, top right). Hollow block represents exercise. Solid block represents the *ad libitum* test meal. Values are mean \pm SEM. * = exercise significantly difference to rest. † = significantly different to t=30 in EX.

6.4.4 Food intake and energy balance.

Mean absolute energy intake and mean relative energy intake for both REST and EX are shown in **figure 6.3a and 6.3b**. There was no significant difference in either absolute energy intake (1035 ± 519 kcal vs. 1053 ± 615 kcal for REST and EX respectively, $t(7) = 0.170$, $p = 0.870$), or relative energy intake (646 ± 463 kcal vs. 513 ± 498 kcal for REST and EX respectively, $t(6) = 1.00$, $p = 0.355$). Total amount of food consumed, in grams, and the macronutrient content of the food consumed, as both absolute intake (grams) and percentage of total energy, are shown in **table 6.3**. There were no differences between REST and EX in any of these values.

(a)



(b)

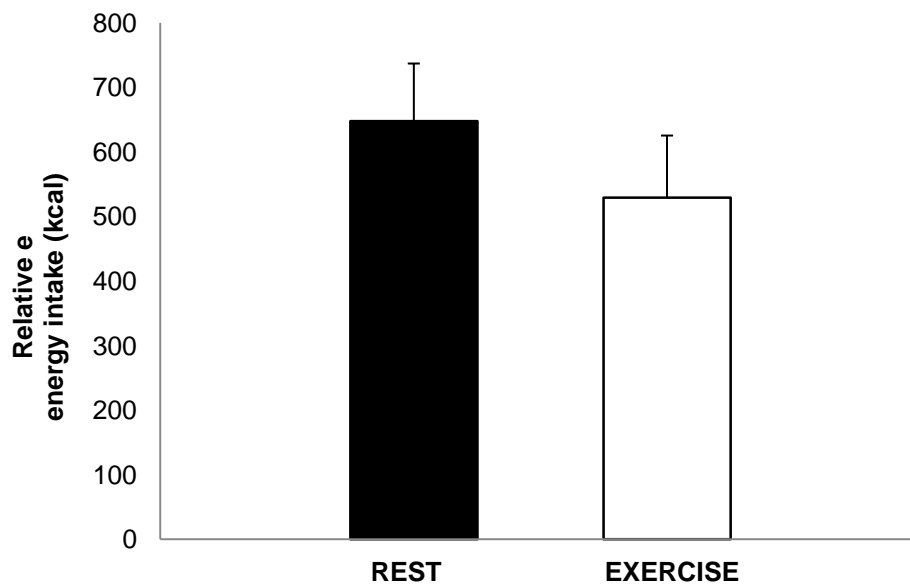


Figure 6.3 – Absolute energy intake (a) and relative energy intake (b), for REST (solid black bar) and EX (empty bar). Values are mean \pm SEM.

	Rest	Exercise
Volume consumed (grams)	869 ± 449	741 ± 341
Carbohydrate (g)	148 ± 66.3	151 ± 102
% energy CHO	59.4 ± 9.0	56.5 ± 8.8
Fat (g)	32.3 ± 24.6	33.4 ± 23.1
% energy fat	24.7 ± 10.3	26.0 ± 11.4
Protein (g)	39.7 ± 23.9	39.8 ± 14.5
% energy protein	15.9 ± 5.6	17.5 ± 6.6

Table 6.3 – Food intake at the *ad libitum* test meal. Values are mean ± SD.

6.4.5 The relationship between appetite-associated hormones, subjective appetite and energy intake

When assessing within-subject, between-condition changes, change from REST scores in $\% \Delta_{\text{EX}}$ VAS and $\% \Delta_{\text{EX}}$ VIMEC, were not significantly or strongly correlated with change from REST in $\% \Delta_{\text{EX}}$ acylated ghrelin, PYY or GLP-1. Within-subject, between-condition percentage changes in energy intake were positively associated with percentage changes in VAS score ($r = 0.755$, $p = 0.016$) and percentage changes in GLP-1 concentration ($r = 0.688$, $p = 0.044$).

6.5 DISCUSSION

The aim of this study was to assess the effect of sprint interval cycling exercise on subjective appetite, food intake and plasma concentration of appetite-associated hormones, in overweight and obese individuals. Just 4 x 30 seconds of maximal effort sprint cycling was sufficient to elicit a transient suppression of subjective appetite, compared with a resting condition. However, as is often the case with exercise-induced appetite suppression, this effect was short-lived, with no significant difference in VAS score or VIMEC score between the exercise and resting conditions 60 minutes post-exercise. The absence of a difference in subjective appetite at this time was allied with no difference in food intake at the *ad libitum* test meal, administered 2 hours after exercise. Mean energy intake was very similar between the two conditions, differing by only 18 kcal (1.7%). When accounting for the energy cost of both rest and exercise, relative energy intake, despite being 20% lower in the exercise trial, was not significantly different between conditions. This finding – a transient suppression of appetite, with no consequent influence on food intake – is in agreement with the findings of previous investigation into the effect of sprint interval exercise on appetite in healthy-weight individuals. (Deighton *et al.*, 2012; Deighton *et al.*, 2013). Further, this response to sprint interval exercise is analogous to the typical response to high-intensity, continuous aerobic exercise (King *et al.*, 2010; King *et al.*, 2011b; King *et al.*, 1997a; Schubert *et al.*, 2013; Thompson *et al.*, 1988). This was despite a very low energy cost of the exercise. In the current study, the mean energy expenditure for the exercise bout (including recovery period between repetitions) was estimated to 152 kcal and the total duration of the bout was 17 minutes, with a total of only 2 minutes worth of effort. Therefore the energy cost and duration of exercise were very low. The protocol utilised by Deighton *et al.* (Deighton *et al.*, 2012) of 6 x 30 seconds maximal effort cycling yielded an energy cost of 142 kcal, although this was estimated, as

opposed to measured, so may have been an underestimate. Irrespective of this, to the author's best knowledge, a suppression of appetite has not previously been observed with such a short exercise bout. Furthermore, this data would suggest the absence of an exercise duration or energy expenditure threshold for exercise-induced appetite suppression.

As stated earlier, food intake at the *ad libitum* test meal did not differ between conditions. This is in line with previous literature, when *ad libitum* food intake measures are obtained ≥ 60 minutes post-exercise (King *et al.*, 2010; King *et al.*, 2011b; King *et al.*, 1997b; Schubert *et al.*, 2013; Thompson *et al.*, 1988). Despite no difference in food and energy intake being a common observation, the calculation of relative energy intake, accounting for the energy expenditure of the exercise bout, often yields a significant energy deficit in an exercise condition, when absolute energy intake is unaffected (Imbeault *et al.*, 1997; King *et al.*, 2010; Lluch *et al.*, 2000; Unick *et al.*, 2010) or even increased (Martins *et al.*, 2007; Pomerleau *et al.*, 2004), post-exercise. This was not the case in the present study, with relative energy intake similar between both the resting and exercise trials. As previously mentioned, the energy expenditure of the sprint interval exercise bout was low, at just 152 kcal. Such a low energy expenditure was evidently insufficient to generate even an acute energy deficit, compared with the resting condition. While it was first considered that the absence of an observed difference was due to lack of statistical power, a power calculation, based on the data collected, suggested that a sample size of 135 participants would be required to observe a statistically significant difference ($\alpha = 0.05$, power of test = 0.8). In addition, there was no evidence of excess post-exercise oxygen consumption (EPOC). EPOC has consistently been shown after high intensity exercise (Phelain *et al.*, 1997) and intermittent supramaximal exercise (Bahr *et al.*, 1992), and therefore may have been expected after even such a short bout as completed in the present study. It is possible that the exercise bout was too short, as it has been shown that

EPOC is, to some respect, dependent on duration of exercise (see Børsheim and Bahr, 2003). It is also possible that the recovery period measure of expired gas, used to calculate energy expenditure, was collected too late to detect any EPOC. This measure was obtained 30 minutes post-exercise. It has previously been shown that EPOC can occur for up to 3 hours post-high-intensity aerobic exercise (Phelain *et al.*, 1997) and for up to 30 minutes after just 1 x 2 minutes of supramaximal exercise (Bahr *et al.*, 1992). While these data would suggest that the timing of the measurement in the present study should have detected any EPOC, it may have been preferable to obtain more than one measurement during this recovery period: one in close proximity to the cessation of exercise (≤ 15 minutes). Nonetheless, the contribution of EPOC to total energy expenditure associated with an exercise bout has been postulated to be low – 6-15% (Laforgia *et al.*, 2006). Therefore, any inaccuracy in this measure is unlikely to cause substantial error in a relative energy balance calculation.

The suppression of subjective appetite was allied with changes in appetite-associated hormones, in favour of an anorexigenic state. Acylated ghrelin was suppressed immediately after exercise and continued to fall, with the lowest concentration observed 30 minutes post-exercise. At this point, plasma concentration was 64% lower than the corresponding time point in the resting trial and had fallen 53% from baseline. While decreases in acylated ghrelin are usually transient, after both continuous (Broom *et al.*, 2009; Broom *et al.*, 2007; King *et al.*, 2010; King *et al.*, 2011a; Shiiya *et al.*, 2011) and interval exercise (Deighton *et al.*, 2012), concentrations remained lower than those of the resting trial for the entire two-hour post-exercise period, until the test meal. To the knowledge of the author, this is the most enduring post-exercise suppression of acylated ghrelin observed. The suppression of acylated ghrelin post-exercise contrasts previous observations of no response (Dall *et al.*, 2002; Kraemer *et al.*, 2004; Martins *et al.*, 2007; Schmidt *et al.*, 2004), or an increase (Larson-Meyer *et al.*, 2012; Russel *et al.*, 2009) in total ghrelin after exercise. This

finding further strengthens the notion that acylated and non-acylated ghrelin respond differently to acute bouts of exercise (Shiia *et al.*, 2011), while also suggesting that acylated ghrelin may be yet more responsive to supramaximal intensity exercise, evidenced by the prolonged suppression observed in the present study.

GLP-1 proved less responsive to exercise. Plasma concentrations increased non-significantly with exercise (by 17%), resulting in values being 15% higher in the exercise condition, versus rest. Interestingly, the GLP-1 concentration continued to increase steadily post-exercise, peaking at t=90, leading to a significantly greater AUC for the entire trial period in the exercise condition, allied with the main effect of condition. Existing literature regarding the effect of exercise on plasma GLP-1 concentration is limited. Martins *et al.* (Martins *et al.*, 2007) reported a significant increase in GLP-1 after intermittent cycling exercise at 65% of estimated maximal heart rate (3 x 17 minutes with 3 minutes recovery), in healthy-weight individuals. While concentration remained higher than corresponding measures in a resting trial at 30 minutes post-exercise, the increase was transient, unlike the sustained increase observed in the present study. Ueda and co-workers showed a sustained but modest, significant increase in GLP-1 concentrations after both moderate- and high-intensity exercise in both lean and obese individuals. (Ueda *et al.*, 2009a; Ueda *et al.*, 2009b). To the best knowledge of the author, this is the first study that has assessed changes in plasma GLP-1 concentration after high-intensity, intermittent exercise. This data would suggest that the response to this form of exercise is similar to that observed with longer duration, continuous exercise of a lower intensity.

It has previously been suggested that prior exercise may sensitise GLP-1 to food ingestion, with an increased acute GLP-1 response to a liquid meal post-exercise (Chanoine *et al.*, 2008). In addition, regular exercise would appear to increase the postprandial secretion of satiety peptides after a standardised test meal (Guelfi *et al.*, 2013; Martins *et al.*, 2010). Here, we witnessed a greater increase in GLP-1 after the

ad libitum test meal in the exercise condition (17% vs. 11% in the resting condition). While this intake was *ad libitum* and not therefore controlled to be the same across both conditions, energy intake varied to such a small degree (1.7%), that it is acceptable to assume that any differing GLP-1 response to the test meal is not due to differences in energy consumed. While this difference is small, it may provide further evidence that prior exercise can sensitise satiation-signalling mechanisms during feeding.

PYY concentration also exhibited a modest response to sprint interval cycling, with a non-significant increase of 23%. Further, all but one of the participants for which PYY concentration data was available, demonstrated an increase in PYY with exercise. This increase was transient, with concentration falling immediately upon ceasing exercise and returning to baseline 30 minutes post-exercise. Deighton *et al.* (Deighton *et al.*, 2012) also did not observe a significant increase in PYY₃₋₃₆ concentration following sprint interval exercise, with an increase of ~30% immediately post-exercise. PYY is commonly responsive to high-intensity, continuous aerobic exercise, of longer duration and greater energy cost (Broom *et al.*, 2009; Larson-Meyer *et al.*, 2012; Martins *et al.*, 2007; Russel *et al.*, 2009; Ueda *et al.*, 2009a; Ueda *et al.*, 2009b). Although, it should be noted that when significant increases in PYY concentrations are observed with such exercise, the magnitude of increase is often similarly modest to the non-significant increase seen in the present study (Broom *et al.*, 2009; Martins *et al.*, 2007; Russel *et al.*, 2009; Ueda *et al.*, 2009b), suggesting that the response to sprint interval exercise may not be dissimilar to continuous, aerobic exercise of a high intensity. It is possible that the failure to observe a significant elevation in the present study is due to lack of statistical power, with a sample size of just 7 for hormone analysis.

These non-uniform changes in appetite-associated hormones led us to examine which change may be the strongest driver of changes in subjective appetite. As

opposed to investigating the more commonly-assessed relationships of between-subject, within-condition differences, it was decided to investigate within-subject changes between the two conditions for each of the variables. This was considered a more appropriate approach to investigating the likely regulatory role of appetite-associated hormones on subjective appetite and energy intake. Within-subject, between-condition percentage changes with exercise for VAS and VIMEC scores were not correlated with percentage change in any of the three hormones. However, unexpectedly, changes in GLP-1 were positively associated with changes in energy intake. Due to the well-established anorexigenic effect of GLP-1, this would suggest a dissociation between hormonal signals of appetite and energy intake with exercise. Considering these data, it would appear that changes in acylated ghrelin, PYY and GLP-1 with exercise may not play a key regulatory role in subjective appetite during the post-exercise period, or in post-exercise food intake.

The exercise-induced suppression of appetite, compared with the resting condition was observed with the VAS method of measurement, but no significant differences were present with the VIMEC measure. Absolute and relative differences failed to reach statistical significance despite being rather large. For instance, VIMEC score dropped by 56%, or 166 kcal, after exercise and the score obtained immediately post-exercise was 76% (414 kcal) lower in the exercise condition, compared with the resting condition. Further, the area under the curve value for the post-exercise, pre-test meal period was 31% lower in the exercise condition. The lack of statistical significance for these differences is due, in part, to large between-subject variation in the VIMEC measure. The mean coefficient of variation for VIMEC measures was 47.4%, compared with 35.2% for VAS measures, indicating a larger spread of values across participants at each measure. The nature of the VIMEC method, offering the participant a considerable choice of food options, lends itself to a larger degree of variability.

However, it may be the case that this leads to a poorer degree of sensitivity of the measure.

Another reason for the failure to observe statistical significance despite considerable absolute and relative differences is lack of statistical power, due to a small sample size. Eight participants completed the study, but we failed to obtain blood samples from one individual, meaning that all hormone analysis was completed for seven participants. Due to the difficulty in recruiting participants from this study population to complete such an intense exercise bout, a larger sample size was not successfully achieved. One participant was excluded due to high resting acylated ghrelin concentrations (>2 SD from the mean) and a further two participants withdrew after the initial pre-testing session.

In conclusion, a single bout of sprint interval exercise, consisting of just 4 x 30 seconds maximal effort cycling was sufficient to elicit a transient suppression of subjective appetite in overweight and obese individuals. This was accompanied by a sustained lowering of the orexigenic hormone acylated ghrelin, to an extent rarely previously observed with continuous submaximal exercise, and more modest increases in anorexigenic hormones. However, changes in appetite-associated hormones did not correlate strongly with changes in appetite, which brings into question the role of such hormones in the regulation of post-exercise appetite. Nonetheless, food intake was not affected by exercise, with no difference between the exercise condition and the resting condition in food intake at the test meal two-hours post-exercise. Further, when accounting for the energy expenditure of exercise, relative energy intake also did not differ between conditions. Therefore, it remains questionable whether high intensity interval exercise could elicit a suppression of appetite sufficient to impact upon short-term food intake and hence favour an acute energy deficit.

6.6 REFERENCES

- Babraj, J., Vollaard, N., Keast, C., Guppy, F., Cottrell, G., and Timmons, J. (2009). Extremely short duration high intensity interval training substantially improves insulin action in young healthy males. *BMC Endocrine Disorders* 9, 3.
- Bahr, R., Grønnerød, O., and Sejersted, O.M. (1992). Effect of supramaximal exercise on excess postexercise O₂ consumption. *Medicine and science in sports and exercise* 24, 66-71.
- Bartlett, J.D., Close, G.L., MacLaren, D.P.M., Gregson, W., Drust, B., and Morton, J.P. (2011). High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: Implications for exercise adherence. *Journal of Sports Sciences* 29, 547-553.
- Bartlett, J.D., Hwa Joo, C., Jeong, T.-S., Louhelainen, J., Cochran, A.J., Gibala, M.J., Gregson, W., Close, G.L., Drust, B., and Morton, J.P. (2012). Matched work high-intensity interval and continuous running induce similar increases in PGC-1 α mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. *Journal of Applied Physiology* 112, 1135-1143.
- Borg, G.A.V. (1973). "Perceived exertion: a note on history and methods". *Medicine and Science in Sports & Exercise* 5, 90-93.
- Børsheim, E., and Bahr, R. (2003). Effect of exercise intensity, duration and mode on post-exercise oxygen consumption. *Sports Med* 33, 1037-1060.
- Boutcher, S.H. (2011). High-intensity intermittent exercise and fat loss. *Journal of Obesity* 2011.
- Broom, D.R., Batterham, R.L., King, J.A., and Stensel, D.J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 296, R29-R35.
- Broom, D.R., Stensel, D.J., Bishop, N.C., Burns, S.F., and Miyashita, M. (2007). Exercise-induced suppression of acylated ghrelin in humans. *J Appl Physiol* 102, 2165-2171.
- Burgomaster, K.A., Heigenhauser, G.J.F., and Gibala, M.J. (2006). Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. *Journal of Applied Physiology* 100, 2041-2047.
- Burgomaster, K.A., Howarth, K.R., Phillips, S.M., Rakobowchuk, M., MacDonald, M.J., McGee, S.L., and Gibala, M.J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *The Journal of Physiology* 586, 151-160.
- Burgomaster, K.A., Hughes, S.C., Heigenhauser, G.J.F., Bradwell, S.N., and Gibala, M.J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *Journal of Applied Physiology* 98, 1985-1990.

Burns, S.F., Broom, D.R., Miyashita, M., Mundy, C., and Stensel, D.J. (2007). A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of Sports Sciences* 25, 635-642.

Chanoine, J.-P., Mackelvie, K.J., Barr, S.I., Wong, A.C.K., Meneilly, G.S., and Elahi, D.H. (2008). GLP-1 and appetite responses to a meal in lean and overweight adolescents following exercise. *Obesity* 16, 202-204.

Dall, R., Kanaley, J., Hansen, T., Moller, N., Christiansen, J., Hosoda, H., Kangawa, K., and Jorgensen, J. (2002). Plasma ghrelin levels during exercise in healthy subjects and in growth hormone-deficient patients. *Eur J Endocrinol* 147, 65-70.

Deighton, K., Barry, R., Connon, C., and Stensel, D. (2012). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*, 1-10.

Deighton, K., Karra, E., Batterham, R.L., and Stensel, D.J. (2013). Appetite, energy intake and PYY₃₋₃₆ responses to energy-matched continuous exercise and submaximal high intensity exercise. *Applied Physiology, Nutrition, and Metabolism*.

Dodd, C.J., Welsman, J.R., and Armstrong, N. (2008). Energy intake and appetite following exercise in lean and overweight girls. *Appetite* 51, 482-488.

George, V.A., and Morganstein, A. (2003). Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite* 40, 43-46.

Guelfi, K.J., Donges, C.E., and Duffield, R. (2013). Beneficial effects of 12 weeks of aerobic compared with resistance exercise training on perceived appetite in previously sedentary overweight and obese men. *Metabolism* 62, 235-243.

Helgerud, J., Hoydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., Simonsen, T., Helgesen, C., Hjorth, N., Bach, R., and Hoff, J. (2007). Aerobic high-intensity intervals improve VO_{2max} more than moderate training. *Medicine & Science in Sports & Exercise* 39, 665-671.

Hill, A.J., and Blundell, J.E. (1982). Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research* 17, 203-212.

Imbeault, P., Saint-Pierre, S., Alm-Ras, N., and Tremblay, A. (1997). Acute effects of exercise on energy intake and feeding behaviour. *British Journal of Nutrition* 77, 511-521.

Kawano, H., Mineta, M., Asaka, M., Miyashita, M., Numao, S., Gando, Y., Ando, T., Sakamoto, S., and Higuchi, M. (2013). Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite* 66, 26-33.

King, J.A., Miyashita, M., Wasse, L.K., and Stensel, D.J. (2010). Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* 54, 492-498.

King, J.A., Wasse, L.K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R.L., and Stensel, D.J. (2011a). Differential acylated ghrelin, peptide YY₃₋₃₆, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology & Metabolism* 96, 1114-1121.

King, J.A., Wasse, L.K., and Stensel, D.J. (2011b). The acute effects of swimming on appetite, food intake, and plasma acylated ghrelin. *Journal of Obesity* 2011.

King, N.A., Burley, V.J., and Blundell, J.E. (1994). Exercise induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition* 48, 715-724

King, N.A., Lluch, A., Stubbs, R.J., and Blundell, J.E. (1997a). High dose exercise does not increase hunger or energy intake in free living males. *Eur J Clin Nutr* 51, 478-483.

King, N.A., Tremblay, A., and Blundell, J.E. (1997b). Effects of exercise on appetite control: implications for energy balance. *Medicine & Science in Sports & Exercise* 29, 1076-1089.

Kissileff, H.R., Pi-Sunyer, F.X., Segal, K., Meltzer, S., and Foelsch, P.A. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition* 52, 240-245.

Kraemer, R.R., Durand, R.J., Acevedo, E.O., Johnson, L.G., Kraemer, G.R., Hebert, E.P., and Castracane, V.D. (2004). Rigorous running increases growth hormone and insulin-like growth factor-i without altering ghrelin. *Experimental Biology and Medicine* 229, 240-246.

Laforgia, J., Withers, R.T., and Gore, C.J. (2006). Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *Journal of Sports Sciences* 24, 1247-1264.

Larson-Meyer, D.E., Palm, S., Bansal, A., Austin, K.J., Hart, A.M., and Alexander, B.M. (2012). Influence of running and walking on hormonal regulators of appetite in women. *Journal of Obesity* 2012, 15.

Little, J.P., Gillen, J.B., Percival, M.E., Safdar, A., Tarnopolsky, M.A., Punthakee, Z., Jung, M.E., and Gibala, M.J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *Journal of Applied Physiology* 111, 1554-1560.

Lluch, A., King, N.A., and Blundell, J.E. (2000). No energy compensation at the meal following exercise in dietary restrained and unrestrained women. *British Journal of Nutrition* 84, 219-225.

Martins, C., Kulseng, B., King, N.A., Holst, J.J., and Blundell, J.E. (2010). The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. *Journal of Clinical Endocrinology & Metabolism* 95, 1609-1616.

Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 193, 251-258.

Marzullo, P., Salvadori, A., Brunani, A., Verti, B., Walker, G.E., Fanari, P., Tovaglieri, I., Medici, C.D., Savia, G., and Liuzzi, A. (2008). Acylated ghrelin decreases during acute exercise in the lean and obese state. *Clinical Endocrinology* 69, 970-971.

Phelain, J.F., Reinke, E., Harris, M.A., and Melby, C.L. (1997). Postexercise energy expenditure and substrate oxidation in young women resulting from exercise bouts of different intensity. *Journal of the American College of Nutrition* 16, 140-146.

Pomerleau, M., Imbeault, P., Parker, T., and Doucet, E. (2004). Effects of exercise intensity on food intake and appetite in women. *Am J Clin Nutr* 80, 1230-1236.

Reid, C.H., CG. Blundell, JE. Stubbs, RJ (1998). What are psychometric assessments of appetite asking: a preliminary multivariate analysis. *International Journal of Obesity* 22, 151.

Russel, R.R., Willis, K.S., Ravussin, E., and Larson-Meyer, E.D. (2009). Effects of endurance running and dietary fat on circulating ghrelin and peptide YY. *Journal of sports science & medicine* 8, 574-583.

Schmidt, A., Maier, C., Schaller, G., Nowotny, P., Bayerle-Eder, M., Buranyi, B., Luger, A., and Wolzt, M. (2004). Acute exercise has no effect on ghrelin plasma concentrations. *Horm Metab Res* 36, 174-177.

Schubert, M.M., Desbrow, B., Sabapathy, S., and Leveritt, M. (2013). Acute exercise and subsequent energy intake. A meta-analysis. *Appetite* 63, 92-104.

Shiia, T., Ueno, H., Toshinai, K., Kawagoe, T., Naito, S., Tobina, T., Nishida, Y., Shindo, M., Kangawa, K., Tanaka, H., Nakazato, M. (2011). Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocrine journal* 58, 335-342.

Sim, A.Y., Wallman, K.E., Fairchild, T.J., and Guelfi, K.J. (2013). High-intensity intermittent exercise attenuates *ad-libitum* energy intake. *International Journal of Obesity advance online publication*.

Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., and Blundell, J.E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr* 84, 405-415.

Thompson, D.A., Wolfe, L.A., and Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medicine & Science in Sports & Exercise* 20, 222-227.

Trapp, E.G., Chisholm, D.J., Freund, J., and Boutcher, S.H. (2008). The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *Int J Obes* 32, 684-691.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., and Fujimoto, S. (2009a). Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *Journal of Endocrinology* 203, 357-364.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., and Fujimoto, S. (2009b). Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J Endocrinol*, JOE-08-0500.

Unick, J.L., Otto, A.D., Goodpaster, B.H., Helsel, D.L., Pellegrini, C.A., and Jakicic, J.M. (2010). Acute effect of walking on energy intake in overweight/obese women. *Appetite* 55, 413-419.

Westerterp-Plantenga, M.S., Verwegen, C.R., Ijeda, M.J., Wijckmans, N.E., and Saris, W.H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* 62, 1345-1354.

Whyte, L.J., Gill, J.M.R., and Cathcart, A.J. (2010). Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. *Metabolism* 59, 1421-1428.

**Matching energy intake to expenditure of isocaloric exercise at
high and moderate intensities**

7.1 ABSTRACT

Background: Those seeking to manage their bodyweight use a variety of strategies, but the most common approaches involve attempting to exercise more and/or consume fewer calories (Weiss *et al.*, 2006). A poor comprehension of the energy cost of exercise and the energy content of food may contribute to weight-gain and the poor success rate of exercise weight-loss interventions.

Purpose: To investigate individuals' ability to consciously match energy intake with energy expenditure after isocaloric exercise at moderate and high intensity.

Method: In a counterbalanced cross-over study design, 14 low- to moderately-active, lean individuals (7 male; mean age 23 ± 3 years; mean BMI 22.0 ± 3.2 kg·m⁻²) completed both a moderate-intensity (60% VO_{2max}, MOD) and a high-intensity (90% VO_{2max}, HIGH) exercise bout on a treadmill, matched for energy expenditure, EE, (450 kcal). Participants were blinded to the intensity and duration of each bout. Thirty minutes post exercise, participants were presented with a buffet, where they were asked to consume food in an attempt to match energy intake with the energy expended during the exercise bout. This was termed the "matching task," providing a matching task energy intake value (EI_{MATCH}). Upon finishing the matching task, a verbal estimate of energy expenditure (EST) was obtained before the participant was allowed to return to the buffet to consume any more food, if desired. This intake was covertly measured and added to EI_{MATCH} to obtain an *ad libitum* intake value (EI_{AD LIB.}).

Results: A significant condition x task interaction showed that, in MOD, EST was significantly lower than EE (298 ± 156 kcal vs. 443 ± 22 kcal, $p = 0.01$). In the HIGH condition, EE, EI_{MATCH} and EST were similar. In both conditions, participants tended to over-eat to a similar degree, relative to EST, with EI_{MATCH} 20% and 22% greater than EST in MOD and HIGH respectively. Between-condition comparisons demonstrated that EI_{MATCH} and EST were significantly lower in MOD, compared with HIGH (374 ± 220

kcal vs. 530 ± 248 kcal, $p = 0.002$ and 298 ± 156 kcal vs. 431 ± 129 kcal, $p = 0.002$ respectively). For both conditions, $EI_{AD\ LIB}$ was approximately 2-fold greater than EE.

Discussion: Participants exhibited a strong ability to estimate exercise energy expenditure after high-intensity exercise. Participants appeared to perceive moderate-intensity exercise to be less energetic than an isocaloric bout of high-intensity exercise. This may have implications for exercise recommendations for weight-loss strategies, especially when casual approaches to exercise and attempting to eat less are being implemented.

7.2 INTRODUCTION

With obesity statistics now demonstrating that 63% of adults and 30% of children in England are overweight or obese (Health and Social Care Information Centre, 2013), many individuals are seeking effective weight-management strategies. Those seeking to manage their bodyweight, whether attempting to lose weight or avoid weight-gain, use a variety of strategies to do so. The most common strategies involve attempting to exercise more and/or consume fewer calories (Weiss *et al.*, 2006). For the effective implementation rather crude weight-loss strategies, such as undertaking more regular exercise, eating less food and eating less fat, a sound appreciation of energy expenditure and energy intake is desirable. It has been extensively demonstrated that individuals are prone to underreporting energy intake when using techniques such as food diaries (Hill and Davies, 2001; Livingstone *et al.*, 1992; Livingstone *et al.*, 1990; Westerterp *et al.*, 1986), with obese individuals likely to underreport to a greater extent (Bandini *et al.*, 1990; Prentice *et al.*, 1986; Schoeller, 1995). A contributing factor to this underreporting may be individuals' poor understanding of the energy content of food (Brindal *et al.*, 2012; Carels *et al.*, 2007; Pettigrew *et al.*, 2013; Polivy, 1976) which, incidentally, has been suggested to be particularly awry in relation to the energy cost of exercise (Blundell and King, 2000; Blundell *et al.*, 2003). Further, this may partly explain why exercise alone can prove an unsuccessful weight-loss strategy (Blundell and King, 2000; King, 1999), with large individual variability in response to increased exercise energy expenditure, when individuals eat *ad libitum* (King *et al.*, 2008).

To the best knowledge of the authors, only two studies have acutely and directly assessed individuals' ability to estimate acute energy expenditure and intake. Harris and George (Harris and George, 2010) asked participants to estimate their energy expenditure after a 60 minute bout of treadmill exercise, at 65% of predicted

maximum heart rate. Fifteen minutes post-exercise, an *ad libitum* buffet meal was provided. The participants were then asked to estimate their energy intake at an *ad libitum* meal. Estimated energy expenditure was significantly greater than the actual energy expenditure of the exercise bout. Conversely, estimated energy intake was significantly lower than actual intake, with participants eating almost twice as many calories as estimated. Willbond and colleagues (Willbond S.M. *et al.*, 2010) conducted a similar study, but after exercise (a 200kcal and a 300kcal bout of treadmill running at 50% $\text{VO}_{2\text{peak}}$), participants were asked to estimate the energy expenditure of the exercise bout and then consume the caloric equivalent from a buffet meal. The energy expenditure of exercise was significantly and substantially overestimated, with estimates 3-4 fold greater than actual expenditure. Intake significantly exceeded expenditure, by 2-3 fold. However, it may be argued that with such low total energy cost of exercise, overcompensation is likely. In addition, it is likely that the perception of energy cost of exercise is dependent on the intensity, as well as the duration of exercise.

Therefore, the aim of this study was to assess individuals' ability to match energy intake with energy expenditure after isoenergetic bouts of moderate- and high-intensity treadmill exercise. It is hypothesised that participants will overestimate the expenditure of both exercise bouts, while underestimating the energy content of food, resulting in a greater intake than expenditure. A secondary aim was to assess *ad libitum* intake after high- and moderate-intensity isoenergetic treadmill exercise.

7.3 METHOD

7.3.1 Participants:

Fourteen healthy-weight, low- to moderately active individuals were recruited primarily from The School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. The characteristics of the participants are shown in **table 7.1**. The criterion for low to moderately active was undertaking a maximum of 3 hours of moderate intensity exercise per week. Those suffering from illness such as cold or flu, those taking medication that was likely to affect appetite or that needed to be taken with food more frequently than once a day, those with food allergies and those suffering from diabetes were excluded from taking part. Ethical approval was obtained from the Ethics Committee of the University of Birmingham.

Age (years)	23 ± 3
BMI (kg·m ⁻²)	22.0 ± 3.2
VO _{2max} (L·min ⁻¹)	3.36 ± 0.67
DEBQ score for restraint	2.0 ± 0.9
IPAQ score (METS)	2207 ± 697

Table 7.1. Participant characteristics. Values are mean ± SD.

7.3.2 Study design:

A within-subject, randomised cross-over study design was utilised, with participants randomly allocated to each of two exercise intensity conditions, termed moderate intensity (MOD – 60% VO_{2max}) and high intensity (HIGH – 90% VO_{2max}).

7.3.3 Preliminary testing:

A single session of pre-testing preceded the study protocol in order to calculate specific exercise intensities to be used for each participant. Participants reported to the

Exercise Metabolism Laboratory, in the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham after an overnight fast. The participant information pack was administered and explained and the participant was given the opportunity to ask any questions regarding the study, prior to providing written consent for their participation. A health questionnaire was completed as a means of a health screening procedure, The International Physical Activity Questionnaire (IPAQ, Appendix 8) was completed as a measure of habitual physical activity and the Dutch Eating Behaviour Questionnaire (DEBQ; (van Strien *et al.*, 1986)) was completed to screen for restrained eating behaviour. Height and weight were then recorded. An incremental exercise test to volitional exhaustion was then completed on a motorised treadmill (H/P/ Cosmos. Nußdorf, Germany) in order to obtain VO_{2max} , and HR_{max} values and to establish the relationship between running speed and rate of oxygen uptake. To achieve this, the test comprised of two components: a constant gradient, steady-state component during which the relationship between running speed and rate of oxygen uptake was calculated; followed by a rapid speed and gradient increase component, from which maximum oxygen uptake (VO_{2max}) was calculated. The test began at a speed of 6 km h⁻¹ and a gradient of 1%. Each stage in the initial section of the test lasted 3 minutes. The speed was increased to 8 km•h⁻¹ at stage 2 and 10 km•h⁻¹ at stage 3. From there on, the speed increased by 1 km•h⁻¹ at each stage with the gradient remaining constant at 1%. This protocol was followed until an RER of 1.00 was reached. At this point, component two of the test commenced. Stages were shortened to 1 minute in duration and with each stage, speed or gradient increased in alternating fashion, by 1 km h⁻¹ and 1% respectively. Participants were adjudged to have reached the end of the test when they voluntarily stopped running, if VO_2 ceased to increase with increasing workload or if it was felt that the participant was struggling to maintain the speed of the treadmill belt. Breath-by-breath measures of exhaled gas, averaged every eight breaths, were recorded using Oxycon Pro (Jaeger, Wuerzburg, Germany) apparatus.

Prior to incremental exercise test, the gas analysers were calibrated using a calibration gas (BOC Gases, Guildford, Surrey, UK) of mixed, known concentrations of O₂ (14.99%) and CO₂ (5.04%) and volume was calibrated using a 3 litre calibration syringe (Jaeger, Wuerzburg, Germany). Exhaled gas was collected throughout the entire test, but submaximal VO₂ values were obtained for each stage during the steady-state component of the test only from air collected during the final minute of the 3 minute stage. VO_{2max} was calculated as the highest average value obtained for any one minute period. From the VO_{2max} value obtained, linear regression was used to calculate an estimate for the speed that would elicit the desired VO₂ for each exercise session, equating to exercise intensities of 60% and 90% VO_{2max}.

7.3.4 Procedures & protocol:

After a minimum period of 3 days after pre-testing, participants returned to the Exercise Metabolism Laboratory after a 10 hour overnight fast for the first of two exercise trials. Trials were separated by a minimum period of 14 days. Participants were provided with a standardised breakfast meal. This consisted of two slices of toast (Thick slice, 50/50 bread, ~90g), margarine (~16g), jam (mixed fruit, ~30g) with a choice of orange or apple juice (~200ml). The approximate energy content of this meal was 415 kcal (71% energy from carbohydrate, 19% from fat and 10% protein), based on the addition of jam and selection of orange juice. Once the breakfast was consumed, the participant began a two-hour rest period before the exercise bout commenced. The participant remained sedentary within the laboratory, leaving them free to watch television, read or use a computer.

At the end of this resting period, the exercise bout commenced. The exercise bout consisted of jogging/running on a motorised treadmill until an energy target of 450 kcal was reached. For two participants, whose VO_{2max} values were lower than 2.5 L•min⁻¹, this target was revised to 400 kcal. This was done to ensure that the HIGH bout was manageable and also to limit between-subject variation in exercise duration.

In the MOD condition, the treadmill was set at a speed estimated to elicit an intensity of 60% $\text{VO}_{2\text{max}}$. In the HIGH condition, the treadmill was set at a speed estimated to elicit an intensity of 90% $\text{VO}_{2\text{max}}$. During both trials, exhaled gas was collected intermittently, with 2 minute samples collected at approximately 10 minute intervals. Breath-by-breath measures of exhaled air, averaged every eight breaths, were recorded using Oxycon Pro (Jaeger, Wuerzburg, Germany) apparatus, allowing for real-time feedback. This allowed for the speed of the treadmill and the duration of the bout to be altered to ensure the target exercise intensity and target energy expenditure were attained. From the exhaled gas collection, VO_2 and RER were recorded and used to calculate energy expenditure. In addition, measures of heart rate were obtained using a heart rate monitor (Polar, S625X; Polar Electro Oy, Kempele, Finland) for the entirety of the bout and ratings of perceived exertion, using the Borg Scale (Borg, 1973), were obtained at 5 minute intervals. Throughout both exercise trials, the participant was blinded to the speed and duration of the bout. The only verbal feedback provided, was to inform the participant that they were approximately half way through the bout.

Upon completing the exercise bout, the participant was free to shower and change, before being escorted to the research kitchen facility to complete the energy matching task (EI_{MATCH}). The participant was presented with an extensive pre-weighed buffet meal (content shown in Appendix 9). They were then given the following verbal instruction: "Consider the exercise bout that you have just completed and the amount of energy that you expended, or the number of calories that you burned. Now, try to match that energy, or number of calories in the food that you consume from the buffet." The participant was informed that, should they wish to eat any more food after the task, they would be free to do so once the matching task was complete. They were then left to complete the task in isolation. When the participant had finished eating, the buffet food was re-weighted and energy intake was calculated using energy density data derived from the manufacturer's nutritional information. The energy matching task was

completed approximately 30 minutes (mean time from cessation of exercise to matching task, 31 ± 4 min.; 29 ± 4 min. for MOD and 32 ± 4 min. for HIGH) after the completion of the exercise bout.

After the energy matching task had been completed, the participant returned to the Exercise Metabolism Laboratory to remain seated until the buffet food had been re-weighed. During this time, they were asked to provide a verbal estimate of the energy expenditure of the exercise bout. After the re-weighing was completed, they were informed that the trial was finished. They were then told that they were free to consume any more food that they wished from the buffet. Participants commenced this second sitting at approximately 60 minutes post-exercise (56 ± 7 min.; 53 ± 4 min. for MOD and 58 ± 7 min. for HIGH). Food intake was covertly recorded, with the buffet food being re-weighed again after the participant had finished eating. The energy intake at this sitting was added to the intake of the energy matching task to provide an *ad libitum* energy intake value ($El_{AD\ LIB.}$).

7.3.5 Measures:

Energy expenditure (EE) was measured, in kcal, for both exercise bouts. This was calculated from exhaled air collected intermittently during the bout. Mean rate of oxygen utilisation (VO_2) and RER were calculated and energy expenditure was estimated using the RER-specific caloric equivalent of oxygen. El_{MATCH} and $El_{AD\ LIB.}$ were measures as described above, from the buffet meal provided and recorded in kcal. The verbal estimate of energy expenditure (EST) was recorded as a further outcome measure.

7.3.6 Statistical analysis:

All values stated are mean values \pm standard deviation (SD) in text and tables and mean \pm standard error of the mean (SEM) in figures. Mean EE, El_{MATCH} and EST values were investigated for energy measures and trial differences using a 3x2 repeated measures factorial ANOVA. Energy measures and trial comparisons of EE

and $El_{AD\ LIB.}$ were assessed by conducting a further 2x2 repeated measures factorial ANOVA. Comparisons of the dietary intakes for the El_{MATCH} and $El_{AD\ LIB.}$ tasks in both conditions were made by conducting separate 2x2 repeated measures factorial ANOVA for each dietary characteristic investigated (total energy density, carbohydrate intake, fat intake and protein intake). Significant interactions and main effects from all ANOVA were further assessed by pairwise comparisons, using Bonferroni post-hoc analysis. Statistical significance level of $p < 0.05$ was in use for all comparisons. All statistical analysis was carried out using the SPSS software programme (SPSS inc., Chicago, Illinois, USA).

7.4 RESULTS

7.4.1 Exercise trials:

Physiological measures of each exercise trial condition are shown in **table 7.2**. As intended, the exercise intensity was significantly different between the two exercise trials. Absolute and relative intensity, represented by absolute VO_2 , absolute heart rate and percentage of $\text{VO}_{2\text{max}}$ and percentage of maximum heart rate was significantly greater in HIGH, compared with MOD (all $p < 0.001$). Duration of exercise was significantly greater for MOD compared with HIGH ($p < 0.001$), while energy expenditure was the same for both conditions.

	MOD	HIGH
VO_2 ($\text{L}\cdot\text{min}^{-1}$)	2.00 ± 0.42	$2.99 \pm 0.59^*$
% $\text{VO}_{2\text{max}}$	60.4 ± 2.9	$91.6 \pm 4.6^*$
Heart rate (bpm)	137 ± 16	$176 \pm 9^*$
% HR_{max}	72 ± 6	$91 \pm 5^*$
Perceived Exertion	10 ± 2	$15 \pm 1^*$
Duration (min)	46.6 ± 8.8	$30.2 \pm 4.9^*$
Energy Expenditure (kcal)	443 ± 22	$444 \pm 21^*$

Table 7.2. Characteristics of exercise. Values are mean \pm SD. * = significant difference between MOD and HIGH, $p < 0.001$.

7.4.2 Energy expenditure, energy intake of the matching task and verbal estimate:

Mean energy expenditure, energy estimate and energy matching task energy intakes are shown in **figure 7.1**. There was a significant condition (exercise intensity) x energy measure (EE, EI_{MATCH} , EST) interaction ($F(2) = 7.903$, $p = 0.002$). Pairwise comparisons for within-condition effects demonstrated that, in the MOD condition, EST was significantly lower than EE (298 ± 156 kcal vs. 443 ± 22 kcal, $p = 0.01$). There was no significant difference between EST and EI_{MATCH} (298 ± 156 kcal vs. 374 ± 220 kcal,

$p = 0.123$). EE and El_{MATCH} were similar. In the HIGH condition, there were no significant differences between EE, El_{MATCH} and EST. Pairwise comparisons for between condition effects showed that El_{MATCH} and EST were both significantly greater after HIGH, compared with MOD (530 ± 248 kcal vs. 374 ± 220 kcal, $p = 0.002$ and 431 ± 129 kcal vs. 298 ± 156 kcal, $p = 0.002$ respectively).

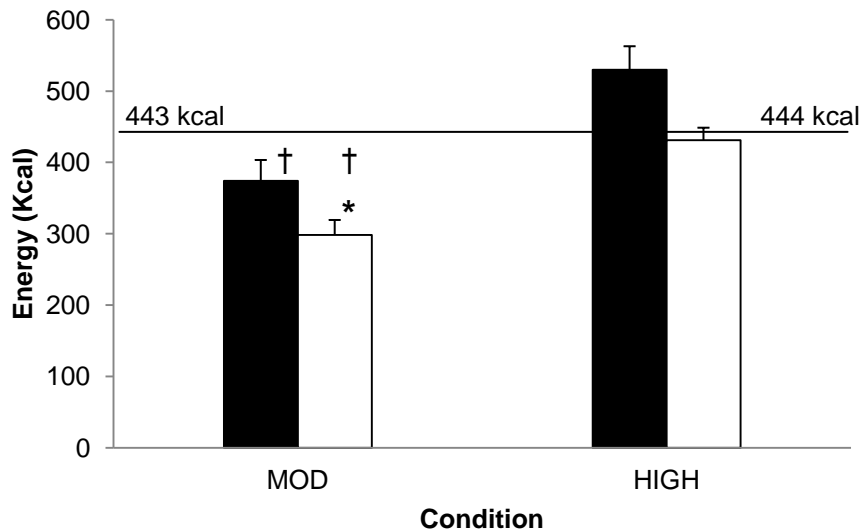


Figure 7.1. Energy expenditure, energy intake at the matching task and verbal EST of energy expenditure. Values are means \pm SEM. Black bars = $El_{(MATCH)}$, white bars = EST. Solid line indicates mean EE of 443 kcal for MOD, 444 kcal for HIGH. * = within-condition effect, significant different to EE. † = between-condition effect, significant different to HIGH.

7.4.3 Ad libitum energy intake:

Ad libitum energy intake for both the MOD and HIGH conditions, along with the energy expenditure of exercise is shown in **figure 7.2**. A significant energy measure main effect was observed, with $El_{AD LIB.}$ significantly greater than EE (914 ± 406 kcal vs. 443 ± 22 kcal, $F(1) = 23.706$, $p < 0.001$). There was no significant interaction, nor condition main effect.

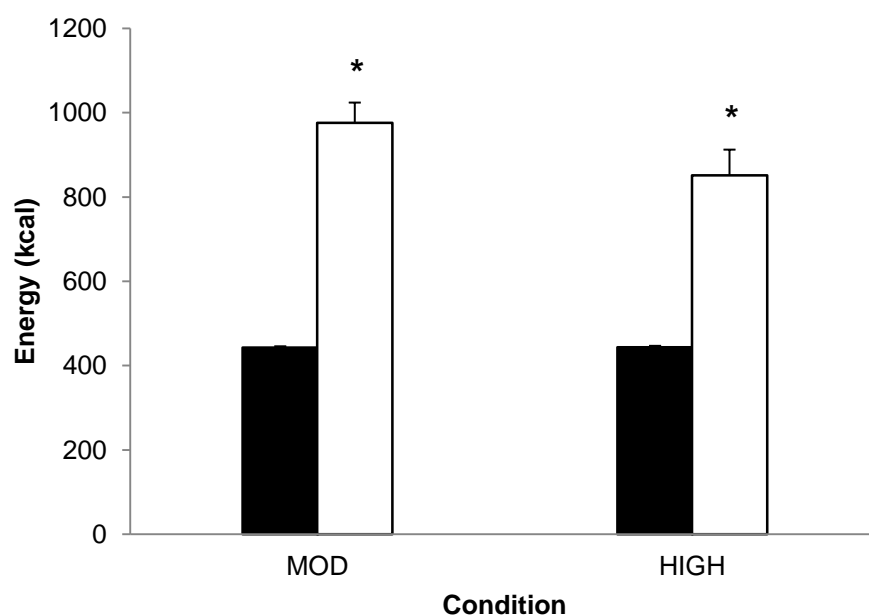


Figure 7.2. Energy expenditure and *ad libitum* energy intake. Values are mean \pm SEM. Filled rectangle = EE, empty rectangle = El_{AD LIB.} * = El_{AD LIB.} significantly different to EE.

7.4.4 Food selection: energy density and macronutrient intake:

The total energy density (expressed as kcal per 100g) and macronutrient content of the meal consumed (expressed in percentage of total energy consumed) are shown in **table 7.3** Energy density of the meal selected did not differ between conditions, however, a task main effect was present, demonstrating that energy density was significantly greater during the *ad libitum* intake, compared with the matching intake ($112 \text{ kcal} \cdot 100\text{g}^{-1}$ vs. $92 \text{ kcal} \cdot 100\text{g}^{-1}$, $F(1) = 11.736$, $p = 0.005$). The percentage of total energy derived from carbohydrate and protein did not differ between condition and task. There was a significant task main effect for percentage of total energy obtained from fat ($F(1) = 7.951$, $p = 0.015$). Pairwise post-hoc comparisons showed that there was a greater percentage of energy from fat consumed in the El_{AD LIB.} task, compared with El_{MATCH} (26.6% vs. 20.0%, $p = 0.021$).

	El_{MATCH}				El_{AD LIB.}			
	ED	CHO (% E)	FAT (% E)	PRO (% E)	ED	CHO (% E)	FAT (% E)	PRO (% E)
MOD	93.5 ± 32.4	59.0 ± 12	19.1 ± 9	21.9 ± 13	120.5 ± 45.6	54.0 ± 9.3	28.5 ± 12.0 ^a	17.5 ± 6.0
HIGH	90.8 ± 27.1	62.5 ± 10.9	19.9 ± 9.3	17.6 ± 5.9	103.3 ± 35.1	57.9 ± 12.1	24.7 ± 11.9	17.4 ± 5.2

Table 7.3 – Dietary characteristics of El_{MATCH} and El_{AD LIB.} intakes for both MOD and HIGH conditions. ED = energy density, CHO = carbohydrate, FAT = fat, PRO = protein, % E = percentage of total energy consumed. a = significant task effect, El_{AD LIB.} greater than El_{MATCH}

7.5 DISCUSSION

The aim of this study was to assess individuals' ability to match energy intake with energy expenditure after isoenergetic bouts of high- and moderate-intensity treadmill exercise. It would appear that individuals accurately match EI with EE after high-intensity and moderate-intensity exercise. In the MOD condition, EI_{MATCH} , consumed at the matching task buffet was very similar to the energy expenditure of the exercise bout (402 ± 220 kcal vs. 443 ± 22 kcal). These values were also not significantly different to each other in the HIGH condition, despite EI_{MATCH} being 23% greater than EE. This is in conflict with the findings of Harris and George (2010) and Willbond *et al.* (Willbond *et al.*, 2010), who both demonstrated poor energy matching ability.

This strong matching ability between EI and EE in the MOD condition was observed despite an undervaluation of the energy cost of exercise. The verbal estimate of energy expenditure of exercise was significantly lower than the exercise EE and EI_{MATCH} . This was not observed in the HIGH condition, with no difference between EST and either EE or EI_{MATCH} . The underestimation of the EE of moderate-intensity exercise was an unexpected finding. It not only contrasted with the hypothesis that EE would be overestimated in both exercise conditions but also contradicted the findings of Willbond and co-workers (Willbon *et al.*, 2010), who observed that the energy cost of treadmill running at an intensity of 50% VO_{2max} was overestimated 2-3 fold. The moderate-intensity exercise bout in the current study was of a considerably greater energy cost (450 kcal) than the two bouts of exercise used in the study of Willbond *et al.* (200 kcal and 300 kcal). With bouts of such low energy cost, overcompensation is much more easily achieved. However, it is suspected that the surprising findings of the current study may have been due to participants altering their behaviour under experimental conditions. It is possible that individuals over-compensated for their expected poor perception of the energy cost of exercise. Unfortunately, there is no means of assessing whether this was the case.

It would appear that an underestimate of the caloric content of food compensated for an undervaluation of the energy of moderate-intensity exercise. El_{MATCH} was, on average, 117 kcal greater than EST. While these two values were not significantly different in either condition, there was a main effect for energy measure, which showed a significant difference between El_{MATCH} and EST, with El_{MATCH} being 28% greater. In the moderate-intensity condition, while EST was significantly lower than EE, this did not transpire into a significantly lower El_{MATCH} than EE, as El_{MATCH} exceeded EST by a mean of over 100 kcal (285 kcal vs. 389 kcal). This undervaluation of the energy content of food is in agreement with previous literature, which has found this to be the case particularly in foods that are considered more “healthy” (Brindal *et al.*, 2012; Carels *et al.*, 2007). The findings of Harris and George (2000) also suggest that the inability to match energy intake with exercise energy expenditure is driven primarily by an undervaluation of the energy content of food; the mean estimate of the caloric intake at a post-exercise *ad libitum* buffet was 435 kcal lower than the mean actual intake. In comparison, the energy content of the exercise bout was overestimated by 129 kcal.

Both EST and El_{MATCH} were significantly greater after high-intensity exercise, compared with moderate-intensity exercise. Mean El_{MATCH} was 159 kcal (33%) greater in the HIGH condition, despite the two exercise bouts being matched for energy cost. This could suggest that individuals perceive shorter, more strenuous bouts of exercise to be more energetic than longer, less strenuous bouts and that perception of the energy cost of exercise may be driven by the intensity of exercise, rather than the duration of exercise. If this is the case, this may provide an argument for the undertaking of sustained, moderate-intensity exercise bouts for those seeking to increase physical activity for weight-management purposes. If such exercise bouts result in an undervaluation of the energy expended, particularly compared with isocaloric bouts at a higher intensity, then this may help produce negative energy balance through the avoidance of overcompensation in post-exercise energy intake.

This may be particularly pertinent for those susceptible to increasing food intake due to using food as a reward. As eating palatable food is a pleasurable experience for the majority of individuals, some use food as a means of reward following behaviour that is deemed an achievement or reward-worthy. One such behaviour may be the undertaking of a bout of exercise. While it would appear that neural responses in areas of the brain associated with the reward system are decreased immediately post-exercise (Evero *et al.*, 2012); Crabtree, D. PhD thesis, University of Birmingham), possibly explaining the “anorexia of exercise” phenomenon, there is now also evidence for increases in reward system activation in the hours after exercise, sensitising it to images of food (Crabtree, D. PhD thesis, University of Birmingham). In addition, Finlayson and colleagues (Finlayson *et al.*, 2011) found that some overweight and obese individuals exhibited increased liking and wanting of food items (components of the reward construct) following exercise. Further, those that did demonstrate this response were those who failed to experience weight-loss with a 12-week exercise programme. If exercise does sensitise individuals to the use or abuse of food as a reward and increase explicit wanting of food, then the perception of a less energetic bout may lower subsequent intake resulting from this response, as the conscious, explicit components of rewarding exercise may be reduced. Therefore, lower-intensity, longer duration bouts may prove preferable to shorter, higher intensity isocaloric bouts when devising exercise regimen to facilitate weight-loss.

One possible explanation for the perceived greater energy cost of shorter, higher-intensity exercise, compared with longer, moderate-intensity exercise, is that it is likely that metabolic rate was slightly higher after high-intensity exercise, due to excess post-exercise oxygen consumption (EPOC). EPOC has been shown to occur after high-intensity exercise (Phelain *et al.*, 1997). A greater metabolic rate after HIGH, compared with MOD, may have contributed to a perception of greater energy cost; although, the only likely perception of EPOC will likely have been a more sustained elevation in heart rate and breathing rate. Further, the EPOC effect was most likely small, especially over a period of just 30 minutes. EPOC has been shown to contribute minimally to the total energy expenditure of exercise

(Laforgia *et al.*, 2006), meaning only a small increase in energy expenditure will have occurred. Another potential explanation is that the participants may not have fully appreciated the difference in duration of each bout. Anecdotally, exercise can feel longer when it is strenuous, with exercisers experiencing a perceived slowing of time when exercising hard. This may have been the case here, with little perceived difference in the duration of the two bouts. It would perhaps have been interesting to have obtained estimates of the duration of exercise, as well as the energy cost.

In both exercise conditions, $El_{AD\ LIB.}$ was significantly greater than exercise EE, resulting in a positive energy balance of $+533 \pm 357$ kcal for MOD and $+408 \pm 448$ kcal for HIGH. Such large positive energy balance values would indicate the absence of a prolonged post-exercise suppression of appetite, or “anorexia of exercise” effect (King *et al.*, 1994). While this phenomenon is commonly observed in the immediate post-exercise period after exercise of $\geq 60\%$ VO_{2max} , (King *et al.*, 1994; Kissileff *et al.*, 1990; Thompson *et al.*, 1988; Ueda *et al.*, 2009; Westerterp-Plantenga *et al.*, 1997), this is not always reflected by a decrease in energy intake (Deighton *et al.*, 2012; George and Morganstein, 2003; King *et al.*, 2011) and rarely persists when an energy intake measure is obtained at ≥ 60 minutes post-exercise (Deighton *et al.*, 2012a; Martins *et al.*, 2007; Thompson *et al.*, 1988). In the current study, it is worth noting that such a suppression appears to be absent, even after undertaking running exercise of an intensity of 90% VO_{2max} – a particularly high intensity of continuous, aerobic exercise that is rarely utilised in such studies. However, it is acknowledged that a true representation of the effect of the exercise bouts on appetite would require a non-exercise control condition. As this was not a primary aim of the current study, such a condition was deemed unnecessary. Similarly, subjective measures of appetite were not recorded. While these would have been integral for a thorough investigation of the effect of the exercise bouts on post-exercise appetite, they were forfeited to ensure that decisions made during the El_{MATCH} task were influenced minimally by thoughts of appetite and hunger. Further, participants were allowed to shower between completing the exercise bout and feeding, and the duration and temperature of the shower were not controlled. Therefore, it is

likely that this will have impacted upon body temperature. As changes in body temperature has been proposed as a mechanism underpinning the post-exercise appetite response (Shorten *et al.*, 2009; White *et al.*, 2005; Halse *et al.*, 2011), showering may have influenced appetite and influenced appetite differential across the two trials.

The substantially greater energy intake when relieved of the constraints of the matching task ($El_{AD\ LIB.}$ intake exceeded El_{MATCH} intake by a mean of 602 ± 358 kcal (91%) in MOD and 322 ± 332 kcal (42%) in HIGH) was due not only to a greater absolute food intake, but also due, in part, to the selection of more energy dense foods. The total energy density of the *ad libitum* intake was $27\text{ kcal}\cdot 100\text{g}^{-1}$ (25%) greater in MOD and $13\text{ kcal}\cdot 100\text{g}^{-1}$ (13%) greater in HIGH, compared with the corresponding matching task intakes. It would appear that this may have been partly driven by a greater fat intake in the *ad libitum* feeding, with the percentage of total energy derived from fat significantly greater in the $El_{AD\ LIB.}$ intake, compared with El_{MATCH} (26.6% vs. 20.0%, data pooled for HIGH and MOD). Therefore, it would seem that individuals attempted to restrict energy intake in the matching task by not only eating less food, but also by successfully selecting less energy dense foods and foods lower in fat, or by avoided high calorie, fatty foods.

In light of the findings of this study, that individuals possess a strong ability to consciously match energy intake with energy expenditure, it may be worth asking the question: if this is the case, then why do people gain weight initially and fail to lose weight when initiating in weight-loss strategies involving increased physical activity? Firstly, it is likely that those that gain weight initially are those that do not exercise regularly, hence an ability to match intake with exercise-induced energy expenditure, whether consciously or not, is irrelevant. However, some exercisers do gain weight and some that begin exercising regularly in an attempt to lose weight fail to do so. It is possible that habitual eating behaviour can override any matching ability. Individuals' varying degree of eating restraint (Elfhag and Linné, 2005; Provencher *et al.*, 2003; van Strien *et al.*, 2009), emotional eating (van Strien *et al.*, 2009) and external eating (Burton *et al.*, 2007) have all been implicated in weight-gain and the pathology of obesity, as well as in the success of attempted weight-loss

(Elfhag and Rössner, 2005; Karlsson *et al.*, 1994). In the current study, *ad libitum* energy intake considerably exceeded the EI_{MATCH} intake and the exercise energy expenditure in both conditions, with large positive energy balances recorded ($+533 \pm 357$ kcal and $+408 \pm 448$ kcal for MOD and HIGH respectively). This suggests that when participants were free to consume as much as they desired, from a buffet-style meal providing considerable external food cues to the participant, little eating restraint was used and a restriction of food intake was not observed.

It should be noted that the participants in the current study were healthy-weight, low-activity level individuals. It may be the case that healthy-weight individuals do possess a strong ability to consciously match energy intake with energy expenditure, hence why they are not overweight. Those that are overweight and obese may exhibit a much poorer matching ability and this may have contributed to their weight-gain. It would be of interest to repeat the current study with overweight and obese participants.

In summary, participants demonstrated a strong ability to consciously match energy intake with exercise-induced energy expenditure after aerobic exercise at both a moderate- and high-intensity. It would appear that an undervaluation of the energy cost of exercise, particularly that of a moderate intensity, was countered by an undervaluation of the energy content of food. Participants perceived exercise of a high intensity to be more energetic than that of isocaloric exercise of a moderate intensity, which may suggest that perception of energy expenditure is driven more by intensity than duration of exercise. This may have implications for the types of exercise bouts recommended during exercise regimes utilised as part of a weight-management strategy. Despite the conscious ability to match energy intake with exercise-induced energy expenditure, participants exhibited little restraint when the restriction of the energy matching task was lifted, resulting in large *ad libitum* intakes and acute positive energy balance. It remains to be seen whether such a sound matching ability is possessed by overweight and obese individuals, as well as the healthy-weight individuals of the current study.

7.6 REFERENCES

- Bandini, L.G., Schoeller, D.A., Cyr, H.N., and Dietz, W.H. (1990). Validity of reported energy intake in obese and nonobese adolescents. *The American Journal of Clinical Nutrition* 52, 421-425.
- Blundell, J.E., and King, N.A. (2000). Exercise, appetite control, and energy balance. *Nutrition* 16, 519-522.
- Blundell, J.E., Stubbs, R.J., Hughes, D.A., Whybrow, S., and King, N.A. (2003). Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proc Nutr Soc* 62, 651-661.
- Borg, G.A.V. (1973). "Perceived exertion: a note on history and methods". *Medicine and Science in Sports & Exercise* 5, 90-93.
- Brindal, E., Wilson, C., Mohr, P., and Wittert, G. (2012). Perceptions of portion size and energy content: implications for strategies to affect behaviour change. *Public Health Nutrition* 15, 246-253.
- Burton, P., Smit, H.J., and Lightowler, H.J. (2007). The influence of restrained and external eating patterns on overeating. *Appetite* 49, 191-197.
- Carels, R.A., Konrad, K., and Harper, J. (2007). Individual differences in food perceptions and calorie estimation: An examination of dieting status, weight, and gender. *Appetite* 49, 450-458.
- Crabtree, D.R. (2012). "The effects of exercise on appetite regulation." Ph.D thesis, University of Birmingham. <http://etheses.bham.ac.uk/3779/>
- Deighton, K., Barry, R., Connon, C., and Stensel, D. (2012a). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*, 1-10.
- Deighton, K., Zahra, J.C., and Stensel, D.J. (2012b). Appetite, energy intake and resting metabolic responses to 60min treadmill running performed in a fasted versus a postprandial state. *Appetite* 58, 946-954.
- Elfhag, K., and Linné, Y. (2005). Gender differences in associations of eating pathology between mothers and their adolescent offspring. *Obesity Research* 13, 1070-1076.
- Elfhag, K., and Rössner, S. (2005). Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain. *Obesity Reviews* 6, 67-85.
- Evero, N., Hackett, L.C., Clark, R.D., Phelan, S., and Hagobian, T.A. (2012). Aerobic exercise reduces neuronal responses in food reward brain regions. *Journal of Applied Physiology* 112, 1612-1619.
- Finlayson, G., Caudwell, P., Gibbons, C., Hopkins, M., King, N., and Blundell, J. (2011). Low fat loss response after medium-term supervised exercise in obese is associated with exercise-induced increase in food reward. *Journal of Obesity* 2011.

George, V.A., and Morganstein, A. (2003). Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite* 40, 43-46.

Halse, R.E., Wallman, K.E., and Guelfi, K.J. (2011). Postexercise water immersion increases short-term food intake in trained men. *Medicine and Science in Sports & Exercise* 43, 632-638.

Harris, C.L., and George, V.A. (2010). Dietary restraint influences accuracies in estimating energy expenditure and energy intake among physically inactive males. *American Journal of Men's Health* 4, 33-40.

Health and Social Care Information Centre (2013). Health Survey for England. Statistics on Obesity, Physical Activity and Diet – England, 2013. <http://www.hscic.gov.uk/searchcatalogue?productid=11194&q=obesity&sort=Relevance&size=10&page=1#top>

Hill, R.J., and Davies, P.S.W. (2001). The validity of self-reported energy intake as determined using the doubly labelled water technique. *British Journal of Nutrition* 85(4), 415-430.

Karlsson, J., Hallgren, P., Kral, J., Lindroos, A.-K., Sjöström, L., and Sullivan, M. (1994). Predictors and effects of long-term dieting on mental well-being and weight loss in obese women. *Appetite* 23, 15-26.

King, J.A., Wasse, L.K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R.L., and Stensel, D.J. (2011). Differential acylated ghrelin, peptide YY₃₋₃₆, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology & Metabolism* 96, 1114-1121.

King, N.A. (1999). What processes are involved in the appetite response to moderate increases in exercise-induced energy expenditure? *Proceedings of the Nutrition Society* 58, 107-113.

King, N.A., Burley, V.J., and Blundell, J.E. (1994). Exercise induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition* 48, 715-724

King, N.A., Hopkins, M., Caudwell, P., Stubbs, R.J., and Blundell, J.E. (2008). Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. *Int J Obes (Lond)* 32, 177-184.

Kissileff, H.R., Pi-Sunyer, F.X., Segal, K., Meltzer, S., and Foelsch, P.A. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition* 52, 240-245.

LaForgia, J., Withers, R.T., and Gore, C.J. (2006). Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *Journal of Sports Sciences* 24, 1247-1264.

Livingstone, M.B., Prentice, A.M., Coward, W.A., Strain, J.J., Black, A.E., Davies, P.S., Stewart, C.M., McKenna, P.G., and Whitehead, R.G. (1992). Validation of estimates of energy intake by weighed dietary record and diet history in children and adolescents. *The American Journal of Clinical Nutrition* 56, 29-35.

Livingstone, M.B., Prentice, A.M., Strain, J.J., Coward, W.A., Black, A.E., Barker, M.E., McKenna, P.G., and Whitehead, R.G. (1990). Accuracy of weighed dietary records in studies of diet and health. *British Medical Journal* 300(6726), 708-712.

Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 193, 251-258.

Pettigrew, S., Rosenberg, M., and Ferguson, R. (2013). Consumers' (in)ability to estimate the energy content of unhealthy foods. *Nutrition & Dietetics*, n/a-n/a.

Phelain, J.F., Reinke, E., Harris, M.A., and Melby, C.L. (1997). Postexercise energy expenditure and substrate oxidation in young women resulting from exercise bouts of different intensity. *Journal of the American College of Nutrition* 16, 140-146.

Polivy, J. (1976). Perception of calories and regulation of intake in restrained and unrestrained subjects. *Addictive Behaviors* 1, 237-243.

Prentice, A.M., Black, A.E., Coward, W.A., Davies, H.L., Goldberg, G.R., Murgatroyd, P.R., Ashford, J., Sawyer, M., and Whitehead, R.G. (1986). High levels of energy expenditure in obese women. *British Medical Journal (Clin. Res. Ed.)* 292(6526), 983-987.

Provencher, V., Drapeau, V., Tremblay, A., Després, J.-P., and Lemieux, S. (2003). Eating Behaviors and Indexes of Body Composition in Men and Women from the Québec Family Study. *Obesity Research* 11, 783-792.

Schoeller, D.A. (1995). Limitations in the assessment of dietary energy intake by self-report. *Metabolism* 44, *Supplement 2*, 18-22.

Shorten, A.L., Wallman, K.E., and Guelfi, K.J. (2009). Acute effect of environmental temperature during exercise on subsequent energy intake in active men. *The American Journal of Clinical Nutrition* 90, 1215-1221.

Thompson, D.A., Wolfe, L.A., and Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medicine & Science in Sports & Exercise* 20, 222-227.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., and Fujimoto, S. (2009). Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *Journal of Endocrinology* 203, 357-364.

van Strien, T., Frijters, J.E.R., Bergers, G.P.A., and Defares, P.B. (1986). The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders* 5, 295-315.

van Strien, T., Herman, C.P., and Verheijden, M.W. (2009). Eating style, overeating, and overweight in a representative Dutch sample. Does external eating play a role? *Appetite* 52, 380-387.

Weiss, E.C., Galuska, D.A., Khan, L.K., and Serdula, M.K. (2006). Weight-Control Practices Among U.S. Adults, 2001–2002. *American Journal of Preventive Medicine* 31, 18-24.

Westerterp-Plantenga, M.S., Verwegen, C.R., Ijeda, M.J., Wijckmans, N.E., and Saris, W.H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* 62, 1345-1354.

Westerterp, K.R., Saris, W.H., van Es, M., and ten Hoor, F. (1986). Use of the doubly labeled water technique in humans during heavy sustained exercise. *Journal of Applied Physiology* 61, 2162-2167.

White, L.J., Dressendorfer, R.H., Holland, E., McCoy, S.C., and Ferguson, M.A. (2005). Increased caloric intake soon after exercise in cold water. *International Journal of Sports Nutrition and Exercise Metabolism* 15, 38-47.

Willbond S.M., Lavoilette M.A., Duval K., and E., D. (2010). Normal weight men and women overestimate exercise energy expenditure. *Journal of sports medicine and physical fitness* 50, 377-384.

General discussion

8.1 General discussion

The rise in the amount of people with excessive bodyweight has led to an alarming global prevalence of obesity and overweight (Finucane *et al.*, 2011). Due to the health implications associated with the disease state of obesity, it may be disconcerting to consider that an adult in England who is not overweight or obese is now part of a ~35% minority (Health and Social Care Information Centre, 2013). Throughout this thesis, the more commonly used term “normal-weight” has been replaced by the term “healthy-weight” when describing a study population; not being overweight or obese is no longer “normal”. With projections showing that the prevalence is set to rise further, there is a need to tackle overweight and obesity with the formulation of effective weight-management strategies. As interventions that combine both exercise and dietary elements have been shown to be the most efficacious for sustained weight-loss (Foster-Schubert *et al.*, 2012; Kerkick *et al.*, 2010) and the avoidance of weight-gain (Artal *et al.*, 2007; Skender *et al.*, 1996), a sensible starting point may be to gain a greater understanding of the effects of acute exercise on short-term food intake and energy balance, addressing both sides of the energy balance equation.

The primary aims of this thesis were:

1. To attempt to develop and validate a novel tool for the measurement of subjective appetite
2. To investigate the effect of exercise on appetite in athletic populations, and in doing so, assess the degree to which appetite responses to exercise may vary in different populations
3. Shed further light on the influence of exercise characteristics, such as duration, energy cost and mode of exercise, on appetite responses to exercise.

A secondary aim was to assess the likely role of appetite-associated hormones in appetite regulation in the post-exercise period, through investigation of the relationships between hormonal changes and changes in subjective appetite and energy intake.

This chapter will address these aims and attempt to answer the relevant research questions.

8.2 A novel tool for the measurement of subjective appetite

This section will evaluate the findings of Chapters 2 and 3, as well as compiling the findings of Chapters 4, 5 and 6, where the BPAR and the VIMEC were used to obtain appetite measures during trials.

In summary, the findings of Chapter 2 and 3 were that the VIMEC was confirmed to be an improvement on its predecessor, the BPAR, demonstrating a stronger ability to predict within-subject changes and between-subject differences in energy intake, while maintaining its efficacy at detecting expected changes in subjective appetite. Moreover, Chapter 3 provided an indication that the VIMEC is a stronger predictor of changes in energy intake than the VAS method, while exhibiting a similar degree of reproducibility. It was concluded that the VIMEC is a valid tool for the measurement of subjective appetite. Further, it may prove preferable to the commonly-used VAS method as a predictor of food intake, particularly when addressing between-subject differences in appetite or expected food intake, either in conjunction with or as a cost-effective, time-efficient and less wasteful alternative to *ad libitum* test meals.

As the studies of Chapters 4, 5 and 6 included VAS measures of subjective appetite and *ad libitum* food intake measures, it is possible to obtain further indications of the efficacy of the BPAR and the VIMEC by investigating the relationship between changes in BPAR/VIMEC and changes in both VAS and energy intake.

Between-subject comparisons showed that VAS scores and BPAR/VIMEC scores were significantly correlated with one another in all conditions, in all three studies, exhibiting moderate-strength correlation (**table 8.1a**). Throughout the three studies, VAS score immediately prior to the test meal was not significantly correlated with energy intake, with

coefficients demonstrating weak-to-moderate strength correlation. While BPAR scores exhibited a weak relationship with energy intake in Chapter 4, the relationship between VIMEC score and energy intake was stronger in Chapter 5, with a significant correlation between the two in the resting condition and non-significant moderate strength correlation demonstrated in the 15 minute and 30 minutes conditions also. In Chapter 6, the correlation between VIMEC and energy intake in the resting condition was strong and statistically significant. Moreover, this correlation was significantly stronger than the corresponding correlation between VAS score and energy intake.

Table 8.1b shows the correlation coefficients for comparisons of between-condition, within-subject percentage changes in BPAR/VIMEC with percentage changes in VAS for all measures obtained in each trial condition and comparison of percentage changes in BPAR/VIMEC score immediately prior to the *ad libitum* test meal with percentage changes in energy intake for Chapters 4, 5 and 6. Changes in BPAR/VIMEC scores were significantly correlated with changes in VAS scores for all but one condition, in all three studies, exhibiting weak- to moderate-strength correlation on the whole. In Chapter 4, both VAS and BPAR proved to be a poor predictor of energy intake. In Chapter 5, both VAS and VIMEC scores immediately prior to the test meal were significantly associated with *ad libitum* intake when assessing changes from the resting trial in the 30 minute trial. However, this relationship was weak-to-moderate in the other two comparison of this study. Only change in VAS score correlated significantly with change in energy intake in Chapter 6. The correlation coefficients obtained for changes in VAS and changes in energy intake, and for changes in VIMEC and changes in energy intake were not significantly different in any condition, in any of the three studies.

The data from Chapters 4, 5 and 6 do not fully substantiate the findings of Chapters 2 and 3. The VIMEC is a stronger predictor of between-subject differences in energy intake than the BPAR and there is evidence that the VIMEC is a stronger predictor than VAS, at least in the resting state. The VIMEC and the BPAR exhibited a similar between-subject correlation with VAS. However, the BPAR did appear a better tool for detecting within-

subject changes in subjective appetite, as indicated by stronger correlation with VAS score. There is evidence that VIMEC is a better predictor of eating behaviour than BPAR, as shown by stronger correlation with within-subject changes in energy intake, but these data would suggest that the VIMEC is no stronger a predictor than VAS.

The relationship between VIMEC score and energy intake does appear to weaken post-exercise. While it is not completely clear as to why this may be the case, it could be that there is simply a dissociation between subjective appetite and energy intake after exercise, as cognitive factors may become increasingly regulatory of eating behaviour during this time. This may be specifically the case with athletic populations, as post-exercise nutrition is considered of high importance for recovery and adaptation to training. This consideration will be discussed further in sections 8.3 (p241) and 8.6 (p254). Nonetheless, further validation of the VIMEC during post-exercise periods may be warranted.

Correlation	Chapter 4			Chapter 5				Chapter 6	
	LOW	MOD	HIGH	REST	15 min	30 min	45 min	REST	EX
VAS vs. BPAR	0.772***	0.671***	0.712***	-	-	-	-	-	-
VAS vs. VIMEC	-	-	-	0.513**	0.412***	0.400**	0.622***	0.699***	0.757***
VAS vs. EI	0.542	0.353	0.240	0.453	0.544	0.472	0.038	0.371	0.477
BPAR vs. EI	0.297	0.249	-0.190	-	-	-	-	-	-
VIMEC vs. EI	-	-	-	0.610*	0.538	0.464	0.218	0.877**†	0.245

Table 8.1a – Product moment correlation coefficients for between-subject correlations for BPAR/VIMEC and VAS score and for BPAR/VAS and energy intake. Data compiled for all analysis from Chapters 4, 5 and 6. * = significant correlation, $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. . † = significant difference between correlation coefficients (for VAS vs. EI correlation and BPAR/VIMEC vs. EI correlation), $p < 0.05$; †† = $p < 0.01$.

Correlation	Chapter 4			Chapter 5			Chapter 6
	HIGH - LOW	HIGH – MOD	MOD - LOW	15 min – REST	30 min - REST	45 min – REST	EX - REST
Changes in VAS vs. changes in BPAR	0.569***	0.605***	0.676***	-	-	-	-
Changes in VAS vs. changes in VIMEC	-	-	-	0.615***	0.217*	0.191	0.328**
Changes in VAS vs. changes in EI	-0.304	-0.356	0.107	0.567	0.626*	0.372	0.755*
Changes in BPAR vs. Changes in EI	-0.197	-0.231	0.452	-	-	-	-
Changes in VIMEC vs. changes EI	-	-	-	0.372	0.761**	-0.002	0.117

Table 8.1b – Product moment correlation coefficients for within-subject correlations for percentage changes in BPAR/VIMEC and percentage changes in VAS score and for percentage changes in BPAR/VIMEC and percentage changes in energy intake. Data compiled for all analysis from Chapters 4, 5 and 6. * = significant correlation, $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

While the majority of the data suggests that VIMEC is effective at detecting differences and changes in subjective appetite, there is some evidence that the VIMEC measure lacks the sensitivity of the VAS method. In Chapter 3, a greater number of within- and between-condition differences reach statistical significance with the VAS than with VIMEC, despite a similar magnitude of difference. Similarly, in Chapter 6, a significant suppression was observed with measures of VAS, but differences in VIMEC scores were not statistically significant. No such discrepancy between the measures was observed in Chapter 5. In the cases of Chapters 3 and 6, inconsistency between measures is likely due to a greater degree of between-subject variability in VIMEC scores, as is highlighted by larger standard error (depicted in figures) and larger coefficient of variation for each measure (as highlighted in section 6.5, p194). The nature of the VIMEC, allowing for extensive food choice and in doing so offering indications of food preference, lends itself to a larger variation than the more restricted application of the VAS method. Therefore, because the magnitude of differences may be large, detecting statistically significant differences will be more difficult. It is likely that this is a trade-off, between a more informative and predictive measure of appetite and food intake, and sensitivity to statistically significant changes in subjective appetite.

The findings of Chapters 2 and 3, supported by the additional data from Chapter 4, 5 and 6 would suggest that the VIMEC is a valid tool for the measure of subjective appetite. It remains to be confirmed whether the strong relationship between VIMEC score and *ad libitum* intake, seen in Chapters 2 and 3, is maintained in the post-exercise period. Further, the efficacy of the VIMEC to monitor subjective appetite and predict eating behaviour must be assessed in a wider variety of populations. Within-subject changes in VIMEC score did not correlate with changes in energy intake in Chapter 6, when the study population consisted of overweight and obese individuals. Farah *et al.* (Farah *et al.*, 2012) found that the ability of a photographic image technique for the measure of desired portion size to detect changes in hunger varied with the weight-status of participants, although they observed a relationship between desired portion size and VAS hunger rating in obese, but

not lean individuals. They also showed that desired portion size matched subjective hunger rating in unrestrained, but not restrained eaters. Chapters 4, 5 and 6 of this thesis used unrestrained eaters, while the participants of Chapters 2 and 3 were not screened for eating restraint. Therefore, it is necessary to investigate the use of the VIMEC further, with restrained eaters and populations of varying weight-status. Nonetheless, the initial development and validation testing of the VIMEC has proved encouraging and further advancement and use of the tool is an exciting proposition.

While the BPAR and VIMEC tools were developed for the purpose of measuring subjective appetite within the sphere of appetite research, it is felt that the VIMEC possesses the potential for additional uses. With a more extensive library of photographic images, the VIMEC could prove to be a useful tool for the retrospective recording of food intake or for measures of habitual intake. Food photography has been used for these purposes previously (Frobisher and Maxwell, 2003; Riley *et al.*, 2007; Subar *et al.*, 2010; Turconi *et al.*, 2005; Venter *et al.*, 2000), but with varied degrees of success. However, the techniques used in these previous studies did not allow for an extensive food choice, or for the creation of a “meal,” as is possible with the VIMEC. This advancement could lead to a greater efficacy with the VIMEC. As weighed food diaries and food frequency questionnaires have been shown to demonstrate questionable validity (Krall and Dwyer, 1987; Muhlheim *et al.*, 1998), allowing participants to recreate their intake using an enhanced version of the VIMEC, with a larger database of food items could improve the accuracy of reporting. The increasing use of technology, especially in the form of “smart phone” apps., to assist with lifestyle interventions (Turner-McGrievy and Tate, 2011; Turner-McGrievy *et al.*, 2013) means that a mobile, interactive device such as a VIMEC app could prove useful, especially when considering that current calorie counter apps do not include photographic images of food, either for the creation of a replica meal of those consumed, or as a reference point for the estimation of portion size.

An alternative approach for the recording of food intake that is becoming increasingly used within weight-management research is to ask participants to photograph the food that

they consume (Higgins *et al.*, 2009; Martin *et al.*, 2012; Small *et al.*, 2009). These images are then received by the researcher and from the image, portion size and intake is estimated. A tool like the VIMEC, with a large database of food items and portion sizes, that can be easily and precisely manipulated and that provides a calorie content output could be used to compare the photographic image with. This may improve the accuracy of estimations of energy content.

A further possible use of the VIMEC is as an educational tool. Many people have a poor grasp of the calorie content of certain foods (Brindal *et al.*, 2012), commonly underestimating the energy content of food regarded as “healthy” (Carels *et al.*, 2007) and overestimating the calories in “unhealthy” food (Pettigrew *et al.*, 2013). Using the VIMEC may be an effective approach to improving understanding of the calorie content of food and meals, allowing users to manipulate a meal (e.g., changing portion sizes and swapping certain food items for alternative options) and observing how this effects total calorie content.

Within our department, similar applications have been explored using the VIMEC. It has proved valuable, particularly when emphasising the effects of consuming energy-dense foods as desert items after a low-calorie main course. We have also used the VIMEC for a task with undergraduate students, emphasising the comparative energy content of food and the energy cost of exercise. Asking participants to select a meal, using the VIMEC, to match the energy cost of a theoretical walk or run proved an enlightening task for the students. It may be of interest to assess the efficacy of the VIMEC at successfully improving comprehension of the energy content of food, relative to the energy expenditure of physical activity, by conducting an intervention study. Perhaps the ability to estimate the energy content of food, or of a diet, before and after educational seminar sessions with and without the use of the VIMEC could be investigated.

8.3 The effect of exercise on appetite in highly-trained athletes

Chapters 4 and 5 investigated appetite responses to exercise in highly-trained male endurance athletes. Chapter 4 showed that subjective appetite was transiently suppressed after high-intensity exercise at 80% $\text{VO}_{2\text{max}}$, compared with exercise of moderate- and low-intensity at 60% and 40% $\text{VO}_{2\text{max}}$ respectively. This suppression was short-lived and did not result in any differences in *ad libitum* food intake 60 minutes post-exercise. PP concentration demonstrated a very modest response to exercise. Small perturbations in PYY concentration with high-intensity exercise were not statistically significant. Chapter 5 aimed to extend these findings by maintaining the high-intensity exercise condition (30 minutes of cycling at 80% $\text{VO}_{2\text{max}}$) and including conditions of a shorter (15 minute) and longer (45 minute) duration. A near identical protocol was used and a resting condition was added. Subjective appetite did not change with exercise in any condition and appetite did not differ between conditions at any point. Food intake 60 minutes post-exercise also did not differ between conditions. PYY was unresponsive to exercise. Acylated ghrelin was transiently suppressed with exercise in all three conditions, with the greatest suppression observed in the 45 minute condition. GLP-1 concentration was increased with exercise in the 30 minute and 45 minute conditions, with concentration remaining elevated during the 60 minute post-exercise period.

The phenomenon of an exercise-induced suppression of appetite, or the “anorexia of exercise” (King *et al.*, 1994) was first stated to occur after high-intensity exercise of 60-70% $\text{VO}_{2\text{max}}$ (King *et al.*, 1994; King *et al.*, 1996; Kissileff *et al.*, 1990; Westerterp-Plantenga *et al.*, 1997), with the majority of this research conducted in obese or overweight sedentary (Kissileff *et al.*, 1990; Westerterp *et al.*, 1986), or healthy-weight low-activity (King *et al.*, 1994; King *et al.*, 1996; Kissileff *et al.*, 1990; Westerterp *et al.*, 1986) individuals. Subsequent work, however, has shown a lack of appetite suppression after exercise at ~70% $\text{VO}_{2\text{max}}$ in physically active individuals (King *et al.*, 2010; King *et al.*, 2011a). Chapter 4 of this thesis showed that exercise at an intensity of 60% $\text{VO}_{2\text{max}}$ was not sufficient to elicit a suppression of appetite in highly-trained endurance males and that, for appetite to be

suppressed, the intensity needed to be 80%VO_{2max}. This data would suggest that exercise-induced suppression of appetite can be experienced in athletic populations, but the intensity required to elicit this response would appear to be higher than for low-activity and recreationally active people.

Despite the observed suppression of subjective appetite after high-intensity exercise, anorexigenic hormones were not affected. There was a small increase in PYY concentration from baseline of 9% in the high-intensity condition, resulting in concentration being 24% and 30% higher than after low- and moderate-intensity exercise respectively. While it is possible that lack of statistical power was responsible for this difference not being statistically significant, a power calculation showed that sample size of 62 participants would be required to observe a statistically significant difference (alpha = 0.05, power of test = 0.5). As an increase in PYY concentration with aerobic exercise is commonly observed in non-athletic populations (Broom *et al.*, 2009; King *et al.*, 2011a; King *et al.*, 2011b; Martins *et al.*, 2007; Ueda *et al.*, 2009b), in an intensity-dependent manner (Ueda *et al.*, 2009a), this data would indicate a potential blunting of this response in athletic populations, or among those familiar with such exercise. This is yet to be directly assessed, either in the form of a cross-sectional investigation, comparing trained with untrained individuals, exercising at the same relative intensity (or a range of intensities), or in the form of a longitudinal study, addressing changes in hormonal responses before and after an exercise programme, in sedentary individuals.

The effect of the duration or energy cost of exercise has not previously been directly investigated. The findings of Chapter 5 would suggest that the duration or energy cost of exercise does not regulate post-exercise appetite, at least not in athletic populations. In previous studies, exercise-induced appetite suppressions have been observed with as little as 30 minutes of continuous exercise (Ueda *et al.*, 2009a) or just a total 3 minutes of maximal effort cycling (6 x 30 sec interval, 23 minutes total, including 5 x 4 minute active recovery at a very low intensity) and with an energy cost of just 142 kcal (Deighton *et al.*, 2012). In addition, no such response has been seen after long, energetic exercise. These observations point towards exercise-induced appetite suppression being independent of

duration or energy cost. This study directly assessed the effect of varying duration (and hence energy cost), while controlling for intensity and the findings confirm these speculations, at least within the range of 15 minutes to 45 minutes of high-intensity aerobic exercise.

However, it is difficult to conclude that duration plays no role in the exercise-induced suppression of appetite phenomenon, as in this instance, no significant post-exercise appetite suppression was observed. This was despite the replication of a study condition that was seen to elicit this response in Chapter 4 (30 minutes of cycling at 80% $\text{VO}_{2\text{max}}$).

Failure to replicate the findings of Chapter 4 in this condition is perplexing. The percentage decrease from baseline seen in VAS score after 30 minutes of cycling at 80% $\text{VO}_{2\text{max}}$ in Chapter 4 was 25%. In contrast, the same condition elicited just a 7% decrease in Chapter 5. The 15 minute and 45 minute conditions did induce a 14% and 11% decrease respectively, but this is still a somewhat blunted response, compared with Chapter 4.

On inspection, it can be seen that the actual intensity of these two comparable bouts differed. Whereas the mean intensity of the bout in Chapter 4 was 80%, as intended, the mean intensity of the bout in Chapter 5 was 76%. This lower intensity was predominantly due to a greater number of participants that struggled to maintain the power output necessary to elicit an intensity of 80% $\text{VO}_{2\text{max}}$, requiring a reduction due cycle ergometer resistance to enable the completion of the bout. This difference in intensity, while small, could explain the different appetite response, especially if a “threshold” intensity for appetite suppression lies between 75% and 80% $\text{VO}_{2\text{max}}$ for such a highly-trained athletic population.

Another possible explanation for this difference is that the studies were conducted with largely different participants and individual variability in appetite response was seen to be considerable. Of the 12 participants of Chapter 5, 2 also partook in the study of Chapter 4. The coefficient of variability values for percentage change in VAS score from baseline, with exercise, for these two identical conditions were 122% and 1064% for Chapter 4 and Chapter 5 respectively. This indicates a huge degree of variability in the appetite response to exercise, especially in Chapter 5; the appetite response immediately post-exercise in

Chapter 5 ranged from a decrease in VAS score of 48% to an increase of 83%. Hence, having different participants, even from a similar population and exhibiting similar characteristics (age, BMI, aerobic capacity, training status), could explain the differing mean responses observed.

While the results of Chapter 5 would suggest that post-exercise appetite responses are not dependent on the duration of exercise, the response of some appetite-associated hormones may be. Acylated ghrelin concentration was decreased with exercise in each of the three exercise conditions, but the greatest decrease was observed in the longest bout. Further, the plot of concentration against duration of exercise indicated that acylated ghrelin concentration continued to fall throughout the period of exercise. GLP-1 concentration increased only in the 30 minute and 45 minute conditions and this increase appeared to have a quadratic polynomial relationship with duration of exercise.

These data would suggest that there may be some duration, or energy cost, dependency in the acylated ghrelin and GLP-1 responses to exercise, but not in the subjective appetite response. This raises the possibility of a dissociation between hormonal and appetite responses to exercise and questions the role of hormonal changes in regulating post-exercise appetite. This will be further investigated and discussed in section 8.6 (p254). The findings of Chapters 4 and 5 would also indicate that the effect of exercise on appetite may differ between different populations, particularly with regard to athletic individuals.

There is evidence from Chapters 4 and 5 that there is a blunting to the exercise-induced appetite-suppression in highly-trained male endurance athletes. While a suppression response was evident after exercise at 80% VO_{2max} in Chapter 4, it was not observed in Chapter 5 with exercise at the same intensity. It could be said that this suppressive effect can be observed, though a greater “threshold intensity” exists for such a response in athletes. It can be speculated that this is due either to athletes being more familiar with high-intensity aerobic exercise or athletes possessing greater exercise capacity. An increased capacity for exercise and greater economy of energy production through regular exercise training in athletes will result in a reduction in metabolic and physiological

perturbation when exercising, not only at the same absolute intensity, but also at the same relative intensity. If changes in appetite with exercise are driven by physiological mechanisms (see section 8.6, p254), then the possible stimuli for such mechanisms are numerous. These could include changes in energy charge of the cell, changes in plasma concentrations of fuels such as glucose and lipids, changes in plasma concentrations of hormones and metabolites associated with exercise and metabolic stress (e.g., insulin, adrenaline, cortisol, lactate etc), changes in blood flow distribution, changes in body temperature and changes in neural drive. (Some of these factors will be discussed further in section 8.6, p254). Such changes, and hence the magnitude of the stimuli are likely to be smaller for highly-trained athletes during sub-maximal exercise, than for non-athletes.

8.4 The effect of different exercise characteristics on appetite responses to exercise

One aim of this thesis was to conduct a progressive, systematic approach investigating the effect of a number of exercise characteristics on the appetite responses to exercise. This section will evaluate the extent to which this aim was achieved.

As discussed in section 8.3, Chapters 4 and 5 would suggest that high-intensity exercise can lead to a transient suppression of appetite that does not influence food intake 60 minutes post-exercise and that any appetite response appears independent of the duration of exercise, or the energy cost.

Having investigated intensity and duration effects on appetite, Chapter 7 looked at these characteristics of exercise from a different perspective. Here, the ability of individuals to consciously match energy intake with the energy expenditure of two isoenergetic bouts of treadmill running: one at a moderate-intensity (60% $\text{VO}_{2\text{max}}$) and one at a high-intensity (90% $\text{VO}_{2\text{max}}$), was assessed. Being matched for energy expenditure, these bouts were of differing duration, with one lasting ~30 minutes, while the other lasted, on average, ~47 minutes. The

findings of the study were that, contrary to the study hypothesis, participants exhibited a strong ability to match intake with expenditure after high-and moderate-intensity exercise, but that higher-intensity, shorter duration exercise is perceived as more energetic than more moderate-intensity, longer duration exercise. This suggests that the perception of the energy cost of exercise is driven more by intensity than by duration. This is not in any way linked to appetite post-exercise. The food intake at the matching task was a conscious attempt to match intake with expenditure of exercise and, when allowed to feed *ad libitum* after the matching task, intake did not differ between the two conditions. However, this differing perception of energy expenditure may play a role in post-exercise energy intake, in certain circumstances. This notion will be explored further in section 8.6 (p254).

It was my initial intention to continue with the systematic approach and investigate the effect of further exercise characteristics on appetite parameters. One possible avenue could have been to distinguish between duration effects and energy cost effects, following the approach suggested in **figure 1.2** (p34) in the Introduction section of this thesis. However, as Chapter 5 showed no difference in subjective appetite and food intake between exercise bouts completed at the same intensity but lasting either 15, 30 or 45 minutes (and hence of differing energy cost), no further investigation was warranted. The influence of the mode of exercise on appetite responses would also have been an interesting research question, with the direct comparison of isoenergetic, intensity-matched ergometer cycling and treadmill running. Gender difference could also have been assessed.

However, it was decided to deviate a little and conclude this line of research within the thesis by addressing the effects of sprint-interval cycle exercise on appetite, food intake and appetite-associated hormones in overweight and obese individuals (Chapter 6). This allowed for the investigation of a different form of exercise (intermittent sprint exercise, as opposed to the continuous aerobic exercise of Chapters 4 and 5), while also allowing for investigation of a different and more clinically relevant population. Unfortunately, due to the time and financial constraints of a PhD programme, direct comparison between this population and the previously-used athletic cohort, by including a separate population group

within this study, was not possible. Also, direct comparison of the forms of exercise was not possible in this instance due to the inability of low-fitness level, overweight and obese individuals to maintain high-intensity, continuous aerobic exercise in order to match the exercise trials of Chapters 4 and 5. However, it was decided that conducting this study was not only in keeping with the latest research trends, but also allowed for mechanistic questions regarding intensity and duration/energy cost to be further investigated, by using an exercise bout of extremes in intensity (very high intensity) and duration/energy cost (very short/low), while also addressing important and clinically relevant questions, namely: 1) Can a low-volume, time-efficient bout of very high-intensity exercise elicit a suppression of appetite in overweight and obese individuals and can this result in a reduction in short-term energy intake? 2) Is there the potential for such exercise bouts within an effective weight-management programme?

It was found that just 4 x 30 seconds maximal effort cycling did elicit a transient suppression of appetite in overweight and obese individuals, with this suppression maintained for 30 minutes post-exercise. Acylated ghrelin was suppressed with sprint interval cycling exercise, with this suppression maintained until the *ad libitum* test meal consumed 60 minutes after exercise. Area-under-the-curve for GLP-1 concentration was higher in the exercise trial, compared with the resting trial. Despite these hormonal responses, there was no difference in *ad libitum* energy intake between the exercise and resting conditions.

These findings, allied with the limited existing literature (Deighton *et al.*, 2012), indicate that the suppression of acylated ghrelin could be greater after sprint, or maximal effort, interval cycle exercise. The enduring length of the suppression was longer in the study of Chapter 6 than in commonly seen after continuous aerobic exercise (Broom *et al.*, 2009; Broom *et al.*, 2007; King *et al.*, 2010; King *et al.*, 2011b; Shiiya *et al.*, 2011; Wasse *et al.*, 2012). Broom *et al.* (Broom *et al.*, 2009) however, did see a sustained suppression after resistance exercise. This data would suggest that the acylated ghrelin response to exercise may be intensity-dependent, with supramaximal exercise eliciting a more enduring

suppression than continuous aerobic exercise. Nevertheless, this did not result in a sustained appetite suppression or reduced food intake in any of these instances. Moreover, with no reduction in food intake and the low energy cost of such low-volume exercise, relative energy intake did not differ between the exercise trial and the resting trial. Deighton *et al.* (Deighton *et al.*, 2012) showed a trend for a greater relative energy intake after sprint interval exercise, compared with continuous aerobic exercise (60 minutes at 65% $\text{VO}_{2\text{max}}$), which was primarily due to the large difference in energy expended during the bouts (~142 kcal for sprint interval exercise vs. ~631 kcal for aerobic exercise). Further, the relative energy intake after exercise in the present study (513 ± 498 kcal) is considerably higher than the corresponding values of Chapter 4 (range of 24 kcal – 200 kcal for the three exercise conditions) and the 30 minute (285 ± 94 kcal) and 45 minute (114 ± 108 kcal) conditions of Chapter 5. This would suggest that, despite causing a transient suppression of appetite, sprint interval cycling exercise is not successful at reducing acute relative energy intake/energy balance. While suggesting such exercise is interesting from a mechanistic perspective, with regards to post-exercise appetite responses, it is questionable whether low-volume, low-energy cost sprint interval exercise is likely to prove an optimal form of exercise for weight-loss strategies. However, before this can be confirmed, longer duration studies, addressing the effect of sprint interval training on long-term appetite, energy balance and bodyweight are required.

8.5 Questioning the regulatory role of appetite-associated hormones on appetite and food intake in the post-exercise period

Throughout Chapters 4, 5 and 6, the effect of differing exercise trials on plasma concentration of appetite-associated hormones was investigated. The effects of exercise on these hormones have been discussed previously in the relevant chapters and collectively here, in sections 8.3 (p241) and 8.4 (p245). However, here I will discuss the relationship

between changes in hormone concentration and changes in both subjective appetite and energy intake.

For each of the Chapters 4, 5 and 6, the relationship between within-subject changes in hormone concentration and corresponding changes in appetite and energy intake were assessed with correlation analysis. This data is shown in **table 8.2**. For ease of presentation and continuity, only VAS appetite scores were used. Significant relationships for within-subject percentage changes in hormone concentration and percentage changes in both subjective appetite and energy intake were rare, with weak or weak-to-moderate strength correlation predominating (**table 8.2**). Only three significant relationships were observed, all of which are somewhat contradictory, occurring in the inverse direction to that which may have been anticipated; changes in the anorexigenic hormones PP and GLP-1 were positively correlated with changes in subjective appetite and energy intake respectively, while the orexigenic hormone acylated ghrelin was negatively correlated with subjective appetite.

Correlation	Chapter 4			Chapter 5			Chapter 6
	HIGH – LOW	HIGH – MOD	MOD – LOW	15 min – REST	30 min – REST	45 min – REST	EX – REST
Changes in %Δ VAS vs. changes in %Δ PP	0.365	-0.032	0.587*	-	-	-	-
Changes in %Δ VAS vs. changes in %Δ PYY	-0.201	-0.479	-0.072	-0.261	0.016	0.038	0.491
Changes in %Δ VAS vs. changes in %Δ GLP-1	-	-	-	-0.386	-0.384	0.041	0.039
Changes in %Δ VAS vs. Changes in %Δ acyl. ghrelin	-	-	-	-0.570*	0.030	0.253	0.286
Changes in EI vs. changes in PP	0.024	-0.036	0.120	-	-	-	-
Changes in EI vs. changes in PYY	0.208	0.146	0.306	-0.436	-0.518	-0.496	0.021
Changes in EI vs. changes in GLP-1	-	-	-	-0.508	-0.384	-0.284	0.688*
Changes in EI vs. changes in acyl. ghrelin	-	-	-	0.507	0.422	0.210	-0.181

Table 8.2 – Product moment correlation coefficients for within-subject correlations of percentage changes in appetite-associated hormones with percentage changes in VAS score and in energy intake. Data compiled for all analysis from Chapters 4, 5 and 6. * = significant correlation, $p < 0.05$.

Of the few studies that have previously conducted such research, changes in appetite-associated hormones have shown mixed associates with subjective appetite or *ad libitum* energy intake (see **table 1.2** in General introduction, p30). PYY has been shown to be a strong predictor of appetite post-exercise in runners, but not walkers (Larson-Meyer *et al.*, 2012). Although, within-subject changes in AUC for PYY were not associated with changes in energy intake in a different study (Ueda *et al.*, 2009a). Unfortunately the former study did not investigate the relationship between changes in hormone concentration and changes in an *ad libitum* energy intake and the latter did not correlate hormonal change with changes in subjective appetite. The findings of Chapters 4, 5 and 6 are in agreement with Ueda *et al.* (Ueda *et al.*, 2009a), suggesting that changes in PYY with exercise are not strongly associated with post-exercise energy intake. Further, our data would also suggest that PYY is not a key regulator of subjective appetite post-exercise, with no significant relationship seen between changes in PYY and changes in VAS scores.

The relationship between changes in acylated ghrelin and both appetite and energy intake has been addressed in a number of studies previously, with mixed findings. No study within the existing literature has shown a correlation between changes in acylated ghrelin and changes in energy intake. No such relationship was observed in the studies of this thesis either. The association between changes in acylated ghrelin and changes in subjective appetite is less clear. Previous studies have shown significant correlations (Broom *et al.*, 2007; Mackelvie *et al.*, 2007), but the lack of such a relationship has also been seen (Broom *et al.*, 2009; Larson-Meyer *et al.*, 2012; Wasse *et al.*, 2012). The compiled findings of the studies within this thesis are somewhat inconclusive, although they suggest that within-subject changes in acylated ghrelin are not strongly associated with changes in subjective appetite.

Changes in GLP-1 were not strongly associated with subjective appetite in highly-trained athletes or overweight and obese individuals. This is not in agreement with the findings of Larson-Meyer *et al.* (Larson-Meyer *et al.*, 2012), who showed that GLP-1 was a strong predictor of appetite in runners, but not walkers. In Chapter 5, no significant

relationships between changes in GLP-1 and energy intake were observed. Paradoxically, within-subject changes in GLP-1 exhibited a strong, positive relationship with energy intake, with a significant, moderate-strength correlation observed in the study of Chapter 6. This is the inverse of the relationship previously seen in the studies of Ueda *et al.* (Ueda *et al.*, 2009a), who showed a strong, negative correlation between change in GLP-1 AUC for the test period and change in energy intake for both exercise trials (high-intensity exercise and moderate-intensity exercise) compared with a resting trial. This latter finding is the more expected, due to the known anorexigenic action of GLP-1. The positive relationship observed in Chapter 6 is rather perplexing. It is possible that the contrasting findings are due to differences in the exercise bout completed, with the sprint interval cycling undertaken in Chapter 6 of this thesis being of a higher intensity than both the moderate- (50% $\text{VO}_{2\text{max}}$) and high-intensity (75% $\text{VO}_{2\text{max}}$) aerobic bouts of recumbent cycling undertaken in the study of Ueda *et al.* (2009a). However, all three trial conditions, across the two studies elicited similar GLP-1 responses to exercise. This suggests that intensity of the exercise was not an important regulator of the GLP-1 response, and while it is possible that the nature of the exercise may have some effect on the post-exercise relationship between GLP-1 concentration and appetite and eating behaviour, there is no clear rationale why this would be the case.

A perhaps more likely reason for the discrepancy is the differing populations studied. The study population of Chapter 6 was overweight and obese men and women, whereas the study of Ueda and colleagues (Ueda *et al.*, 2009a) was conducted in young, healthy-weight males. It is possible that different populations show a differing relationship between changes in appetite-associated hormones and both subjective appetite and *ad libitum* intake. Overweight and obese individuals may possess a blunting to GLP-1 post-exercise, which would explain the dissociation between changes in GLP-1 concentration and energy intake in Chapter 6. There is evidence that this cohort exhibit higher fasting concentrations of GLP-1 (Ranganath *et al.*, 1996), which can suggest a blunting, while the overweight and obese also demonstrate a blunted GLP-1 response to feeding (Lugari *et al.*, 2004; Ranganath *et*

al., 1996; Verdich *et al.*, 2001). Also, Larson-Meyer *et al.*, (2012) demonstrated that runners, but not walkers, exhibited a strong, negative relationship between changes in GLP-1 concentration and subjective appetite, post-exercise. Moreover, Martins and co-workers (Martins *et al.*, 201) showed that this GLP-1 response to feeding was sensitised by exercise training in overweight individuals. These data support the notion of a possible blunting to post-exercise alterations in GLP-1 concentration in the overweight and obese, and hence a dissociation between changes in GLP-1 and food intake.

From assessing the accumulated data from three studies of this thesis, it is not easy to draw firm conclusions regarding the relationship between within-subject changes in appetite-associated hormones and both subjective appetite and energy intake, and hence speculating about the regulatory role of these hormones post-exercise. It would appear that any changes in PYY are not strongly associated with changes in appetite or energy intake, and hence PYY is an unlikely key regulator of post-exercise appetite. A lack of a significant increase in PYY concentration post-exercise in any exercise trial (vs. baseline or resting trial), even when an appetite suppression was present substantiates this claim. A similar observation can be made for PP. The role of acylated ghrelin is less clear. The present data, allied with that of previous studies would suggest that acylated ghrelin does not play a key regulatory role in post-exercise food intake, due to a lack of a relationship between changes in these two measures. However, the relationship between changes in acylated ghrelin and changes in subjective appetite remains unclear. Further investigation, assessing this relationship in the post-exercise period may be warranted. The data from this thesis would suggest that GLP-1 does not play a key regulatory role in post-exercise appetite and food intake, although this does contradict existing literature to some degree. Further investigation is again required to elucidate this relationship.

It should also be acknowledged that assessing the influence or relationship of a single appetite-associated hormone on or with appetite and food intake is likely to prove a somewhat moot exercise. It is probable that changes in appetite and food intake are influenced by perturbations in the entire profile of appetite-associated hormones, rather than

being strongly influenced by alterations in just one hormone. This may explain the lack of strong relationships between any single hormone and measures of appetite and food intake in this thesis. Future research should attempt to address the exercise-induced changes in a range of appetite-associated hormones and, if possible, assess relationships between perturbations in the concentrations of these hormones and appetite measures using large-scale multiple regression models. However, it is acknowledged that for such analysis, a large sample is required and the economic cost of obtaining such data from a single study would be very high.

8.6 What does regulate post-exercise appetite and food intake?

Section 8.5 would indicate that peripheral endocrine signals are perhaps not key regulators of post-exercise appetite and may not be the primary cause of any exercise-induced suppression of appetite. If this is the case, it remains necessary to ask the question: what, then, does regulate post-exercise appetite and energy intake, and what is the mechanism, or mechanisms by which an appetite suppression transpires?

Peripheral endocrine signals are not the only physiological responses to exercise that have been postulated to effect appetite. One proposed explanation for exercise-induced appetite suppression is the redistribution of blood flow during exercise away from the gastrointestinal region, towards the muscles (Blundell *et al.*, 2003). Splanchnic blood flow has been shown to reduce considerably during moderate- to high-intensity exercise (Rowell *et al.*, 1964) and it is thought that this may limit the blood volume available to the gut for the circulation of the orexigenic hormone ghrelin (Burns *et al.*, 2007). However, evidence of increases in other gut-derived hormones with exercise, such as the satiety peptides would contradict this theory. In addition, if a redistribution of blood flow acts as a means by which the action of appetite-associated hormones is altered, then the findings of section 8.5 – that these hormones may not play an important regulatory role in post-exercise changes in appetite – again dispute the importance of reduced splanchnic blood flow.

An increase in body temperature during exercise is another possible mechanism by which appetite could be suppressed. Exercise in the heat has been shown to elicit a greater suppression of appetite than exercise conducted in a thermoneutral environment (Shorten *et al.*, 2009). Exercise in cold environments has been seen to stimulate appetite and food intake, compared with exercise at a neutral temperature (White *et al.*, 2005; Crabtree, D. PhD Thesis, University of Birmingham), while cold water immersion immediately post-exercise can stimulate post-exercise feeding (Halse *et al.*, 2011). Further, the anecdotal increase in hunger post-swimming, compared with other, land-based forms of exercise has been explained by a reduced increase in body temperature during swimming. However, King and colleagues (King *et al.*, 2011b) did observe a transient suppression in appetite during and immediately after prolonged, moderate-intensity swimming, with no difference in post-exercise *ad libitum* energy intake, compared with resting, in the hours following. The mechanism by which changes in body temperature may influence appetite has been linked to a greater redistribution of blood flow during exercise in hot environments, with an increase in flow to the skin in order to dissipate heat and hence further decrease splanchnic blood flow (Rowell *et al.*, 1971). In contrast, exercise in the cold results in a decrease in blood flow to the periphery and a consequent maintenance of flow to the gastrointestinal region. Following the aforementioned theory of blood flow availability for the circulation of ghrelin, this could explain the effect of temperature on appetite responses to exercise. However, increases in plasma acylated ghrelin after exercise in the heat was not correlated with increases in skin temperature (a marker of skin blood flow) (Crabtree, D. PhD Thesis, University of Birmingham). In addition, as the study of Shorten *et al.* (Shorten *et al.*, 2009) observed a decrease in ghrelin concentration with exercise at a higher temperature, one might also have expected to see a lower circulating concentration of PYY, as PYY is also secreted from the gastrointestinal tract. However, this was not the case, with PYY largely unaffected by exercise at different temperatures; in fact, PYY concentration was slightly increased after exercise in the heat, compared with exercise in a thermoneutral environment. This again questions the role of blood flow redistribution in the post-exercise appetite

response and would suggest that any effect of body temperature acts independent of changes in blood flow.

As discussed in section 1.4 (p21), peripheral endocrine signals act upon central appetite-governing regions of the brain (see **figure 1.1**, p23). It is plausible that any exercise-induced alterations in appetite are mediated here. Only recently has the effect of exercise on the central regulation of appetite been investigated. Primarily, this has been done by using functional magnetic resonance imaging (fMRI) techniques to assess blood flow to areas of the brain that are associated with the central reward system and the hedonic properties of food, such as the orbitofrontal cortex (Kringelbach, 2005), the insula (Simmons *et al.*, 2005) and the amygdale (Siep *et al.*, 2009) during the post-exercise period. For an in depth discussion of the roles of these brain areas in the reward sensation and hedonics of food and consequences for food intake and weight-status, please see these comprehensive reviews: (Berridge, 2009; Kenny, 2011). There is evidence that acute (Evero *et al.*, 2012) and chronic (Cornier *et al.*, 2012) exercise can reduce the neural responses in these areas to visual food cues, while a region of the brain associated with feelings of satiation and satiety, the dorsolateral prefrontal cortex, has also been shown to be responsive to exercise (Montenegro *et al.*, 2012). A bout of strenuous running downregulated activity within reward areas of the brain when participants were shown photographic images of food immediately post-exercise (Crabtree, D. PhD Thesis, University of Birmingham). This was coupled with a suppression of appetite, an increase in acylated ghrelin and a decrease in total PYY concentration. Furthermore, the activity of reward regions of the brain increased in the hours after exercise and this was matched by a rebound increase in subjective appetite. It is possible that appetite and food intake post-exercise are regulated via changes in sensitivity of reward areas of the brain to food cues.

Other central factors could also regulate post-exercise changes in appetite, such as changes in brain blood flow and hence delivery of peripheral signals to the arcuate nucleus, changes in receptor affinity for the signals or changes in the downstream signalling of hunger and/or satiation. These mechanisms have not yet been investigated. Also, as

different regions of the hypothalamus regulate both appetite and thermoregulation, any effect of change in core temperature on appetite may take place here, with “cross-talk” between appetite and thermoregulation command centres.

Changes in the hedonic perception of foods can be considered a cognitive response to exercise, resulting in autonomous changes to feeding behaviour in the post-exercise period. It is plausible that other behavioural responses, governed by volitional cognition play a key role in food intake, if not subjective appetite, after exercise (King *et al.*, 2007). As stated in Chapter 1, the thesis is not directly concerned with the cognitive influences of behavioural responses in food intake to exercise. However, as eating is governed entirely by behaviour (Blundell *et al.*, 2005), these factors cannot be overlooked and volitional changes to behaviour undoubtedly play a role in post-exercise food intake.

As touched upon in section 8.5 (p248), powerful volitional changes in behaviour can override less potent physiological stimuli. This may be particularly evident in specific populations, such as highly-trained athletes. Current dogma recommends immediate post-exercise feeding to facilitate recovery (Burke, 1997; Jentjens and Jeukendrup, 2003), maximise adaptations to training (Tipton and Wolfe, 2004) and maintain immune function (Gleeson *et al.*, 2001). An increased capacity for glucose uptake and hence glycogen resynthesis after exercise (Goodyear *et al.*, 1991; Ivy and Kuo, 1998) and an increased potential for protein synthesis in the post-exercise period when amino acid delivery is adequate (Tipton *et al.*, 2007) are both well established and publicised responses that encourage the athlete to consume food within close proximity to the cessation of exercise. So much so, that it is very plausible that food intake choices after exercise are governed entirely by a desire to take advantage of a metabolic “window” for feeding and to maximise recovery and adaptations to training, with physiological stimuli and any perceived levels of hunger or satiety overridden and ignored. It would perhaps be of interest to investigate this theory through qualitative research, gaining a greater understanding of what drives the post-exercise feeding of athletes.

It is likely that volitional behavioural choices influence post-exercise intake in populations other than athletes. The environment, or society, exerts a potent effect on eating behaviour. The high abundance of food, the extensive marketing of food (including post-exercise recovery drinks and foods) and high hedonic value of energy-dense foods favour feeding and over-consumption of food. This development of a society that promotes food consumption and hence a state of positive energy balance has been termed the obesogenic environment (Swinburn *et al.*, 1999). If such factors are so prevalent and powerful, then it is likely that they also play a role in regulating eating behaviour, and hence energy intake in the post-exercise period. The aforementioned desire of athletes to fuel post-exercise, by consuming extensively marketed recovery supplements, is an example of such environmental influences on post-exercise eating behaviour. Other examples in non-athletic populations could include consumption of similar items, targeted at recreational exercisers or food product sale (such as vending machines) in exercise venues, such as gyms and swimming pools.

A further example of a potential influence on volitional behavioural choices is the feeling of having “earned” food, particularly energy-dense “treats” after exercise. This may be particularly prevalent in restrained eaters, who’s eating behaviour is influenced by their perceived energy balance status (Herman and Mack, 1975). However, a poor understanding of the relative rate of energy expenditure during exercise and the rate of intake during feeding can lead to over-eating and energy surplus after exercise.

Chapter 7 investigated individuals’ ability to consciously match energy intake with the energy expenditure of isocaloric exercise at a moderate and high intensity. Contrary to what was hypothesised, participants demonstrated a strong ability to consciously match energy intake with exercise-induced energy expenditure, with an undervaluation of the energy cost of exercise, particularly that of a moderate intensity offset by an undervaluation of the energy content of food. This would suggest that over-eating post-exercise because an exerciser thought that they had expended more energy than they actually had, is unlikely. However, it is possible that participants altered their behaviour under experimental conditions, perhaps

over-compensating for an expected imbalance in their perception of energy cost of exercise and energy content of food. The participants in this study exhibited varying degrees of eating restraint, as measured using the Dutch Eating Behaviour Questionnaire (van Strien *et al.*, 1986). The data was analysed with the addition of eating restraint as a between-subject factor (high restraint (score > 2.2, n = 6) vs. low (n = 8) restraint) and strong matching ability was seen in both groups. It would be of interest to repeat this study in a larger population of restrained versus unrestrained eaters, as this ability to match may be an important regulator of post-exercise intake amongst those that are consciously attempting to achieve an energy deficit in order to lose weight, or to achieve energy balance to avoid weight gain, but doing so without access to quantitative measures of both energy cost and content of energy in food. While rather crude, this approach to weight-management is adopted by many individuals attempting to manage their bodyweight (Weiss *et al.*, 2006).

8.7 Summary of thesis findings

In summary, the findings of this thesis were as follows:

1. The novel tool for the measure of subjective appetite – The Visual Meal Creator – is a valid measure of subjective appetite and proved a strong predictor of food intake at rest. It remains to be confirmed whether this is the case after exercise interventions. Nonetheless, the VIMEC may be considered a useful tool for appetite research, either in addition or as an alternative to existing methods and techniques.
2. The exercise-induced suppression of appetite effect was observed in highly-trained athletes, although there appears a blunting to this response. This could be due to a reduction in physiological perturbations during exercise in athletes or ingrained post-exercise volitional eating behaviours.
3. The exercise-induced suppression of appetite appears independent of the duration or energy cost of exercise.

4. PP and PYY were largely unresponsive to exercise. Acylated ghrelin and GLP-1 concentrations were responsive to exercise; however, there appears a dissociation between changes in appetite-regulating hormones and both subjective appetite and food intake in the post-exercise period.
5. Sprint-interval cycle exercise, in the form of just 4 x 30 seconds maximal effort cycling, was sufficient to elicit a transient appetite suppression in overweight and obese individuals. While this suppression was more enduring than is often observed after continuous aerobic exercise, food intake was unaffected. In addition, relative energy intake, and hence acute energy balance was no different to that of a resting condition, which questions the appropriateness and value of low-volume, low-energy cost exercise for weight-management strategies.
6. Individuals demonstrated a strong ability to match energy intake with the energy cost of moderate- and high-intensity exercise. With moderate-intensity exercise, this appears to be due to a slight underestimate of the energy content of food compensating for and under-valuation of the energy cost of exercise. Further, the perception of the energy cost of exercise appears to be driven by the intensity, rather than the duration of exercise, which may have implications for weight-management interventions, especially when casual approaches to exercise and attempting to eat less are being implemented.

8.8 Future research

It would be fair to comment that this thesis has formulated more questions than it has answered. From the findings and observations, a number of possible future directions of research can be suggested:

1. It would be desirable to extend the validation of the VIMEC to a large scale intervention study, further investigating its strength as a predictor of food intake. Prior to this, it would perhaps be beneficial to increase the library of food items, including,

in particular, some more commonly-consumed breakfast items; the lack of such items is considered a current limitation when obtaining measures in the morning. It may also be wise to pursue some of the potential additional uses of the VIMEC.

2. It could prove informative to continue the systematic approach of Chapters 4 and 5, principally investigating the concept of the exercise-induced suppression of appetite effect differing in different populations. Direct comparisons of different population cohorts (e.g. sedentary vs. moderately-active vs. highly active/trained) within the same study would be an interesting next step. Investigating the effect of differing modes of exercise (cycling vs. running vs. swimming) on appetite responses, as was initially intended within this PhD programme, may also be of interest.
3. The regulatory role of appetite-associated hormones in the post-exercise period certainly requires further attention. It has perhaps been assumed that changes in these hormone concentrations are key regulators of any exercise-induced suppression of appetite, yet recent data, including data from this thesis questions this belief. If there is truly a dissociation between changes in appetite-associated hormone concentration and subjective appetite, it is certainly worth investigating why such a dissociation occurs after exercise. Due to individual variability in circulating concentrations of these hormones, as well as variability in response to exercise, a larger scale study would be warranted, with sufficient sample size and statistical power to conduct multiple regression analysis. This would be a more robust measure of the relationship between hormones and subjective appetite or food intake, assessing the degree to which changes in the former predict changes in the latter. However, due to the cost of measuring appetite-associated hormones, such an investigation would be very expensive to conduct.
4. As with any investigation of acute responses to exercise in appetite and food intake, only a small “snap shot” of energy balance is obtained. For an understanding of likely effects on body weight and composition, long-term interventions and monitoring are required. Therefore, any findings from acute studies, such as potentially prolonged

appetite suppression after supramaximal exercise, should be investigated further, using such exercise bouts in chronic exercise intervention studies and addressing not only changes in acute appetite and energy balance, but also in body weight and composition changes. Investigating the efficacy of differing forms of exercise, perhaps of differing intensity but matched for energy cost, at promoting compliance to exercise and changes in *ad libitum* food intake and body weight and composition would be worthwhile.

8.9 Thoughts, speculation and practical implications

I would like to conclude this chapter, and the thesis, by relaying some of my own personal thoughts and speculations regarding the appetite response to exercise and the potential role of such responses in weight-management strategies.

8.9.1 Can an exercise-induced suppression of appetite be used as a tool to promote acute energy deficit and assist weight-loss?

There is little evidence that an exercise-induced suppression of appetite can result in an acute reduction in food intake. It would appear that the response is lacking in potency and is too transient. However, a reduction in relative energy intake, or energy balance, can be observed acutely, if the energy cost of the exercise bout is sufficient. This is due to the absence of a compensatory response in appetite to the energy cost of exercise; appetite is rarely seen to be increased after high-intensity exercise, even after particularly energetic exercise bouts. This would suggest that the exercise-induced appetite suppression effect *per se* is not a useful tool that can be utilised and manipulated to assist with achieving an energy deficit as part of a weight-management strategy, although it may assist with the limitation of an energy surplus and weight gain.

However, before accepting this outright, it was worth considering a scenario where a short-lived, transient suppression of appetite may impact upon energy balance. Supposing an individual is following an energy restricted diet in order to lose weight. Major barriers to

such a diet include, unsurprisingly, hunger and snacking (Tu and Barchard, 1993). Therefore, it is likely that dieters will experience hunger between meals and become tempted to snack. Hence, the energy-restricted diet will be compromised. Imagine a scenario where this individual is due to consume a meal in around one hour, but they are now hungry and want to snack on a calorie-dense snack item, such as a cereal bar. If there was a means by which appetite could be suppressed, just until meal time, without consuming calories to do so, then this would be ideal. Could an exercise bout prove beneficial in this instance? Chapter 6 showed that just 4 x 30 seconds maximal effort bouts of cycling was sufficient to cause and appetite suppression that lasted at 30 minutes, with appetite scores still non-significantly lower than the resting trial at 60 minutes post-exercise. Interestingly, one participant was only able to complete two of the four sprints, yet their subjective appetite was still suppressed. It would be interesting to see if this is a robust finding. What's more, is even just a single 30 second maximal effort sprint sufficient to elicit this response? If so, such a short, time-efficient micro-bout of exercise may be able to act as a "snack replacement" in the scenario describe here. While such a bout would result in a very small energy cost, this can be added to the energy saved, if it prevents a snack from being consumed. This suggestion requires access to some mode of exercise, ideally a cycle ergometer and performing a micro-bout of exercise may not always be possible or convenient, depending on the time, location and situation, hence, the feasibility of this recommendation is highly questionable. Yet, it is a scenario in which an exercise-induced appetite suppression effect may result in a benefit to short-term energy balance.

8.9.2 Losing weight is difficult; the key for society is to prevent weight-gain.

Achieving clinically significant, sustained weight-loss is difficult. Through the work of my thesis I have seen that creating acute energy deficit with exercise is not easily achieved. By definition, clinically significant weight-loss is a loss of $\geq 5\%$ bodyweight, whereas weight maintenance is considered a bodyweight change of $\leq 3\%$. The American College of Sports Medicine Stand Point suggests that the weekly energy deficit required to promote weight-

loss is ~3500-7000 kcal week⁻¹ (Jakicic *et al.*, 2001), whereas successful weight maintenance requires a more modest deficit of 1200-2000 kcal week⁻¹ (Donnelly *et al.*, 2009). With fewer barriers to remaining weight stable than to losing weight, attempting to remain weight stable should be a more achievable goal. Prevention rather than cure should be the way forward.

8.9.3 Exercise for the avoidance of weight-gain.

In the investigation of this thesis, acute bouts of exercise failed to elicit a sustained suppression of appetite and in no trial condition was a mean negative energy balance for the trial period achieved. Given these observed relationships between exercise and subsequent appetite, food intake and acute energy balance, it could be argued that the likely key role of exercise alone, in the absence of dietary restriction, is in assisting with the avoidance of weight-gain. It has been demonstrated that an acute exercise bout can elicit a substantial, short-term shift of energy balance in favour of energy deficit compared with doing no exercise, as long as the energy expenditure of the exercise bout is considerable (>450 kcal, in the case of the findings of this thesis). While such a shift in energy balance (without the further influence of dietary restriction limiting energy intake) may be insufficient to promote weight-loss, when repeated regularly, it is likely to prevent sustained, prolonged periods of positive energy balance, leading to weight-gain. However, this cannot be substantiated from the acute studies of this thesis. Importantly, in keeping with the trends of previous research, in no instance did exercise increase post-exercise appetite and food intake. Therefore, energetic bouts of exercise should prove an effective method for increasing energy expenditure and shifting the energy balance in favour of deficit, without triggering an acute compensatory increase in energy intake. Thus, exercise can compliment an energy restriction diet to assist in creating a more substantial energy deficit to promote weight-loss. Transient suppressions of appetite were observed with high-intensity aerobic bouts of continuous exercise, and after low-volume sprint interval exercise, although this did not impact upon post-exercise food intake, just 60 minutes after exercise. It would appear that the overall energy cost of the exercise is a vital determinant of acute post-exercise energy

balance and therefore, the intensity should be such that it does not limit the longevity of the exercise bout due to the onset of fatigue and in doing so result in a low energy expenditure. Therefore, whether attempting to avoid weight-gain or facilitate weight-loss, the total energy cost of exercise is key.

8.10 References

- Artal, R., Catanzaro, R.B., Gavard, J.A., Mostello, D.J., and Friganza, J.C. (2007). A lifestyle intervention of weight-gain restriction: diet and exercise in obese women with gestational diabetes mellitus. *Applied Physiology, Nutrition, and Metabolism* 32, 596-601.
- Berridge, K.C. (2009). 'Liking' and 'wanting' food rewards: Brain substrates and roles in eating disorders. *Physiology & Behavior* 97, 537-550.
- Blundell, J.E., King, N.A., and Bryant, E. (2005). Interactions among physical activity, food choice and appetite control: health messages in physical activity and diet. In: Caero N. Norgan NG Ellison GTH. eds. *Childhood obesity* London: Taylor & Francis, 135-148.
- Blundell, J.E., Stubbs, R.J., Hughes, D.A., Whybrow, S., and King, N.A. (2003). Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proc Nutr Soc* 62, 651-661.
- Brindal, E., Wilson, C., Mohr, P., and Wittert, G. (2012). Perceptions of portion size and energy content: implications for strategies to affect behaviour change. *Public Health Nutrition* 15, 246-253.
- Broom, D.R., Batterham, R.L., King, J.A., and Stensel, D.J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 296, R29-R35.
- Broom, D.R., Stensel, D.J., Bishop, N.C., Burns, S.F., and Miyashita, M. (2007). Exercise-induced suppression of acylated ghrelin in humans. *J Appl Physiol* 102, 2165-2171.
- Burke, L.M. (1997). Nutrition for post-exercise recovery. *Australian journal of science and medicine in sport* 29, 3-10.
- Burns, S.F., Broom, D.R., Miyashita, M., Mundy, C., and Stensel, D.J. (2007). A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of Sports Sciences* 25, 635-642.
- Carels, R.A., Konrad, K., and Harper, J. (2007). Individual differences in food perceptions and calorie estimation: An examination of dieting status, weight, and gender. *Appetite* 49, 450-458.
- Cornier, M.-A., Melanson, E.L., Salzberg, A.K., Bechtell, J.L., and Tregellas, J.R. (2012). The effects of exercise on the neuronal response to food cues. *Physiology & Behavior* 105, 1028-1034.
- Crabtree, D.R. (2012). "The effects of exercise on appetite regulation." Ph.D thesis, University of Birmingham. <http://etheses.bham.ac.uk/3779/>
- Deighton, K., Barry, R., Connon, C., and Stensel, D. (2012). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*, 1-10.
- Donnelly, J.E., Blair, S.N., Jakicic, J.M., Manore, M.M., Rankin, J.W., Smith, B.K., and null (2009). American College of Sports Medicine Position Stand. Appropriate physical activity

intervention strategies for weight loss and prevention of weight regain for adults. *Medicine and science in sports and exercise* 41, 459-471.

Evero, N., Hackett, L.C., Clark, R.D., Phelan, S., and Hagobian, T.A. (2012). Aerobic exercise reduces neuronal responses in food reward brain regions. *Journal of Applied Physiology* 112, 1612-1619.

Farah, N.M.F., Brunstrom, J.M., and Gill, J.M.R. (2012). Using a novel computer-based approach to assess the acute effects of exercise on appetite-related measures. *Appetite* 58, 196-204.

Finucane, M.M., Stevens, G.A., Cowan, M.J., Danaei, G., Lin, J.K., Paciorek, C.J., Singh, G.M., Gutierrez, H.R., Lu, Y., Bahalim, A.N., *et al.* (2011). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *The Lancet* 377, 557-567.

Foster-Schubert, K.E., Alfano, C.M., Duggan, C.R., Xiao, L., Campbell, K.L., Kong, A., Bain, C.E., Wang, C.-Y., Blackburn, G.L., and McTiernan, A. (2012). Effect of diet and exercise, alone or combined, on weight and body composition in overweight-to-obese postmenopausal women. *Obesity* 20, 1628-1638.

Frobisher, C., and Maxwell, S.M. (2003). The estimation of food portion sizes: a comparison between using descriptions of portion sizes and a photographic food atlas by children and adults. *Journal of Human Nutrition and Dietetics* 16, 181-188.

Gleeson, M., Lancaster, G.I., and Bishop, N.C. (2001). Nutritional Strategies to Minimise Exercise-Induced Immunosuppression in Athletes. *Canadian Journal of Applied Physiology* 26, S23-S35.

Goodyear, L.J., Hirshman, M.F., and Horton, E.S. (1991). Exercise-induced translocation of skeletal muscle glucose transporters. *American Journal of Physiology - Endocrinology And Metabolism* 261, E795-E799.

Halse, R.E., Wallman, K.E., and Guelfi, K.J. (2011). Postexercise water immersion increases short-term food intake in trained men. *Medicine and Science in Sports & Exercise* 43, 632-638.

Health and Social Care Information Centre (2013). Health Survey for England. Statistics on Obesity, Physical Activity and Diet – England, 2013. <http://www.hscic.gov.uk/searchcatalogue?productid=11194&q=obesity&sort=Relevance&size=10&page=1#top>

Herman, C.P., and Mack, D. (1975). Restrained and unrestrained eating. *Journal of personality* 43, 647-660.

Higgins, J.A., LaSalle, A.L., Zhaoxing, P., Kasten, M.Y., Bing, K.N., Ridzon, S.E., and Witten, T.L. (2009). Validation of photographic food records in children: are pictures really worth a thousand words? *Eur J Clin Nutr* 63, 1025-1033.

Ivy, J.L., and Kuo, C.H. (1998). Regulation of GLUT4 protein and glycogen synthase during muscle glycogen synthesis after exercise. *Acta Physiologica Scandinavica* 162, 295-304.

Jakicic, J.M., Clark, K., Coleman, E., Donnelly, J.E., Foreyt, J., Melanson, E., Volek, J., Volpe, S.L., and null (2001). American College of Sports Medicine position stand. Appropriate intervention strategies for weight loss and prevention of weight regain for adults. *Medicine and science in sports and exercise* 33, 2145-2156.

Jentjens, R., and Jeukendrup, A. (2003). Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports Med* 33, 117-144.

Kenny, Paul J. (2011). Reward mechanisms in obesity: New insights and future directions. *Neuron* 69, 664-679.

Kerksick, C., Wismann-Bunn, J., Fogt, D., Thomas, A., Taylor, L., Campbell, B., Wilborn, C., Harvey, T., Roberts, M., La Bounty, P., *et al.* (2010). Changes in weight loss, body composition and cardiovascular disease risk after altering macronutrient distributions during a regular exercise program in obese women. *Nutr J* 9, 1-19.

King, J.A., Miyashita, M., Wasse, L.K., and Stensel, D.J. (2010). Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* 54, 492-498.

King, J.A., Wasse, L.K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R.L., and Stensel, D.J. (2011a). Differential acylated ghrelin, peptide YY₃₋₃₆, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology & Metabolism* 96, 1114-1121.

King, J.A., Wasse, L.K., and Stensel, D.J. (2011b). The acute effects of swimming on appetite, food intake, and plasma acylated ghrelin. *Journal of Obesity* 2011.

King, N.A., Burley, V.J., and Blundell, J.E. (1994). Exercise induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition* 48, 715-724

King, N.A., Caudwell, P., Hopkins, M., Byrne, N.M., Colley, R., Hills, A.P., Stubbs, J.R., and Blundell, J.E. (2007). Metabolic and behavioral compensatory responses to exercise interventions: barriers to weight loss[ast]. *Obesity* 15, 1373-1383.

King, N.A., Snell, L., Smith, R.D., and Blundell, J.E. (1996). Effects of short-term exercise on appetite responses in unrestrained females. *European Journal of Clinical Nutrition* 50, 663-667.

Kissileff, H.R., Pi-Sunyer, F.X., Segal, K., Meltzer, S., and Foelsch, P.A. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition* 52, 240-245.

Krall, E.A., and Dwyer, J.T. (1987). Validity of a food frequency questionnaire and a food diary in a short-term recall situation. *Journal of the American Dietetic Association* 87, 1374-1377.

Kringelbach, M.L. (2005). The human orbitofrontal cortex: linking reward to hedonic experience. *Nat Rev Neurosci* 6, 691-702.

Larson-Meyer, D.E., Palm, S., Bansal, A., Austin, K.J., Hart, A.M., and Alexander, B.M. (2012). Influence of running and walking on hormonal regulators of appetite in women. *journal of obesity* 2012, 15.

- Lugari, R., Dei Cas, A., Ugolotti, D., Barilli, A.L., Camellini, C., Ganzerla, G.C., Luciani, A., Salerni, B., Mittenperger, F., Nodari, S., Gnudi, A., and Zandomeneghi, R. (2004). Glucagon-like peptide 1 (GLP-1) secretion and plasma dipeptidyl peptidase IV (DPP-IV) activity in morbidly obese patients undergoing biliopancreatic diversion. *Hormone and Metabolism Research* 36, 111-115.
- Mackelvie, K.J., Meneilly, G.S., Elahi, D., Wong, A.C.K., Barr, S.I., and Chanoine, J.-P. (2007). Regulation of appetite in lean and obese adolescents after exercise: Role of acylated and desacyl ghrelin. *J Clin Endocrinol Metab* 92, 648-654.
- Martin, C.K., Correa, J.B., Han, H., Allen, H.R., Rood, J.C., Champagne, C.M., Gunturk, B.K., and Bray, G.A. (2012). Validity of the remote food photography method (RFPM) for estimating energy and nutrient intake in near real-time. *Obesity* 20, 891-899.
- Martins, C., Morgan, L.M., Bloom, S.R., and Robertson, M.D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 193, 251-258.
- Montenegro, R.A., Okano, A.H., Cunha, F.A., Gurgel, J.L., Fontes, E.B., and Farinatti, P.T.V. (2012). Prefrontal cortex transcranial direct current stimulation associated with aerobic exercise change aspects of appetite sensation in overweight adults. *Appetite* 58, 333-338.
- Muhlheim, L.S., Allison, D.B., Heshka, S., and Heymsfield, S.B. (1998). Do unsuccessful dieters intentionally underreport food intake? *International Journal of Eating Disorders* 24, 259-266.
- Pettigrew, S., Rosenberg, M., and Ferguson, R. (2013). Consumers' (in)ability to estimate the energy content of unhealthy foods. *Nutrition & Dietetics*, n/a-n/a.
- Ranganath, L.R., Beety, J.M., Morgan, L.M., Wright, J.W., Howland, R., and Marks, V. (1996). Attenuated GLP-1 secretion in the obese: cause or consequence? *Gut* 38, 916-919.
- Riley, W.T., Beasley, J., Sowell, A., and Behar, A. (2007). Effects of a web-based food portion training program on food portion estimation. *Journal of Nutrition Education and Behavior* 39, 70-76.
- Rowell, L.B., Blackmon, J.R., and Bruce, R.A. (1964). Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. *The Journal of clinical investigation* 43, 1677-1690.
- Rowell, L.B., Detry, J.R., Profant, G.R., and Wyss, C. (1971). Splanchnic vasoconstriction in hyperthermic man--role of falling blood pressure. *Journal of Applied Physiology* 31, 864-869.
- Shiia, T., Ueno, H., Toshinai, K., Kawagoe, T., Naito, S., Tobina, T., Nishida, Y., Shindo, M., Kangawa, K., Tanaka, H., *et al.* (2011). Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocrine journal* 58, 335-342.
- Shorten, A.L., Wallman, K.E., and Guelfi, K.J. (2009). Acute effect of environmental temperature during exercise on subsequent energy intake in active men. *The American Journal of Clinical Nutrition* 90, 1215-1221.
- Siep, N., Roefs, A., Roebroek, A., Havermans, R., Bonte, M.L., and Jansen, A. (2009). Hunger is the best spice: An fMRI study of the effects of attention, hunger and calorie content on food reward processing in the amygdala and orbitofrontal cortex. *Behavioural Brain Research* 198, 149-158.

Simmons, W.K., Martin, A., and Barsalou, L.W. (2005). Pictures of appetizing foods activate gustatory cortices for taste and reward. *Cerebral Cortex* 15, 1602-1608.

Skender, M.L., Goodrick, G.K., Del Junco, D.J., Reeves, R.S., Darnell, L., Gotto Jr, A.M., and Foreyt, J.P. (1996). Comparison of 2-year weight loss trends in behavioral treatments of obesity: diet, exercise, and combination interventions. *Journal of the American Dietetic Association* 96, 342-346.

Small, L., Sidora-Arcoleo, K., Vaughan, L., Creed-Capsel, J., Chung, K.-Y., and Stevens, C. (2009). Validity and reliability of photographic diet diaries for assessing dietary intake among young children. *ICAN: Infant, Child, & Adolescent Nutrition* 1, 27-36.

Subar, A.F., Crafts, J., Zimmerman, T.P., Wilson, M., Mittl, B., Islam, N.G., McNutt, S., Potischman, N., Buday, R., Hull, S.G., *et al.* (2010). Assessment of the accuracy of portion size reports using computer-based food photographs aids in the development of an automated self-administered 24-hour recall. *Journal of the American Dietetic Association* 110, 55-64.

Swinburn, B., Egger, G., and Raza, F. (1999). Dissecting obesogenic environments: The development and application of a framework for identifying and prioritizing environmental interventions for obesity. *Preventive Medicine* 29, 563-570.

Tipton, K.D., Elliott, T.A., Cree, M.G., Aarsland, A.A., Sanford, A.P., and Wolfe, R.R. (2007). Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *American Journal of Physiology - Endocrinology And Metabolism* 292, E71-E76.

Tipton, K.D., and Wolfe, R.R. (2004). Protein and amino acids for athletes. *Journal of Sports Sciences* 22, 65-79.

Tu, K.-S., and Barchard, K. (1993). An assessment of diabetes self-care barriers in older adults. *Journal of Community Health Nursing* 10, 113-118.

Turconi, G., Guarcello, M., Berzolari, F.G., Carolei, A., Bazzano, R., and Roggi, C. (2005). An evaluation of a colour food photography atlas as a tool for quantifying food portion size in epidemiological dietary surveys. *Eur J Clin Nutr* 59, 923-931.

Turner-McGrievy, G., and Tate, D. (2011). Tweets, Apps, and Pods: Results of the 6-month Mobile Pounds Off Digitally (Mobile POD) randomized weight-loss intervention among adults. *Journal of medical Internet research* 13, e120.

Turner-McGrievy, G.M., Beets, M.W., Moore, J.B., Kaczynski, A.T., Barr-Anderson, D.J., and Tate, D.F. (2013). Comparison of traditional versus mobile app self-monitoring of physical activity and dietary intake among overweight adults participating in an mHealth weight loss program. *Journal of the American Medical Informatics Association* 20, 513-518.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., and Fujimoto, S. (2009a). Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *Journal of Endocrinology* 203, 357-364.

Ueda, S.-y., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., and Fujimoto, S. (2009b). Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J Endocrinol*, JOE-08-0500.

van Strien, T., Frijters, J.E.R., Bergers, G.P.A., and Defares, P.B. (1986). The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders* 5, 295-315.

Venter, C.S., MacIntyre, U.E., and Vorster, H.H. (2000). The development and testing of a food portion photograph book for use in an African population. *Journal of Human Nutrition and Dietetics* 13, 205-218.

Verdich, C., Toubro, S., Buemann, B., Madsen, L.J., Holst, J.J., and Astrup, A. (2001). The role of postprandial release of insulin and incretin hormones in meal-induced satiety: effect of obesity and weight reduction. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity* 25, 1206-1214.

Wasse, L.K., Sunderland, C., King, J.A., Miyashita, M., and Stensel, D.J. (2012). The influence of vigorous running and cycling exercise on hunger perceptions and plasma acylated ghrelin concentrations in lean young men. *Applied Physiology, Nutrition, and Metabolism* 38, 1-6.

Weiss, E.C., Galuska, D.A., Khan, L.K., and Serdula, M.K. (2006). Weight-control practices among u.s. adults, 2001–2002. *American Journal of Preventive Medicine* 31, 18-24.

Westerterp-Plantenga, M.S., Verwegen, C.R., Ijeda, M.J., Wijckmans, N.E., and Saris, W.H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* 62, 1345-1354.

Westerterp, K.R., Saris, W.H., van Es, M., and ten Hoor, F. (1986). Use of the doubly labeled water technique in humans during heavy sustained exercise. *Journal of Applied Physiology* 61, 2162-2167.

White, L.J., Dressendorfer, R.H., Holland, E., McCoy, S.C., and Ferguson, M.A. (2005). Increased caloric intake soon after exercise in cold water. *International Journal of Sports Nutrition and Exercise Metabolism* 15, 38-47.

Appendix 1 – Written consent form, Chapter 2



Consent Form



A novel tool for the measure of subjective appetite: a validation.

Research Centre: Exercise Metabolism Laboratory at the School of Sport and Exercise Sciences Researchers: Prof. Asker Jeukendrup, Mr Adrian Holliday

Participant ID Number:

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.

☐

2. I understand that my participation is voluntary and that I am free to withdraw up to 3 months after my testing period has finished.

☐

3. I understand that my data and personal information will be saved on the work computer of the lead researcher and hard copies will stored in a locked filing cabinet, accessible only by the lead researchers and will be kept for a period of 10 years, unless I ask to have this information deleted.

☐

4. I agree to take part in the above study.

☐

Name of Participant

Signature

Date

Researcher

Signature

Date

Appendix 2 – General Health Questionnaire

The University of Birmingham School of Sport and Exercise Sciences General Health Questionnaire

Name:

Address:

.....

.....

Phone:

Name of the responsible investigator for the study:

.....

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1.	You are.....	Male	Female
2.	What is your exact date of birth? Day..... Month.....Year..... So your age is..... Years		
3.	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		

4.	Are you currently taking any medication?	YES	NO
5.	Has your doctor ever advised you not to take vigorous exercise?	YES	NO
6.	Has your doctor ever said you have “heart trouble”?	YES	NO
7.	Has your doctor ever said you have high blood pressure?	YES	NO
8.	Have you ever taken medication for blood pressure or your heart?	YES	NO
9.	Do you feel pain in your chest when you undertake physical activity?	YES	NO
10.	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
11.	Has your doctor (or anyone else) said that you have a raised blood cholesterol?	YES	NO
12.	Have you had a cold or feverish illness in the last month?	YES	NO
13.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14.	a) Do you suffer from back pain b) if so, does it ever prevent you from exercising?	YES YES	NO NO
15.	Do you suffer from asthma?	YES	NO
16.	Do you have any joint or bone problems which may be made worse by exercise?	YES	NO
17.	Has your doctor ever said you have diabetes?	YES	NO
18.			

	Have you ever had viral hepatitis?	YES	NO
19.	If you are female, to your knowledge, are you pregnant?	YES	NO
20.	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
21.	Are you accustomed to vigorous exercise (an hour or so a week)?	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signed:

Date:

Appendix 3 – Food items, with nutritional information, of the Birmingham Photographic Appetite Rating

Food Item	Energy Density (kcal•100g⁻¹)	Carbohydrate (grams)	Fat (grams)	Protein (grams)
Banana	94.8	23.2	0.3	1.2
Scrambled egg	146.0	0	1.8	12.5
Rice (plain)	141.9	30.6	1	2.7
Jelly beans	382.6	93.5	0.3	0.1
Chocolate raisins	399.1	58.1	16.4	4.5
Cous Cous (roast veg. flavour)	139.5	25.5	1.5	5.6
Tortilla chips	366.9	36.5	21.2	7.1
Popcorn (sweet)	450.0	57.5	21.8	5.9
Noodles	161.2	28.3	2.4	6.2
Bacon	339.6	0	20.5	14.4
Rice (biryani)	184.9	32.4	4	4.4
Tomato soup	39.4	5.5	1.7	0.5
Porridge oats	359.0	60	8	11
Chocolate buttons	528.6	55.6	31.5	6
Cous Cous (plain)	112.0	23	0.2	3.8
Chicken breast pieces (BBQ flavour)	140.4	5.7	1.9	24.8
Strawberry yoghurt	89.3	15.5	1	4.3
Potato wedges	806	27	8	3
Mixed vegetables	192.5	8.9	1.1	3.4
Fries	288.0	38	14	3

Appendix 4 – The Dutch Eating Behaviour Questionnaire

Please answer ALL questions. Circle the appropriate response.

When you have put on weight do you eat less than you usually do?	not relevant	never	seldom	sometimes	often	very often
Do you try to eat less at mealtimes than you would like to eat?		never	seldom	sometimes	often	very often
How often do you refuse food or drink offered to you because you are concerned about your weight?		never	seldom	sometimes	often	very often
Do you watch exactly what you eat?		never	seldom	sometimes	often	very often
Do you deliberately eat foods that are slimming?		never	seldom	sometimes	often	very often
When you have eaten too much, do you eat less than usual the following day?	not relevant	never	seldom	sometimes	often	very often
Do you deliberately eat less in order not to become heavier?		never	seldom	sometimes	often	very often
How often do you try not to eat between meals because you are watching your weight?		never	seldom	sometimes	often	very often
How often in the evenings do you try not to eat because you are watching your weight?		never	seldom	sometimes	often	very often
Do you take your weight into account with what you eat?		never	seldom	sometimes	often	very often

If food tastes good to you, do you eat more than usual?		never	seldom	sometimes	often	very often
If food smells good, do you eat more than usual?		never	seldom	sometimes	often	very often
If you smell something delicious, do you have a desire to eat it?		never	seldom	sometimes	often	very often
If you have something delicious to eat, do you eat it straight away?		never	seldom	sometimes	often	very often
If you walk past a baker, do you have a desire to buy something delicious?		never	seldom	sometimes	often	very often
If you walk past a snackbar or café, do you have a desire to buy something delicious?		never	seldom	sometimes	often	very often
If you see others eating, do you also have a desire to eat?		never	seldom	sometimes	often	very often
Can you resist eating delicious		never	seldom	sometimes	often	very

foods?	often				
Do you eat more than usual, when you see others eating?	never	seldom	sometimes	often	very often
When preparing a meal, are you inclined to eat something?	never	seldom	sometimes	often	very often

Do you have a desire to eat when you are irritated?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you have nothing to do?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you are depressed or discouraged?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you are feeling lonely?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you somebody lets you down?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you are cross?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you are something unpleasant is about to happen?		never	seldom	sometimes	often	very often
Do you get the desire to eat when you are anxious, worried or tense?		never	seldom	sometimes	often	very often
Do you have a desire to eat when things are going against you and when things have gone wrong?		never	seldom	sometimes	often	very often
Do you have a desire to eat when you are frightened?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you are disappointed?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you are emotionally upset?	not relevant	never	seldom	sometimes	often	very often
Do you have a desire to eat when you are bored or restless?	not relevant	never	seldom	sometimes	often	very often

Appendix 5 – Food and drink items, with nutritional information, of the *ad libitum* breakfast meal for Chapter 5

Food	Energy density (kcal•100g⁻¹)	Carbohydrate (grams•100g⁻¹)	Fat (grams•100g⁻¹)	Protein (grams•100g⁻¹)
Cornflakes (Kellogg's)	372	84.0	0.9	7.0
Milk (semi-skimmed)	49	5.0	1.7	3.4
Sugar	400	100	0	0
Bread	225	41.2	2.3	9.9
Margarine	354	2.8	38	0.1
Jam	253	63.3	0.7	0.7
Apple	49	11.6	0.1	0.4
Banana	95	20.9	0.3	1.2
Orange	37	8.5	0.1	1.1
Orange juice	42	9.1	0.1	0.5
Apple juice	44	10.4	0.1	0.1

Appendix 6 – Food and drink items, with nutritional information, of the buffet meal for Chapter 6

Food item	Energy density (kcal•100g⁻¹)	Carbohydrate (grams•100g⁻¹)	Fat (grams•100g⁻¹)	Protein (grams•100g⁻¹)
Mixed leaf salad	19	1.5	0.5	2.2
Savoury rice	122	25.4	1	2.9
Strawberry yoghurt	80	12.6	1	5.3
Apple	49	11.6	0.1	0.4
Banana	95	20.9	0.3	1.2
Chocolate biscuit	520	62.4	27.7	5.2
Cookies	508	67.0	23.9	6.2
Bread	253	63.3	0.7	0.7
Chicken breast	148	0.1	2.2	32
Cheese (red Leicester)	399	0.1	23.8	33.7
Ham	118	0.9	2.8	22.3
Mini sausage roll	422	26.7	31.1	8.7
Mini blueberry muffins	293	65.2	8.1	6.3
Pasta	357	73.1	1.7	12.3
Pasta sauce	105	22.4	0.2	3.5
Cereal bar	391	72.8	8.8	5.1
Strawberry jam	253	63.3	0.7	0.7
Salad dressing (balsamic)	316	13.8	28.8	0.4
Salad dressing (honey and mustard)	366	15.4	33	1
Crisps (ready salted)	538	47.4	36.8	4.3
Jelly beans	365	90.3	0.4	0.1
Margarine	354	2.8	38	0.1
Mayonnaise	298	6.5	29.8	0.7
Orange juice	42	9.1	0.1	0.5
Apple juice	44	10.4	0.1	0.1
Apple and blackcurrant squash	2	0.2	0.1	0.1
Pepsi	44	11.1	0	0

Appendix 7 – Food items, with nutritional information, of the Visual Meal Creator

Food Item	Energy Density (kcal•100g ⁻¹)	Carbohydrate (grams)	Fat (grams)	Protein (grams)
Pasta and sauce	142	38.2	1.0	4.9
Rice	351	77.6	1.0	7.1
Baked potato	138	28.8	0.2	3.8
Sausage	254	16.0	15.9	11.7
Pork steak	226	0.6	16.1	19.5
Salmon	197	<0.5g	11	24
Baked beans	81	13.9	0.5	5.2
Mixed vegetables	193	8.9	1.1	3.4
Fries	288	38	14	3
Chicken breast pieces (BBQ flavour)	140	5.7	1.9	24.8
Steak pie	327	27.9	18.5	11.5
Cous Cous (roast veg. flavour)	144	27.4	1.2	5.3
Leaf salad	19	1.4	0.5	2.2
Cheese and pickle sandwiches	243	29.3	9.3	10.2
Ham Sandwiches	209	31.4	3.9	11.3
Margarita pizza	266	30.3	9.9	12.7
Chicken curry	170	20.6	4.7	11.0
Crisps	481	58.4	23.4	6.8
Sweets	344	83.2	0.1	2.5
Blueberry muffins	408	51.4	20.0	5.4
Cookies	496	64.3	23.9	5.9
Cranberries	325	77.5	0.3	0.1
Banana	94.8	23.2	0.3	1.2
Chocolate buttons	513	63.0	26.5	5.4

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for

Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No →

Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include travelling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐ No vigorous job-related physical activity



Skip to question 4

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐

No moderate job-related physical activity



Skip to question 6

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐

No job-related walking



Skip to PART 2: TRANSPORTATION

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐

No travelling in a motor vehicle



Skip to question 10

9. How much time did you usually spend on one of those days **travelling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐

No bicycling from place to place



Skip to question 12

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐

No walking from place to place



***Skip to PART 3:
HOUSEWORK, HOUSE
MAINTENANCE, AND
CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐

No vigorous activity in garden or yard



Skip to question 16

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐

No moderate activity in garden or yard



Skip to question 18

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐

No moderate activity inside home



***Skip to PART 4:
RECREATION, SPORT AND
LEISURE-TIME PHYSICAL
ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐

No walking in leisure time



Skip to question 22

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐

No vigorous activity in leisure time



Skip to question 24

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐

No moderate activity in leisure time



Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

Appendix 9 – Food and drink items, with nutritional information, of the buffet meal for Chapter 7

Food item	Energy density (kcal•100g⁻¹)	Carbohydrate (grams•100g⁻¹)	Fat (grams•100g⁻¹)	Protein (grams•100g⁻¹)
Mixed leaf salad	19	1.5	0.5	2.2
Savoury rice	122	25.4	1	2.9
Strawberry yoghurt	80	12.6	1	5.3
Apple	49	11.6	0.1	0.4
Banana	95	20.9	0.3	1.2
Chocolate biscuit	520	62.4	27.7	5.2
Cookies	508	67.0	23.9	6.2
Bread	253	63.3	0.7	0.7
Chicken breast	148	0.1	2.2	32
Cheese (red Leicester)	399	0.1	23.8	33.7
Ham	118	0.9	2.8	22.3
Mini sausage roll	422	26.7	31.1	8.7
Mini blueberry muffins	293	65.2	8.1	6.3
Boiled potatoes	75	17.8	0.3	1.5
Pasta	357	73.1	1.7	12.3
Pasta sauce	105	22.4	0.2	3.5
Tuna	113	0.1	0.5	27
Cereal bar	391	72.8	8.8	5.1
Strawberry jam	253	63.3	0.7	0.7
Salad dressing (balsamic)	316	13.8	28.8	0.4
Salad dressing (honey and mustard)	366	15.4	33	1
Crisps)	538	47.4	36.8	4.3
Jelly beans	365	90.3	0.4	0.1
Margarine	354	2.8	38	0.1
Mayonnaise	298	6.5	29.8	0.7
Orange juice	42	9.1	0.1	0.5
Apple juice	44	10.4	0.1	0.1
Apple and blackcurrant squash	2	0.2	0.1	0.1
Pepsi	44	11.1	0	0