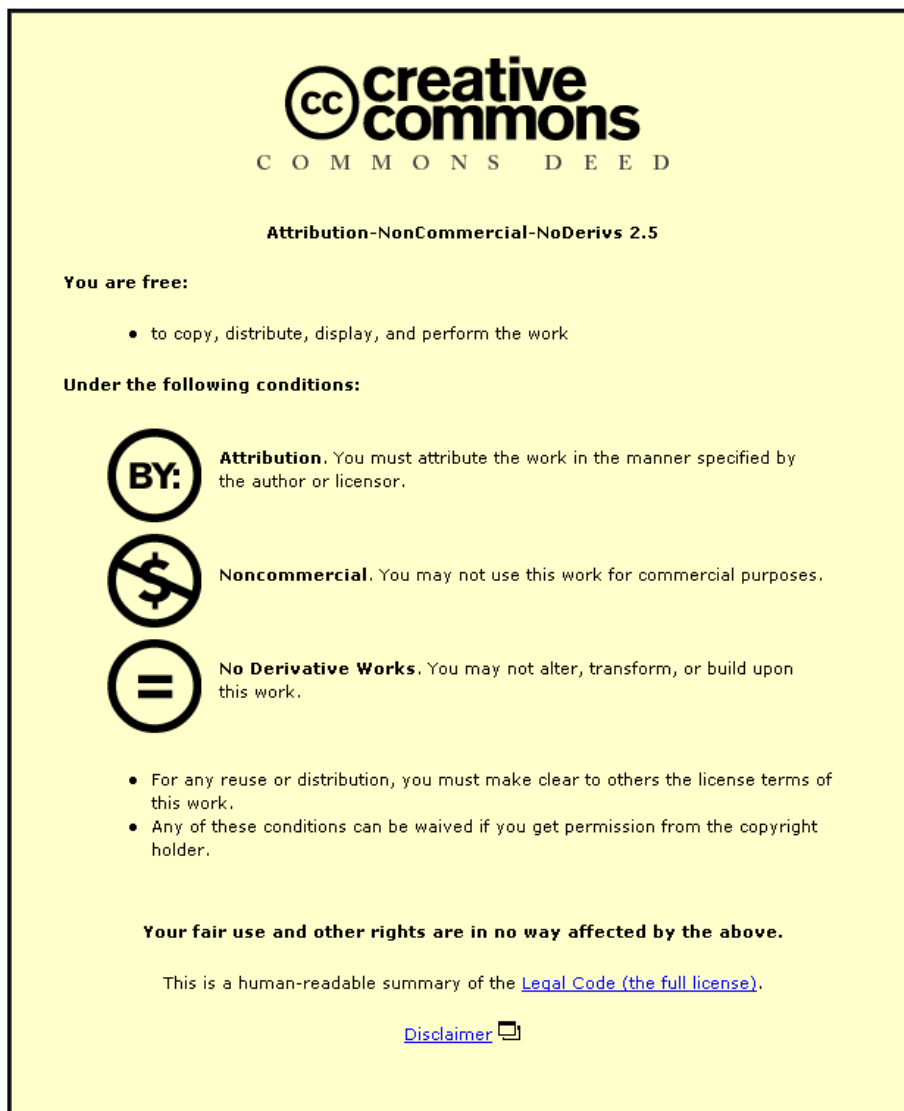




This item was submitted to Loughborough University as a PhD thesis by the author and is made available in the Institutional Repository (<https://dspace.lboro.ac.uk/>) under the following Creative Commons Licence conditions.



**CC creative commons**  
COMMONS DEED

**Attribution-NonCommercial-NoDerivs 2.5**

**You are free:**

- to copy, distribute, display, and perform the work

**Under the following conditions:**

**BY:** **Attribution.** You must attribute the work in the manner specified by the author or licensor.


**Noncommercial.** You may not use this work for commercial purposes.

**No Derivative Works.** You may not alter, transform, or build upon this work.

- For any reuse or distribution, you must make clear to others the license terms of this work.
- Any of these conditions can be waived if you get permission from the copyright holder.

**Your fair use and other rights are in no way affected by the above.**

This is a human-readable summary of the [Legal Code \(the full license\)](#).

[Disclaimer](#) 

For the full text of this licence, please go to:  
<http://creativecommons.org/licenses/by-nc-nd/2.5/>

**ACUTE EFFECTS OF EXERCISE ON APPETITE, FOOD INTAKE AND  
CIRCULATING CONCENTRATIONS OF GASTROINTESTINAL  
HORMONES**

by

**Kevin Deighton**

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of  
Philosophy of Loughborough University

August 2013

## ABSTRACT

Recent years have witnessed significant research into the acute effects of exercise on appetite, energy intake and gut hormone responses. The experiments in this thesis have further investigated this topic by examining the appetite, acylated ghrelin, peptide YY and energy intake responses to energy deficits induced via different exercise protocols and food restriction. To achieve this, 48 young healthy males (mean (SD): age 23 (3) years, body mass index 23.7 (2.7) kg.m<sup>-2</sup>, maximum oxygen uptake 52.9 (9.8) mL.kg<sup>-1</sup>.min<sup>-1</sup>) were recruited into four studies.

In study one, 60 min of treadmill running at 70% of VO<sub>2</sub> max did not stimulate any increases in appetite or daily energy intake regardless of whether the exercise was performed after breakfast or in the fasted state. In study two, six 30 s Wingate tests stimulated increases in appetite during the subsequent hours compared with 60 min of cycling at 68% of VO<sub>2</sub> max. Differences in appetite appeared to be unrelated to changes in plasma acylated ghrelin concentrations and did not influence ad libitum energy intake. Subsequently, endurance exercise resulted in a significantly greater negative daily energy balance than sprint exercise due to a larger exercise energy expenditure. Study three revealed that appetite and energy intake did not differ from a resting control trial after either ten, 4 min cycling bouts at 85–90% of VO<sub>2</sub> max separated by 2 min of rest or 60 min of constant cycling at 60% of VO<sub>2</sub> max. This occurred despite elevated PYY<sub>3-36</sub> concentrations during the hours after exercise. Finally, study four showed that an energy deficit of ~1475 kJ stimulated increases in appetite when induced via food restriction but not when achieved by an acute bout of exercise. This was associated with differences in plasma PYY<sub>3-36</sub> concentrations but did not appear to be related to changes in circulating levels of acylated ghrelin and did not influence energy intake.

This thesis has shown that appetite perceptions do not differ from a resting control trial during the hours after continuous endurance exercise. Alternatively, supramaximal cycling exercise and subtle reductions in food intake stimulated increases in appetite during the subsequent hours. Such increases in appetite do not appear to be related to changes in acylated ghrelin but may be influenced by plasma PYY<sub>3-36</sub> concentrations. Despite differences in appetite, daily energy intake was unaffected by all interventions.

**Key words:** exercise, appetite, energy intake, energy balance, compensation, acylated ghrelin, peptide YY, energy expenditure, high intensity intermittent exercise, hormones

## ACKNOWLEDGEMENTS

Firstly, I would like to thank Dr David Stensel for providing me with the opportunity to undertake this PhD and for the support and encouragement that he has provided throughout the duration of my studies. David's calming influence and friendly nature has provided a great working environment and I truly value the friendship that we have developed during the last few years. I am looking forward to remaining in contact with David in the future, whether this is for research collaborations or simply to 'catch up' over a cup of herbal tea.

I also thank Dr Rachel Batterham and her colleagues at University College London for their continued collaboration and for performing the PYY<sub>3-36</sub> analysis for the experiments contained within this thesis. Additionally, I am grateful to Professor Myra Nimmo and Dr Jonathan Folland for their encouragement and critique at my first and second year panel meetings, respectively.

Thank you to all of the volunteers that participated in my research. Without the sacrifice and commitment of these individuals this work would not have been possible and I am extremely grateful for their help. I am also grateful to the students that have assisted with the experimental testing during my PhD: Jessica Zahra, Ruth Barry, Charlotte Connon, Hannah Bateson, Rachel Malcolm, Jessica Douglas and Harriet Pryke.

My family and friends have been invaluable, not only during the completion of this thesis but also during the preceding 22 years. I am eternally grateful to my parents for providing me with every opportunity to fulfil my potential and for their constant love and support. I also thank my friends for maintaining my sanity during recent years and providing countless good times. Finally, thank you Desi for your love, support and constant belief in me.

## PREFACE

The findings presented in this thesis have been peer reviewed as follows:

### **Published Articles**

Deighton K, Zahra JC and Stensel DJ (2012). Appetite, energy intake and resting metabolic responses to 60 min treadmill running performed in a fasted versus a postprandial state. *Appetite* 58: 946-954.

Deighton K, Barry R, Cannon CE and Stensel DJ (2013). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *European Journal of Applied Physiology* 113: 1147-1156.

Deighton K, Karra E, Batterham RL and Stensel DJ (2013). Appetite, energy intake and PYY<sub>3-36</sub> responses to energy-matched continuous exercise and submaximal high intensity exercise. *Applied Physiology, Nutrition, and Metabolism* 38: 947-952.

Deighton K and Stensel DJ (In Press). Creating an acute energy deficit without stimulating increases in appetite: is there an optimal exercise protocol? *Proceedings of the Nutrition Society*.

### **Conference Presentations**

Deighton K, Zahra JC and Stensel DJ. The influence of running in a fed versus a fasted state on appetite, energy intake and resting energy expenditure in men (oral presentation). *BASES Annual Conference 2011*, University of Essex. Published in: *Journal of Sports Sciences* (2011), 29 (S2): S31.

Deighton K, Barry R, Cannon CE and Stensel DJ. Appetite, acylated ghrelin and 24 hour energy intake responses to low volume sprint interval exercise versus prolonged endurance exercise (oral presentation). *Nutrition Society Winter Meeting 2011*, Royal College of Physicians, London. Published in: *Proceeding of the Nutrition Society* (2011), 70: E350.

Deighton K, Douglas J, Pryke H and Stensel DJ. Modest food restriction stimulates increases in appetite that do not occur in response to an equivalent exercise-induced

energy deficit (oral presentation). *Annual Congress of the European College of Sport Science 2013*, Barcelona. Published in: *European Journal of Sport Science* (In Press).

Deighton K and Stensel DJ. Creating an acute energy deficit without stimulating increases in appetite: is there an optimal exercise protocol? *Nutrition Society Summer Meeting 2013*, Newcastle University (oral presentation).

Deighton K, Karra E, Batterham RL and Stensel DJ. Appetite, energy intake and PYY<sub>3-36</sub> responses to energy-matched continuous exercise and submaximal high intensity exercise (oral presentation). *BASES Annual Conference 2013*, University of Central Lancashire. Published in: *Journal of Sports Sciences* (In Press)

### **Awards**

Routledge Recently Qualified Researcher Oral Presentation Award, BASES Annual Conference 2011.

Nutrition Society Postgraduate Competition Winner 2013.

## TABLE OF CONTENTS

	<b>Page</b>
<b>Abstract</b>	<b>i</b>
<b>Acknowledgements</b>	<b>ii</b>
<b>Preface</b>	<b>iii</b>
<b>Table of contents</b>	<b>v</b>
<b>List of tables</b>	<b>xi</b>
<b>List of figures</b>	<b>xii</b>
<b>List of abbreviations</b>	<b>xiv</b>
<b>Chapter I</b>	
<b>Introduction</b>	<b>1</b>
<b>Chapter II</b>	
<b>Literature review</b>	
2.1 Introduction	<b>4</b>
2.2 Physiological regulation of appetite and energy intake	<b>4</b>
2.2.1 Central regulation of appetite and energy intake	<b>4</b>
2.2.2 Peripheral regulation of appetite and energy intake	<b>6</b>
2.2.2.1 Tonic signals	<b>8</b>
2.2.2.2 Episodic signals	<b>9</b>
2.3 Ghrelin	<b>12</b>
2.3.1 The discovery of ghrelin	<b>12</b>
2.3.2 Structure, production and secretion	<b>12</b>
2.3.3 Physiological functions	<b>14</b>
2.3.4 Acute effects of ghrelin on appetite and energy intake	<b>14</b>
2.3.5 Ghrelin and chronic energy homeostasis	<b>16</b>
2.4 Peptide YY	<b>18</b>
2.4.1 The discovery of PYY	<b>18</b>
2.4.2 Structure, production and secretion	<b>18</b>
2.4.3 Physiological functions	<b>19</b>
2.4.4 Acute effects of PYY on appetite and energy intake	<b>19</b>
2.4.5 Peptide YY and chronic energy homeostasis	<b>22</b>
2.5 The acute effects of exercise on appetite and food intake	<b>24</b>

2.5.1 Appetite and energy intake assessment	24
2.5.2 Relevance	25
2.5.3 Exercise-induced anorexia	26
2.5.4 Appetite responses in the immediate post-exercise period (0 – 120 min)	26
2.5.5 Energy intake responses in the immediate post-exercise period (0 – 120 min)	28
2.5.6 Appetite responses beyond a single test meal (2 – 9 h)	30
2.5.7 Energy intake responses beyond a single test meal (2 – 9 h)	31
2.5.8 Macronutrient intake	32
2.5.9 Manipulating exercise protocols to produce the most beneficial responses	32
2.6 The acute effects of exercise on ghrelin and PYY	33
2.6.1 Relevance	33
2.6.2 Ghrelin	34
2.6.3 Peptide YY	37
2.7 Summary	40

## **Chapter III**

### **General methods**

3.1 Participants	41
3.2 Anthropometry	42
3.3 Heart rate measurement	43
3.4 Ratings of perceived exertion	43
3.5 Preliminary exercise tests	43
3.5.1 Preliminary running tests	43
3.5.2 Preliminary cycling test	44
3.6 Criteria for maximum oxygen uptake	45
3.7 Expired gas analysis	45
3.8 Calculation of energy expenditure	45
3.9 Physical activity and dietary control	46
3.10 Assessment of Appetite	46
3.11 Standardised test meals	47
3.12 Ad libitum buffet meals	48



3.13 Ad libitum pasta meal	49
3.14 Power	50
3.15 Environmental temperature and humidity	50
3.16 Blood sample collection	51
3.17 Blood sample analysis	52
3.17.1 Estimation of changes in plasma volume	52
3.17.2 Acylated ghrelin	52
3.17.3 Total PYY	53
3.17.4 PYY <sub>3-36</sub>	53
3.17.5 Precision of analysis	53
3.18 Statistical analysis	53

## **Chapter IV**

### **Appetite, energy intake and resting metabolic responses to 60 min treadmill running performed in a fasted versus a postprandial state**

4.1 Introduction	55
4.2 Methods	58
4.2.1 Participants	58
4.2.2 Preliminary trial	58
4.2.3 Experimental protocol	58
4.2.4 Standardised breakfast and ad libitum food intake	59
4.2.5 Statistical analysis	59
4.3 Results	61
4.3.1 Exercise responses	61
4.3.2 Baseline parameters	61
4.3.3 Appetite	63
4.3.4 Energy and macronutrient intake	64
4.3.5 Energy expenditure and substrate utilisation	67
4.3.6 Relative energy intake	69
4.4 Discussion	70

## **Chapter V**

### **Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise**

5.1 Introduction	74
5.2 Methods	76
5.2.1 Participants	76
5.2.2 Preliminary trial	76
5.2.3 Experimental protocol	76
5.2.4 Endurance exercise session	79
5.2.5 Sprint interval exercise session	79
5.2.6 Net energy expenditure of exercise	79
5.2.7 Standardised breakfast, ad libitum buffet meals and overnight food bag	80
5.2.8 Blood sampling	80
5.2.9 Biochemical analysis	80
5.2.10 Statistical analysis	80
5.3 Results	81
5.3.1 Exercise responses	81
5.3.2 Appetite	81
5.3.3 Energy and macronutrient intake	83
5.3.4 Relative energy intake	85
5.3.5 Plasma acylated ghrelin and total PYY concentrations	85
5.3.6 Resting energy expenditure and substrate utilisation	89
5.3.7 Correlations	90
5.4 Discussion	91

## **Chapter VI**

### **Appetite, energy intake and PYY<sub>3-36</sub> responses to energy-matched continuous exercise and submaximal high intensity exercise.**

6.1 Introduction	95
6.2 Methods	97
6.2.1 Participants	97
6.2.2 Preliminary trial	97
6.2.3 Experimental protocol	97
6.2.4 Steady state exercise session	98

6.2.5 High intensity intermittent exercise session	98
6.2.6 Net energy expenditure of exercise	98
6.2.7 Standardised test meals and ad libitum meal	98
6.2.8 Blood sampling	99
6.2.9 Biochemical analysis	99
6.2.10 Statistical analysis	99
6.3 Results	101
6.3.1 Exercise responses	101
6.3.2 Appetite	101
6.3.3 Energy intake	103
6.3.4 Plasma PYY <sub>3-36</sub> concentrations	103
6.4 Discussion	107

## **Chapter VII**

### **Appetite, gut hormone and energy intake responses to moderate energy deficits induced via exercise and food restriction.**

7.1 Introduction	111
7.2 Methods	113
7.2.1 Participants	113
7.2.2 Preliminary trials	113
7.2.3 Experimental protocol	113
7.2.4 Energy deficits	114
7.2.5 Ad libitum meal	114
7.2.6 Blood sampling	115
7.2.7 Biochemical analysis	115
7.2.8 Statistical analysis	115
7.3 Results	117
7.3.1 Exercise responses	117
7.3.2 Appetite	117
7.3.3 Energy intake	117
7.3.4 Plasma acylated ghrelin concentrations	119
7.3.5 Plasma PYY <sub>3-36</sub> concentrations	123
7.3.6 Correlations	123
7.4 Discussion	126

## **Chapter VIII**

### **General Discussion**

8.1 Introduction	<b>130</b>
8.2 Appetite	<b>132</b>
8.3 Energy intake	<b>133</b>
8.4 Acylated ghrelin	<b>135</b>
8.5 Peptide YY	<b>136</b>
8.6 Limitations and future directions	<b>138</b>

<b>References</b>	<b>139</b>
-------------------	------------

### **Appendices**

Appendix A: Informed consent form	<b>174</b>
Appendix B: Health screen questionnaire	<b>175</b>
Appendix C: Physical activity questionnaire	<b>177</b>
Appendix D: Three-factor eating questionnaire	<b>178</b>
Appendix E: Food preference questionnaire for Chapter 4	<b>182</b>
Appendix F: Food preference questionnaire for Chapter 5	<b>184</b>
Appendix G: Food preference questionnaire for Chapters 6 & 7	<b>187</b>
Appendix H: Appetite visual analogue scales	<b>188</b>

## LIST OF TABLES

	<b>Page</b>
4.1 Baseline appetite, resting energy expenditure and resting respiratory exchange ratio values in the control, fasted and postprandial trials	<b>62</b>
4.2 Ad libitum energy intake during the control, fasted and postprandial trials	<b>65</b>
4.3 Macronutrient intake in the control, fasted and postprandial trials	<b>66</b>
5.1 Time-averaged area under the curve values for appetite perceptions in the control, endurance and sprint trials	<b>83</b>
5.2 Ad libitum energy intake during the control, endurance and sprint trials	<b>84</b>
5.3 Macronutrient intake in the control, endurance and sprint trials	<b>84</b>
6.1 Exercise responses during steady state exercise and high intensity intermittent exercise	<b>101</b>
6.2 Time-averaged area under the curve values for overall appetite perceptions in the control, steady state exercise and high intensity intermittent exercise trials	<b>103</b>
6.3 Time-averaged area under the curve values for absolute and delta PYY <sub>3-36</sub> concentrations in the control, steady state exercise and high intensity intermittent exercise trials	<b>106</b>
7.1 Time-averaged area under the curve values for overall appetite perceptions in the control, Ex-Def and Food-Def trials	<b>118</b>
7.2. Time-averaged area under the curve values for absolute and delta PYY <sub>3-36</sub> concentrations in the control, Ex-Def, and Food-Def trials.	<b>125</b>
8.1 Summary of the study protocols presented within the experimental chapters of this thesis	<b>131</b>

## LIST OF FIGURES

	<b>Page</b>
2.1 A simplified schematic of the hypothalamic nuclei involved in energy homeostasis	<b>5</b>
2.2 Tonic and episodic appetite-regulating signals acting upon neural circuits to influence energy homeostasis	<b>7</b>
2.3 A schematic diagram of the gastrointestinal tract illustrating the major storage sites and putative functions of gut hormones	<b>10</b>
2.4 The structure of acylated ghrelin	<b>13</b>
2.5 Temporal profile of plasma ghrelin after the ingestion of preloads containing 7.5 %, 16 %, and 33 % of estimated total daily energy expenditure	<b>16</b>
2.6 Diurnal profile of plasma total PYY concentrations in response to feeding	<b>22</b>
4.1 Schematic representation of the protocol for the experimental trials	<b>60</b>
4.2 Appetite ratings in the control, fasted and postprandial exercise trials	<b>63</b>
4.3 Energy expenditure in the control, fasted and postprandial exercise trials	<b>67</b>
4.4 Respiratory exchange ratio values in the control, fasted and postprandial exercise trials	<b>68</b>
5.1 Schematic representation of the protocol for the experimental trials	<b>78</b>
5.2 Appetite ratings in the control, endurance and sprint exercise trials	<b>82</b>
5.3 Absolute and delta acylated ghrelin concentrations in the control, endurance and sprint exercise trials	<b>87</b>
5.4 Absolute and delta total PYY concentrations in the control, endurance and sprint exercise trials	<b>88</b>
5.5 Resting energy expenditure and respiratory exchange ratio in the control, endurance and sprint exercise trials	<b>89</b>
6.1 Schematic representation of the protocol for the experimental trials	<b>100</b>
6.2 Appetite ratings in the control, steady state and high intensity intermittent exercise trials	<b>102</b>
6.3 Absolute and delta PYY <sub>3-36</sub> concentrations in the control, steady state and high intensity intermittent exercise trials	<b>105</b>
7.1 Schematic representation of the protocol for the experimental trials	<b>116</b>
7.2 Appetite ratings in the control, Ex-Def and Food-Def trials	<b>118</b>
7.3 Absolute and delta acylated ghrelin concentrations including all participants in the control, Ex-Def and Food-Def trials	<b>120</b>
7.4 Absolute and delta acylated ghrelin concentrations after the removal of outliers in the control, Ex-Def and Food-Def trials	<b>121</b>

7.5 Absolute and delta acylated ghrelin concentrations for one of the outlying participants in the control, Ex-Def and Food-Def trials	<b>122</b>
7.6 Absolute and delta PYY <sub>3-36</sub> concentrations in the control, Ex-Def and Food-Def trials	<b>124</b>

## LIST OF ABBREVIATIONS

The following abbreviations are used throughout multiple chapters of this thesis. Where they appear in text, they have been defined in the first instance:

- ANOVA (analysis of variance)
- AUC (area under the curve)
- BMI (body mass index)
- ES (effect size)
- GHS-R (growth hormone secretagogue receptor)
- GOAT (ghrelin-O-acyltransferase)
- HIIE (high intensity intermittent exercise)
- PFC (prospective food consumption)
- PYY (peptide YY)
- REI (relative energy intake)
- RER (respiratory exchange ratio)
- RPE (rating of perceived exertion)
- SD (standard deviation)
- SEM (standard error of the mean)
- VO<sub>2</sub> max (maximum oxygen uptake)



# CHAPTER I

## Introduction

Overweight and obesity are defined by a body mass index (BMI) of 25 to 29.9 kg.m<sup>-2</sup> and 30 kg.m<sup>-2</sup> or greater, respectively, and characterised by an excess accumulation of body fat. Recent decades have witnessed a global increase in BMI to the extent that 22.5 % of adults worldwide were estimated to be overweight in 2008, with an additional 11.8 % qualifying as obese (Finucane et al. 2011). These conditions are associated with an increased prevalence of several chronic diseases (Bray 2004), which has resulted in the classification of overweight and obesity as one of the top five global risk factors for mortality and one of the top ten risk factors for morbidity (World Health Organisation 2009).

This issue is also of great domestic relevance as the prevalence of overweight and obesity in England is considerably higher than the global average with 37 % of adults estimated to be overweight and an additional 26 % estimated to be obese in 2010 (NHS Information Centre 2012). The associated health consequences also yield a significant economic burden as overweight and obesity are estimated to account for approximately five billion pounds of spending within the NHS and incur a total societal cost of £14 billion each year (Royal College of Physicians 2013). Reversing the current ‘obesity epidemic’ therefore represents a major priority from both a public health and economic perspective.

For weight loss to occur, a sustained negative energy balance is required and is typically achieved by decreasing energy intake (i.e. dieting) and/or increasing energy expenditure (i.e. exercising). However, recent media articles have reported that exercise stimulates compensatory increases in appetite and food intake that prevent weight loss (Time Magazine 2009) and actually increase body fat (The Daily Telegraph 2009).

Contrary to these media articles, King and colleagues (2008) recently reported that 12 weeks of supervised aerobic exercise significantly reduced body weight and fat mass in overweight and obese participants without any dietary intervention. Furthermore, a dose-response relationship has been observed between exercise volume and weight loss (Jakicic et al. 2008), and exercise has consistently been demonstrated to facilitate the

long-term maintenance of weight loss (Curioni & Lourenço 2005; Franz et al. 2007; Klem et al. 1997).

The issue of compensation has also been comprehensively investigated throughout the past two decades with an abundance of studies examining the appetite and energy intake response to exercise. In opposition to the aforementioned media articles, the majority of studies have demonstrated that compensatory increases in appetite and energy intake do not occur during the hours after exercise (Blundell & King 1999; Blundell et al. 2003; Martins et al. 2008). Furthermore, although a compensatory increase in energy intake begins after several days of consecutive exercise, this compensation is only partial (Stubbs et al. 2004), and a failure to fully compensate for the energy expenditure of exercise appears to explain the dose-response relationship observed between exercise training and reductions in body fat (Elder & Roberts 2007).

Recently, several authors have suggested that exercise protocols may be manipulated to further minimise compensatory increases in appetite and energy intake after exercise. In this regard, preliminary findings from Cheng and colleagues (2009) suggest that exercise most effectively lowers appetite perceptions when performed after breakfast consumption, rather than in the fasted state. Additionally, several authors have postulated that high intensity intermittent exercise (HIIE) may promote greater weight loss than traditional endurance exercise due to greater reductions in appetite during the post-exercise period (Boutcher 2011; Heydari et al. 2012; Trapp et al. 2008; Tremblay et al. 1994). However, despite such postulations, the appetite and energy balance responses to HIIE have not yet been investigated.

Recent years have also witnessed significant advances in our understanding of the signalling processes involved in the regulation of appetite and energy homeostasis. In this regard, several gut hormones have been identified that provide key brain regions with information regarding the acute nutritional state of the body and stimulate appropriate changes in appetite perceptions and feeding responses (Murphy & Bloom 2006). Of these hormones, acylated ghrelin has received explicit attention as the only gut peptide known to stimulate appetite, whereas peptide YY (PYY) has received significant interest as a prominent mediator of satiety and chronic energy homeostasis (Karra & Batterham 2010). Current evidence suggests that these peptides may also mediate the appetite response to exercise as several studies have reported a temporal

association between appetite, acylated ghrelin and PYY during and after an acute exercise bout (King et al. 2010a; 2010b; 2011a). However, this is not a universal finding (Broom et al. 2007; Cheng et al. 2009; Wasse et al. 2013) and further research is required to elucidate the relationship between these variables in response to exercise.

Subsequently, the aims of this thesis were two-fold. The primary objective of this thesis was to establish the most effective methods of inducing an acute energy deficit without stimulating compensatory increases in appetite and energy intake. The secondary objective of this thesis was to investigate the mediating influence of changes in plasma acylated ghrelin and PYY concentrations as determinants of these appetite and feeding responses.

The first experiment in this thesis (Chapter 4) investigated the appetite and energy intake response to exercise when performed in either a fasted or postprandial state. The second experiment (Chapter 5) compared the effects of traditional endurance exercise and sprint interval exercise on appetite, energy intake and plasma acylated ghrelin and total PYY responses. Study three (Chapter 6) extended the findings of the previous experiment by examining the appetite, energy intake and PYY<sub>3-36</sub> responses to high volume submaximal intermittent exercise and continuous exercise of equal duration and energy expenditure. Finally, experiment four (Chapter 7) investigated the sensitivity of the appetite-regulating system by examining the appetite, energy intake, acylated ghrelin and PYY<sub>3-36</sub> responses to subtle energy deficits induced via exercise or food restriction.

## **CHAPTER II**

### **Literature Review**

#### **2.1 Introduction**

The following literature review will initially focus on the physiological regulation of appetite and provides information detailing the peripheral signals involved in energy homeostasis as well as the central integration of these signals. This contains a particular focus on the roles of ghrelin and PYY in the regulation of appetite and energy homeostasis as these peptides are measured throughout the experimental chapters of this thesis. Subsequently, the acute effects of exercise on appetite and energy intake responses are thoroughly discussed and the final section of the review will discuss the effects of exercise on the gut hormones ghrelin and PYY.

#### **2.2 Physiological regulation of appetite and energy intake**

##### **2.2.1 Central regulation of appetite and energy intake**

The hypothalamus and brainstem are thought to be the main brain regions responsible for the regulation of energy homeostasis. In this regard, these brain centres integrate a number of central and peripheral signals that provide information about the acute nutritional state and adiposity of the body (Murphy & Bloom 2006).

The hypothalamus can be subdivided into several nuclei that each consist of a collection of neurones with specific functions (Neary et al. 2004) (Figure 2.1). The integration of energy-related signals is thought to primarily occur within the arcuate nucleus (ARC), which receives inputs from other hypothalamic nuclei and brain regions as well as directly from peripherally circulating hormones that cross the incomplete blood-brain barrier at the median eminence. The ARC integrates signals of energy balance via distinct, but adjacent, neurones that contain either the orexigenic neuropeptides agouti-related peptide (AgRP) and neuropeptide Y (NPY) or the anorexigenic neuropeptide pro-opiomelanocortin (POMC) (Schwartz et al. 2000; Wynne et al. 2005a).

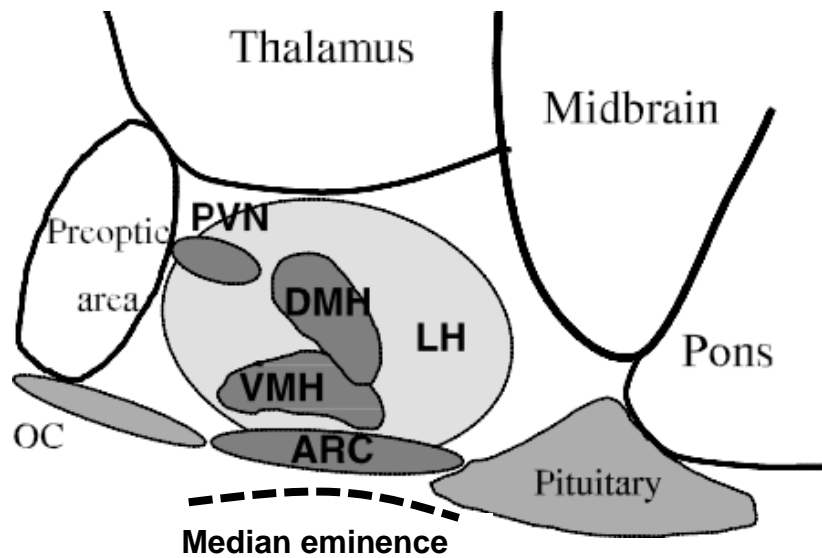


Figure 2.1. A simplified schematic of the hypothalamic nuclei involved in energy homeostasis (lateral view of the rat brain). Including the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), lateral hypothalamus (LH), and optic chiasm (OC). Adapted from Neary et al. (2004).

Stimulation of ARC neurones induces the transmission of neuropeptides to other hypothalamic nuclei, particularly the paraventricular nucleus (PVN). In this regard, stimulation of POMC neurones increases the expression of the anorexigenic neuropeptide  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), which acts on MC3 and MC4 receptors in the PVN to reduce appetite. In contrast, AgRP blocks the actions of  $\alpha$ -MSH by exerting antagonistic effects on MC3 and MC4 receptors (Wynne et al. 2005a) and NPY acts to stimulate feeding predominantly through the activation of Y1 and Y5 receptors in the PVN (Neary et al. 2004).

The nucleus of the solitary tract (NTS) also represents a key area for the integration of signals concerning the nutritional state of the body. The NTS is located within the brainstem and receives hormonal inputs from the circulation via the incomplete blood-brain barrier at the area postrema, in addition to neural inputs from vagal afferent nerves located in the gastrointestinal tract. Signals from this brain region are integrated with those from the ARC and other hypothalamic nuclei in the PVN, which

subsequently regulates thyroid and sympathetic nervous system activity as well as signalling to higher brain centres to influence behaviour (Murphy & Bloom 2006; Wynne et al. 2005a).

Although the ARC, NTS and PVN have prominent roles in the physiological regulation of appetite, many additional integrated and redundant pathways also exist (Schwartz et al. 2000). A discussion of these additional pathways is beyond the scope of this thesis but the reader is directed to Wynne et al. (2005a) for a more comprehensive review.

### **2.2.2 Peripheral regulation of appetite and energy intake**

Episodic and tonic hormones within the peripheral circulation are integrated by brain centres to influence energy homeostasis. Tonic hormones produce long-term signals in proportion to body fat mass, whereas episodic hormones change acutely in response to feeding episodes. A simplified schematic demonstrating the integration of tonic and episodic appetite-regulating signals is presented in Figure 2.2.

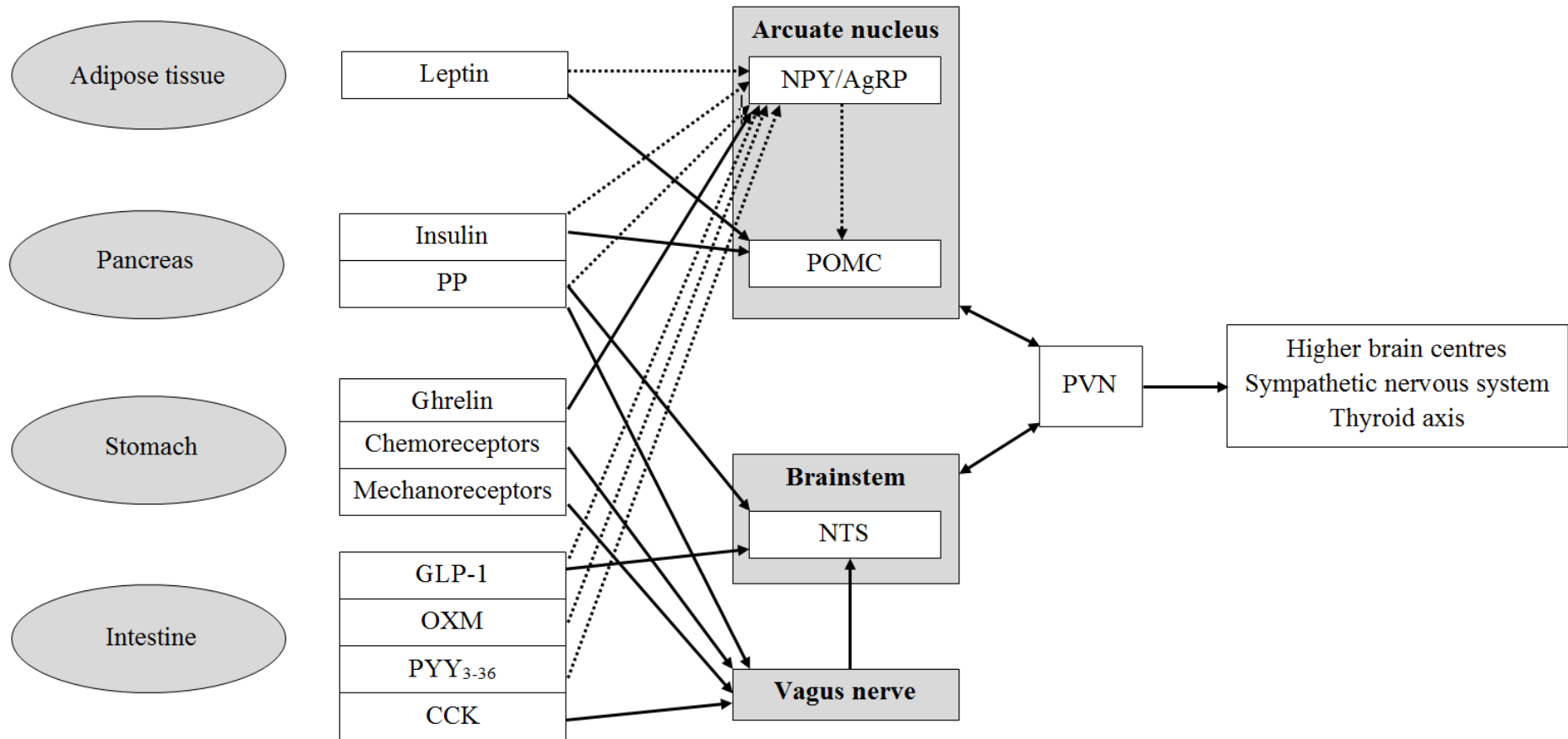


Figure 2.2. Tonic and episodic appetite-regulating signals acting upon neural circuits to influence energy homeostasis. Adapted from Murphy & Bloom (2006) and Neary et al. (2004). Solid lines indicate stimulatory effects and dashed lines indicate inhibitory effects. AgRP, agouti-related peptide; CCK, cholecystokinin; GLP-1, glucagon-like-peptide 1; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; OXM, oxyntomodulin; POMC, pro-opiomelanocortin; PP, pancreatic polypeptide; PVN, paraventricular nucleus; PYY, peptide YY.

### **2.2.2.1 Tonic signals**

In 1953, Kennedy postulated that the hypothalamic control of food intake was regulated by a circulating factor that provided information regarding the extent of body fat stores (Kennedy 1953). The discovery of the hormone leptin provided a major breakthrough in support of this theory (Zhang et al. 1994). In accordance with its role as an adiposity negative-feedback signal, leptin is primarily secreted from fat cells and circulates in plasma at concentrations proportional to body fat mass (Considine et al. 1996). Furthermore, leptin exerts anorexigenic effects in the ARC of the hypothalamus via direct inhibition of NPY and AgRP neurones and stimulation of POMC neurones (Sahu 2003). The importance of leptin in the regulation of energy homeostasis in humans is emphasised by the observation that individuals with congenital leptin deficiency exhibit extreme childhood onset obesity and hyperphagia. Furthermore, the treatment of such individuals with recombinant leptin reverses this phenotype (Farooqi et al. 2002). However, the vast majority of obese individuals do not exhibit leptin deficiency and have very high circulating leptin concentrations in proportion with body adiposity (Considine et al. 1996). This suggests a state of leptin resistance in obesity, thereby reducing the anorexigenic effects of this hormone.

The only other circulating hormone currently regarded as a tonic appetite regulator is insulin. Although insulin is not secreted directly from adipocytes, insulin concentrations circulate in proportion with body fat mass (Woods & Seeley 1998). The administration of insulin into the third ventricle of the brain has been shown to reduce food intake and body weight in rodents (Air et al. 2002), while central administration of insulin antibodies increases food intake and body weight (McGowan et al. 1992). Furthermore, peripheral infusion of insulin reduces food intake in rats in the absence of hypoglycaemia (Nicolaidis & Rowland 1976). Insulin is believed to induce such anorectic effects via the inhibition of NPY and AgRP neurones and via the stimulation of POMC neurones in the ARC of the hypothalamus (Murphy & Bloom 2004; Wynne et al. 2005a). However, in accordance with peripheral insulin resistance (Schwartz & Porte 2005), obesity may induce insulin resistance in the hypothalamus (De Souza et al. 2005), which would reduce the anorexigenic effects of this peptide.



### **2.2.2.2 Episodic signals**

Although leptin and insulin have important roles in the tonic regulation of appetite, small changes in the circulating levels of these hormones cannot explain the preprandial increases and postprandial decreases in appetite that govern meal initiation and termination. Alternatively, such acute appetite-regulation appears to be predominantly mediated by neuroendocrine signalling from the gut (Morton et al. 2006). Satiety and satiety exemplify the acute regulation of appetite and are defined as the processes that induce meal termination and maintain feelings of fullness after eating, respectively (Benelam 2009).

The process of satiety appears to be largely governed by mechanoreceptors and chemoreceptors in the stomach that transmit information to the NTS via the vagal nerve (Janssen et al. 2011). The gut hormone cholecystokinin (CCK) also appears to be involved in meal termination (Harrold et al. 2012) as it is rapidly released from intestinal I cells into the circulation in response to the presence of nutrients in the small intestine. Circulating concentrations of CCK peak at approximately 25 min after meal initiation and are particularly sensitive to meals that are rich in either fat or protein (Harrold et al. 2012; Paik et al. 2007). The anorexigenic effects of CCK have been demonstrated in man as peripheral infusion of CCK immediately prior to meal initiation significantly reduced food intake via earlier meal termination (Kissileff et al. 1981). The role of CCK in meal termination has also been demonstrated in rats as intermittent infusion upon meal initiation acutely decreased food intake. However, compensatory increases in meal frequency occurred which prevented a decrease in total daily energy intake (West et al. 1984). Although this evidence suggests that CCK may not play a role in post-meal satiety, the temporal pattern of CCK release suggests a more prolonged role as concentrations remain elevated for up to five hours after feeding (Liddle et al. 1985). It is therefore possible that the short-term functioning of CCK during infusion studies reflects the rapid half-life of this hormone, which is approximately 1 – 2 min (Wynne et al. 2005a). Increases in CCK also affect digestion by stimulating enzyme release from the pancreas and gall bladder, as well as increasing intestinal mobility and inhibiting gastric emptying (Murphy & Bloom 2006; Wynne et al. 2005a). The anorexigenic effects of CCK are thought to be mediated primarily via CCK1 receptors on the vagal nerve.

Several other gut peptides are also sensitive to nutrient ingestion and act as episodic mediators of hunger and satiety. In this regard, the release of peptide YY<sub>3-36</sub> (PYY<sub>3-36</sub>), pancreatic polypeptide (PP), oxyntomodulin (OXM) and glucagon-like-peptide-1 (GLP-1) appear to increase satiety, whereas ghrelin acts to stimulate hunger. Figure 2.3 provides a schematic representation of the gastrointestinal tract, illustrating where these hormones are concentrated and their physiological functions.

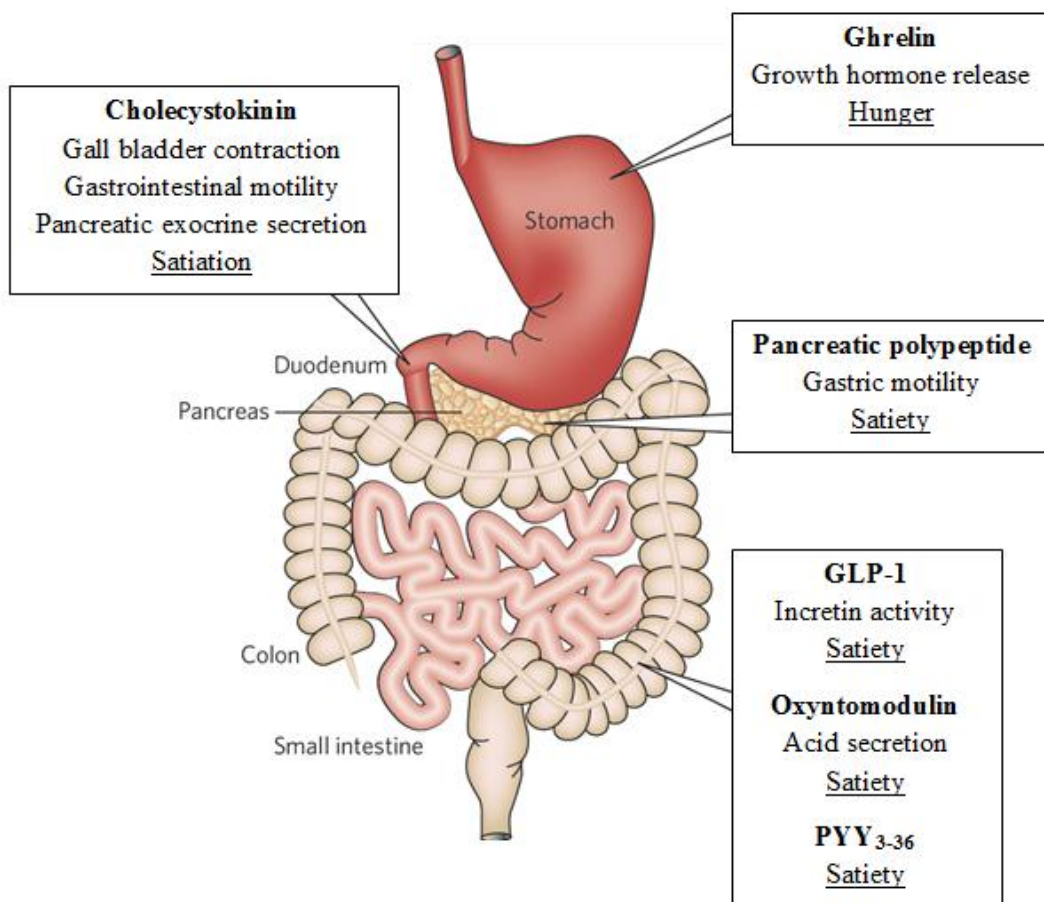


Figure 2.3. A schematic diagram of the gastrointestinal tract illustrating the major storage sites and putative functions of gut hormones. GLP-1, glucagon-like-peptide-1; PYY, peptide YY. Adapted from Murphy & Bloom (2006).

Pancreatic polypeptide is a 36 chain amino acid that is released from F-cells in the pancreas in response to nutrient ingestion and remains elevated for up to six hours after a meal (Karra & Batterham 2010; Track et al. 1980). The anorexigenic effects of PP have been demonstrated in mice as peripheral infusion stimulated an acute decrease in subsequent food intake and repeated peripheral infusions induced a significant decrease in body weight (Asakawa et al. 2003). Furthermore, data from human studies suggests that elevations in PP in response to pancreatic tumours may contribute to the reduction in appetite associated with this condition as a pathophysiological dose of PP reduced food intake by approximately 11 % at an ad libitum meal presented one hour later (Jesudason et al. 2007). Such anorectic effects of PP are thought to be primarily mediated by the Y4 receptor in the hypothalamus, brainstem and vagal nerve (Asakawa et al. 2003; Larhammar 1996). Pancreatic polypeptide also exhibits several digestive functions as increases in this hormone have been shown to inhibit gastric emptying, gallbladder motility and pancreatic exocrine secretion (Kojima et al. 2007).

Oxyntomodulin and GLP-1 are synthesised from different post-translational processing of the pre-proglucagon precursor molecule in the L-cells of the intestine (Murphy & Bloom 2006). Oxyntomodulin is a 37 chain amino acid that is released into the circulation after feeding in proportion to caloric load (Karra & Batterham 2010). Acute peripheral administration of OXM has been shown to reduce appetite and food intake in both normal weight (Cohen et al. 2003) and obese humans (Wynne et al. 2005b), with the duration of this inhibitory effect lasting for up to 12 hours. The anorexigenic effects of OXM appear to be maintained in response to repeated administrations as subcutaneous infusion prior to meals for 4 weeks stimulated a weight loss of ~ 2.3 kg compared with ~ 0.5 kg in a control group (Wynne et al. 2005b). Although these effects appear to be largely mediated by a decrease in food intake, evidence suggests that OXM may also increase voluntary physical activity in obese humans (Wynne et al. 2006). The anorectic effects of OXM are believed to be mediated primarily via the GLP-1 receptor in the hypothalamic ARC (Dakin et al. 2004) but this hormone may also act by suppressing ghrelin levels (Cohen et al. 2003). Increases in OXM after feeding are also thought to act to inhibit gastric acid secretion (Murphy & Bloom 2006).

Glucagon-like-peptide-1 is also released into the circulation from intestinal L-cells in response to nutrient ingestion. In addition to its primary function as an incretin hormone, GLP-1 has also been demonstrated to exert anorectic effects and delay gastric

emptying (Harrold et al. 2012). In this regard, peripheral infusion of GLP-1 has been demonstrated to significantly inhibit food intake in a dose-dependent manner in both lean and obese humans (Verdich et al. 2001). Such appetite-suppressing effects appear to be mediated primarily by GLP-1 receptors in the ARC and NTS (Turton et al. 1996; Yamamoto et al. 2003). However, it must be acknowledged that GLP-1 appears to elicit only small changes in appetite and energy intake when infused at physiological doses (Flint et al. 2001).

The experimental work in this thesis included the measurement of the gut hormones ghrelin and PYY. Ghrelin has received explicit attention within the scientific literature as the only known orexigenic gut peptide, whereas PYY has received significant interest as a prominent mediator of postprandial satiety and chronic energy homeostasis. The following sections of this review will describe the structure and function of these peptides in greater detail, with particular emphasis on their roles in energy homeostasis.

## **2.3 Ghrelin**

### **2.3.1 The discovery of ghrelin**

After the identification of the growth hormone secretagogue receptor (GHS-R) in 1996 (Howard et al. 1996), a search for its endogenous ligand was actively undertaken as a potential treatment for growth hormone deficiency. Surprisingly, despite the GHS-R being expressed primarily in the brain, the endogenous ligand was detected in the stomach. The subsequently purified peptide was named 'ghrelin' based on "ghre" meaning "grow" in reference to its ability to stimulate growth hormone release (Kojima et al. 1999).

### **2.3.2 Structure, production and secretion**

Ghrelin is a 28 chain amino acid peptide that is produced from a pre-proghrelin precursor and is unique as the only mammalian hormone that requires post-translational acylation with a medium chain fatty acid to exert its biological functions (Lim et al. 2011). During acylation, octanoic acid is covalently linked to the serine 3 residue of ghrelin via an ester bond (Figure 2.4). This reaction is catalysed by a member of the membrane-bound acyl-transferase superfamily that has been subsequently named ghrelin-O-acyltransferase (GOAT) (Yang et al. 2008).

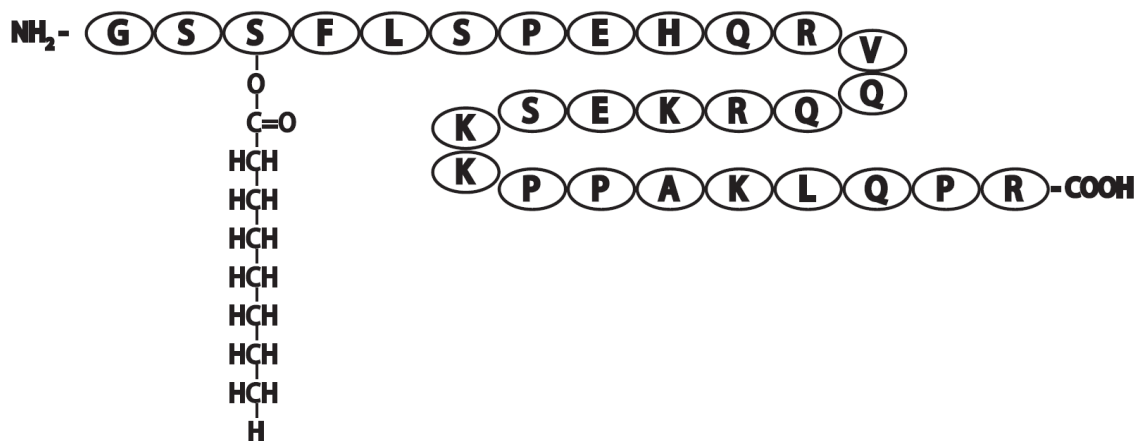


Figure 2.4. The structure of acylated ghrelin. Adapted from Sato et al. (2012).

In accordance with the functional relationship between ghrelin and GOAT, these peptides exhibit similar tissue distributions with levels being highest in the stomach and lower levels in the intestine gradually decreasing from the duodenum to the colon (Yang et al. 2008). Ghrelin production in the stomach occurs in specific X/A cells of the oxyntic glands of the gastric mucosa and is responsible for approximately 50 – 70 % of systemic ghrelin production (Kojima & Kangawa 2005). Staining of these cells with an antibody specific to the acylated form of ghrelin confirmed that acylation occurs within the cell before secretion into the bloodstream (Hosoda et al. 2000).

Although acylation occurs within the cell, Hosoda et al. (2000) demonstrated that only 21 % of ghrelin in rat stomach is acylated, with the remaining 79 % representing des-acyl ghrelin. This effect is likely to be explained by the greater abundance of ghrelin than GOAT within ghrelin-producing cells (Yang et al. 2008). Acylated ghrelin concentrations are further reduced in the circulation as a result of deacylation by a number of esterases including thioesterase (Satou et al. 2010) and butyrylcholinesterase (De Vriese et al. 2004). Subsequently, acylated ghrelin represents only ~ 10 % of total ghrelin within the peripheral circulation of humans (Hosoda et al. 2004; Marzullo et al. 2008).

Feeding has been established as the most important factor regulating ghrelin secretion as circulating concentrations increase in response to short-term fasting and decrease

with food intake. The mechanisms mediating this effect are currently unknown but appear to involve nutrient sensing as gastric distension alone does not influence plasma ghrelin concentrations (Tschöp et al. 2000).

### **2.3.3 Physiological functions**

The first recognised effect of ghrelin was the stimulation of growth hormone release from the anterior pituitary via binding to the GHS-R. In this regard, ghrelin appears to be a more potent stimulus for growth hormone release than growth hormone releasing hormone (Arvat et al. 2001). Ghrelin also influences digestion by stimulating gastric acid secretion and gastric motility (Masuda et al. 2000), and decreases blood pressure and circulating concentrations of thyroid stimulating hormone in humans (Kluge et al. 2010; Nagaya et al. 2001a). Ghrelin has also been shown to inhibit apoptosis in cardiomyocytes and endothelial cells in vitro (Baldanzi et al. 2002), and may influence memory and learning (Carlini et al. 2008) as well as immune function and inflammation (Baatar et al. 2011). Despite such multifaceted effects, the role of ghrelin in energy homeostasis has received particular attention and is discussed in the following sections.

### **2.3.4 Acute effects of ghrelin on appetite and energy intake**

During the last decade, ghrelin has received explicit attention as the only peripheral hormone known to increase appetite through the circulation. Such orexigenic effects were first demonstrated by Wren et al. (2000) as central and peripheral ghrelin administration stimulated an increase in 24 h food intake in free-living rats. Subsequent experiments have confirmed these findings and identified that the orexigenic actions of ghrelin are solely mediated by the acylated form of this peptide, which binds to the GHS-R on the vagal nerve and hypothalamic nuclei to stimulate food intake (Chen et al. 2004; Date et al. 2002; Nakazato et al. 2001; Neary et al. 2006).

These findings have also been extended to humans. In normal weight men and women, intravenous ghrelin infusion at a dose of  $5 \text{ pmol.kg}^{-1}.\text{min}^{-1}$  for a duration of 270 min significantly increased appetite and stimulated a 28 % increase in energy intake during an ad libitum lunch meal provided towards the end of the infusion. Analysis of food diaries completed for the remainder of the day revealed a tendency for a sustained increase in food intake that resulted in a 24 h energy intake that was approximately 22 %

higher than a saline infusion control trial (Wren et al. 2001). Such increases in appetite and energy intake have subsequently been replicated in obese participants (Druce et al. 2005) and in response to subcutaneous administration of ghrelin (Druce et al. 2006). Although these findings stimulated great interest in ghrelin as a potential therapeutic target for the prevention of obesity, it must be acknowledged that the doses infused in the aforementioned studies produced supraphysiological plasma ghrelin concentrations.

Further support for ghrelin as a physiological mediator of appetite stems from observations of the secretory pattern of this peptide as circulating concentrations rise preprandially and fall postprandially (Cummings et al. 2001; 2002). Furthermore, this pattern of ghrelin release occurs in the absence of time- and food-related cues and with participants initiating meals voluntarily (Cummings et al. 2004). Frequent blood sampling during the study of Cummings et al. (2004) also revealed a close temporal pattern between circulating ghrelin concentrations and hunger perceptions. This relationship is further strengthened by the finding that the postprandial suppression of ghrelin is proportional to caloric intake (Callahan et al. 2004).

However, although ghrelin undoubtedly possesses orexigenic properties, other authors have questioned the influence of physiological changes in ghrelin on acute appetite and energy intake responses. In this regard, Lippl and colleagues (2012) performed a randomised double blind, crossover study in which either ghrelin or saline was infused intravenously, with the infusion beginning 45 min after the consumption of a standardised breakfast meal and ending 1 h after the completion of a spontaneously requested ad libitum lunch meal. A dose of  $1 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  of ghrelin was employed in this study, which is approximately 6 % of the dose previously administered by Wren and colleagues (2001). Despite the low dose, ghrelin infusion increased plasma acylated ghrelin concentrations to supraphysiological levels but did not influence appetite perceptions, the timing of meal request or energy intake at the ad libitum meal. This study demonstrates the need for caution when interpreting the findings of early infusion studies that administered large doses of exogenous ghrelin.

The importance of ghrelin as a signal for meal initiation has been further questioned by the finding that the recovery of ghrelin in response to different caloric preloads does not predict spontaneous meal request (Callahan et al. 2004) (Figure 2.5). Furthermore, closer inspection of the findings of Cummings et al. (2004) revealed that one out of the

six participants did not exhibit a preprandial rise in ghrelin prior to meal initiation despite demonstrating a similar inter-meal interval and hunger ratings as the other participants.

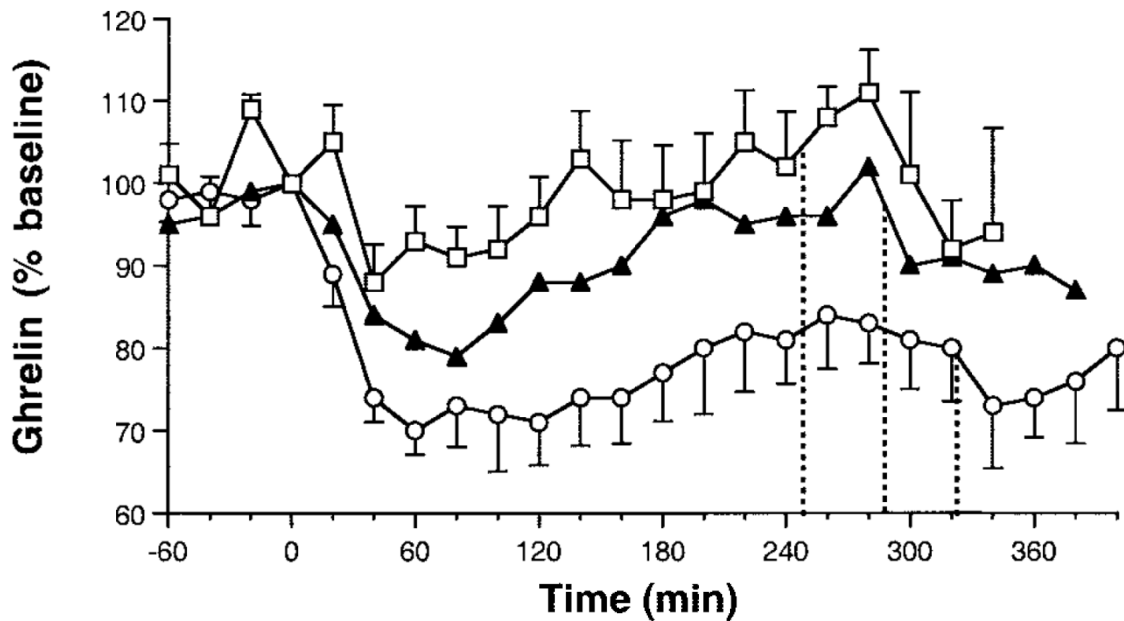


Figure 2.5. Temporal profile of plasma ghrelin after the ingestion of preloads containing 7.5 % (squares), 16 % (triangles), and 33 % (circles) of estimated total daily energy expenditure. Preloads were administered at 0 min. Dotted lines indicate voluntary meal request. Values are mean (SEM). N = 10. Adapted from Callahan et al. (2004).

### 2.3.5 Ghrelin and chronic energy homeostasis

Ghrelin has also been observed to influence chronic energy homeostasis as repeated central and peripheral administration of exogenous ghrelin significantly increased body weight and fat mass in rats. Furthermore, this effect appears to be a result of a decrease in fat oxidation as well as an increase in food intake (Kamegai et al. 2001; Tschöp et al. 2000).

In humans, ghrelin responds to chronic changes in energy balance as circulating concentrations are inversely related to adiposity, with low levels in obese populations and high levels in patients with anorexia nervosa or cachexia (Nagaya et al. 2001b; Otto et al. 2001; Shiiya et al. 2002; Tschöp et al. 2001). The sensitivity of ghrelin to chronic



changes in energy balance has also been highlighted by Ravussin and colleagues (2001) who reported a down-regulation of circulating ghrelin concentrations in response to 100 days of overfeeding and an up-regulation in response to an energy deficit induced via 93 days of exercise.

Although these findings support the role of ghrelin as a regulator of chronic energy homeostasis, the observation that circulating concentrations are lower in obese populations suggests that ghrelin does not have a causal role in weight gain. Furthermore, Ravussin et al. (2001) found no relationship between baseline ghrelin concentrations and subsequent body weight change in response to chronic changes in energy balance.

Changes in ghrelin levels may however, contribute to the difficulty of weight loss maintenance as circulating concentrations increase in response to reductions in body mass achieved by either diet or exercise interventions (Leidy et al. 2007; Purnell et al. 2007; Scheid et al. 2011). Furthermore, ghrelin levels were found to be significantly reduced after gastric bypass surgery; an effect that was postulated as a potential mechanism underlying successful weight loss maintenance after this procedure (Cummings et al. 2002). However, despite the initial excitement generated by this finding, subsequent authors have found significant increases or no change in plasma ghrelin concentrations in response to gastric bypass surgery despite similar reductions in appetite and body weight (Tymitz et al. 2011).

Initial studies involving ghrelin and GHS-R knockout mice also demonstrated a major physiological role of ghrelin in the regulation of feeding as these mice were found to have a marked resistance to obesity induced by a high fat diet (Wortley et al. 2005; Zigman et al. 2005). However, subsequent authors have reported that ghrelin and GHS-R knockout mice exhibit similar fasting-induced hyperphagia and diet-induced obesity as wild-type mice (Sun et al. 2008). The reasons for such conflicting findings are unclear but may be influenced by the purer genetic background of the knockout mice or the later onset of the high fat diet in the latter study. Although food intake was unaffected in the study of Sun and colleagues (2008), ghrelin and GHS-R knockout mice exhibited significantly lower blood glucose concentrations in response to 50 % calorie restriction compared with wild-type mice. These findings have been further supported by Zhao et al. (2010) as GOAT knockout mice did not exhibit any defects in

food intake but failed to maintain blood glucose levels in response to seven days of 60 % calorie restriction. Subsequently, after 7 – 8 days of calorie restriction, GOAT knockout mice were moribund and euthanized by the experimenters. However, infusion of either acylated ghrelin or growth hormone prevented a decrease in blood glucose concentrations in GOAT knockout mice. These findings suggest that increases in circulating ghrelin concentrations in response to calorie restriction function primarily to maintain blood glucose concentrations rather than to stimulate appetite and food intake. However, although these knockout studies suggest a minimal role of ghrelin in the physiological regulation of appetite and food intake, these results must be interpreted with caution as compensatory mechanisms may occur during development in knockout mice to ensure that food intake pathways remain stable in adulthood. In this regard, the ablation of NPY and AgRP neurones in neonate mice does not appear to affect feeding behaviour but ablation in adulthood results in rapid starvation (Luquet et al. 2005).

## **2.4 Peptide YY**

### **2.4.1 The discovery of PYY**

Peptide YY was initially isolated from porcine intestinal extracts by Tatemoto and Mutt in (1980). Since the biological function of this peptide was unknown upon discovery, it was named PYY due to the presence of a tyrosine residue (amino acid abbreviation, Y) at each terminus of its amino acid structure (Tatemoto & Mutt 1980).

### **2.4.2 Structure, production and secretion**

Peptide YY is derived from the precursor pre-pro PYY and exists in two known endogenous forms: the intact 36 amino acid peptide PYY<sub>1-36</sub>, and the truncated 34 amino acid peptide PYY<sub>3-36</sub>. These peptides are predominantly synthesised and secreted from L-cells located in the distal gastrointestinal tract with concentrations increasing from the pylorus to the rectum (Adrian et al. 1985a). Peptide YY<sub>3-36</sub> is produced via the cleavage of tyrosine and proline residues from the N-terminus of PYY<sub>1-36</sub> by the enzyme dipeptidyl peptidase IV (DPPIV) (Mentlein et al. 1993). The amino acid sequences of PYY<sub>1-36</sub> and PYY<sub>3-36</sub> are as follows:

PYY<sub>1-36</sub>: YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY

PYY<sub>3-36</sub>: --IKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY

The truncation of PYY is thought to occur both within L-cells and upon secretion into the circulation (Eberlein et al. 1989; Mentlein 1999), which produces plasma concentrations that consist of ~65 % PYY<sub>3-36</sub> and ~35 % PYY<sub>1-36</sub> (Batterham et al. 2006). The truncation of PYY markedly changes the three-dimensional conformation of this peptide, which alters its receptor specificity and biological effects. In this regard, PYY<sub>1-36</sub> is thought to bind with similar affinity to all of the functional Y receptor subtypes in humans (Y1-5) but PYY<sub>3-36</sub> preferentially binds to the Y2 receptor (Cabrele & Beck-Sickinger 2000; Keire et al. 2000; Nygaard et al. 2006).

Feeding is the most important factor regulating PYY secretion, with concentrations increasing within 15 min of food ingestion, reaching a peak at approximately 90 min and remaining elevated for up to 6 hours (Adrian et al. 1985a). The immediate postprandial rise in PYY occurs before ingested nutrients have reached the nutrient-sensing L-cells of the distal gastrointestinal tract and therefore suggests a neural or hormonal mechanism in this initial release. Alternatively, the sustained release after feeding is thought to be due to the direct stimulation of L-cells by intraluminal gut contents (Imamura 2002).

#### **2.4.3 Physiological functions**

Peripheral administration of PYY in man has been demonstrated to delay gastric emptying and inhibit secretions from the stomach, pancreas and gallbladder (Adrian et al. 1985b; Allen et al. 1984; Hoentjen et al. 2001; Savage et al. 1987). In addition to changes in digestive functions, PYY infusion has been found to increase systolic and diastolic blood pressure (Adrian et al. 1986) and may also influence renal function (Playford et al. 1995). Despite such multifaceted effects, PYY has attracted particular attention as a mediator of appetite and energy homeostasis (Karra et al. 2009).

#### **2.4.4 Acute effects of PYY on appetite and energy intake**

The anorectic effects of PYY were first demonstrated in rats as a single intraperitoneal injection of PYY<sub>3-36</sub> significantly reduced food intake under ad libitum conditions and in response to a 24 h fast (Batterham et al. 2002). Although this finding was initially questioned by some authors, the anorexigenic effects of PYY<sub>3-36</sub> have subsequently been confirmed in several studies and it has become clear that the habituation of rodents to handling and injection is required for these effects to be observed (Challis et

al. 2003; Chelikani et al. 2005; Cox & Randich 2004; Halatchev et al. 2004; Neary et al. 2005; Pittner et al. 2004; Riediger et al. 2004).

The anorectic effects of PYY have also been replicated in humans. In normal weight men and women, intravenous PYY<sub>3-36</sub> infusion at a dose of 0.8 pmol.kg<sup>-1</sup>.min<sup>-1</sup> for a duration of 90 min resulted in a significant decrease in hunger scores and a 36 % reduction in energy intake at an ad libitum buffet provided two hours after termination of the infusion. Analysis of food diaries completed for the remainder of the day revealed a continued inhibition of food intake during the 12 h after infusion without any compensatory increases thereafter, which resulted in a 33 % lower 24 h energy intake compared with a saline infusion control trial (Batterham et al. 2002). These findings have subsequently been extended to obese participants (Batterham et al. 2003) and a dose-response relationship has been demonstrated (le Roux et al. 2006a). Such reductions in food intake do not occur with an equivalent dose of PYY<sub>1-36</sub>, which suggests that the appetite suppressing effects of PYY are primarily mediated by the truncated form of this peptide (Sloth et al. 2007). Although these studies demonstrate anorectic effects of PYY<sub>3-36</sub>, it must be acknowledged that the infused doses produced supraphysiological plasma PYY concentrations. In contrast, a lower dose of 0.2 pmol.kg<sup>-1</sup>.min<sup>-1</sup> does not appear to influence food intake in normal weight and obese men, which suggests that pharmacological doses of exogenous PYY<sub>3-36</sub> are required to influence appetite and food intake (Degen et al. 2005; le Roux et al. 2006a; Sloth et al. 2007).

The anorectic effects of PYY<sub>3-36</sub> are thought to be mediated primarily by Y2 receptors (Y2R) in the ARC of the hypothalamus (Karra & Batterham 2010). In this regard, the appetite suppressing effects of exogenous PYY<sub>3-36</sub> are attenuated with the administration of Y2R antagonists and abolished in Y2R knockout mice (Abbott et al. 2005a; Acuna-Goycolea & van den Pol 2005; Batterham et al. 2002; Scott et al. 2005). The mediating influence of the Y2 receptor also explains the greater potency of PYY<sub>3-36</sub> as an appetite suppressant compared with PYY<sub>1-36</sub> (Sloth et al. 2007). The binding of PYY to the Y2R appears to suppress appetite by inhibiting NPY neurones, which decreases orexigenic signalling and also disinhibits POMC neurones to increase anorexigenic outputs (Batterham et al. 2002; 2006; Challis et al. 2003; Riediger et al. 2004). Other authors have suggested that the vagus nerve also contributes to the anorectic effects of PYY as vagotomy abolishes the effects of peripherally administered

PYY<sub>3-36</sub> in rats (Abbott et al. 2005b; Koda et al. 2005). However, abolition of vagal signalling in mice has failed to reproduce these findings (Halatchev & Cone 2005; Talsania et al. 2005). The role of the vagus nerve in mediating the anorectic effects of PYY<sub>3-36</sub> therefore remains uncertain.

More recently, authors have suggested that PYY may influence appetite and food intake via brain reward centres in addition to hypothalamic pathways. Using blood-oxygen-level-dependant magnetic resonance imaging, Batterham et al. (2007) demonstrated that PYY<sub>3-36</sub> infusion produced the largest change in brain activity in the left caudolateral orbital frontal cortex (OFC), a brain region implicated in reward processing. Furthermore, the change in OFC signalling explained 77 % of the variance in caloric intake after PYY<sub>3-36</sub> infusion. These findings suggest that exogenous PYY may suppress energy intake by decreasing the rewarding aspects of food in addition to altering homeostatic brain centres. Such reductions in OFC activity after PYY<sub>3-36</sub> infusion were recently confirmed by De Silva and colleagues (2011) but were unrelated to subsequent food intake. Further research is required regarding the influence of changes in brain reward centres on appetite and food intake.

The secretory pattern of endogenous PYY also supports a role of this peptide in appetite and food intake regulation as circulating concentrations decrease with fasting and increase with feeding in humans (Chan et al. 2006a). Furthermore, significant correlations have been observed between postprandial increases in circulating PYY and decreases in appetite perceptions (Guo et al. 2006; Stoeckel et al. 2008). This relationship is further strengthened by the diurnal profile of plasma PYY, which exhibits a 24 h meal-driven response with postprandial peaks occurring in proportion with the caloric content of the meal (Hill et al. 2011) (Figure 2.6).

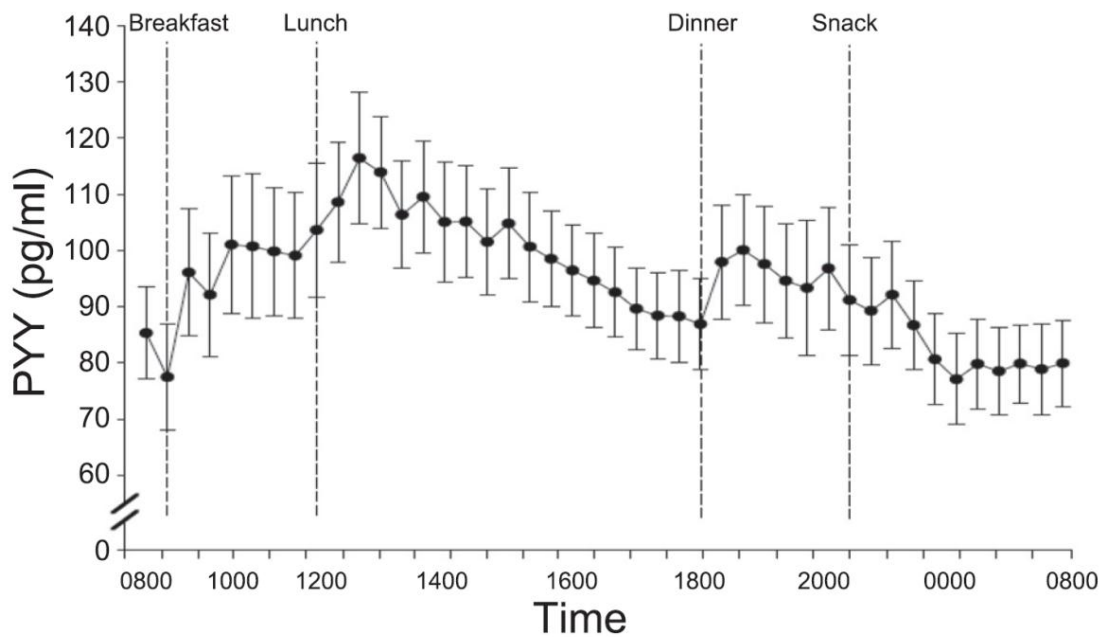


Figure 2.6. Diurnal profile of plasma total PYY concentrations in response to feeding. Values are mean (SEM). N = 11. Adapted from Hill et al. (2011).

Peptide YY is thought to be particularly crucial for regulating the appetite response to protein ingestion as enhanced satiety in response to meals of high protein content is associated with greater postprandial increases in PYY (Batterham et al. 2006; Belza et al. 2013). Furthermore, the satiating effects of protein appear to be abolished in mice that are deficient for either PYY or the Y2R (Batterham et al. 2006). Although Pedersen et al. (2013) recently reported that PYY levels were associated with enhanced satiety during oligofructose intake; Gibbons et al. (2013) found that PYY levels were not associated with changes in appetite and energy intake in response to isoenergetic meals of varying fat and carbohydrate content. This has also been previously demonstrated in obese but not lean individuals (Batterham et al. 2006), which suggests that the PYY response to fat and carbohydrate may depend on the weight status of the participant.

#### 2.4.5 Peptide YY and chronic energy homeostasis

Peptide YY also appears to influence chronic energy homeostasis as continuous and repeated peripheral infusions of PYY<sub>3-36</sub> has been shown to significantly reduce body weight and adiposity in rodents (Batterham et al. 2002; Chelikani et al. 2006; Pittner et

al. 2004; Vrang et al. 2006). Furthermore, this effect appeared to be a result of an increase in whole body fat oxidation and maintenance of metabolic rate as well as a reduction in food intake (Adams et al. 2006; van den Hoek et al. 2007).

In humans, PYY also responds to chronic changes in energy balance as postprandial increases in circulating concentrations are blunted in obese participants; a response that is associated with reduced postprandial satiety (Batterham et al. 2003; 2006; Korner et al. 2005; le Roux et al. 2006a; Stock et al. 2005). In contrast, although some authors have reported depressed fasting PYY concentrations in obesity (Alvarez Bartolomé et al. 2002; Batterham et al. 2003; Guo et al. 2006; le Roux et al. 2006a), this is not a universal finding (Cahill et al. 2011; Korner et al. 2005; Pfluger et al. 2007). The mechanisms underlying a blunted PYY response in obesity are currently unclear but research in obese mice suggests that circulating concentrations are suppressed as a result of impaired postprandial secretion, rather than synthesis, of PYY (Chandarana et al. 2011; le Roux et al. 2006a). Regardless of the mechanisms mediating this effect, it seems plausible that a blunted PYY and satiety response to food consumption may contribute to the maintenance or exacerbation of obesity.

The development of PYY knockout mice has provided further support for this peptide as a physiological regulator of energy homeostasis. In this regard, PYY knockout mice have been shown to be hyperphagic and obese compared with wild-type littermates (Batterham et al. 2006; Boey et al. 2006). Furthermore, daily intraperitoneal administration of PYY<sub>3-36</sub> reversed this phenotype and the subsequent cessation of PYY<sub>3-36</sub> injections stimulated weight regain (Batterham et al. 2006).

These findings are substantiated by experiments involving transgenic mice that moderately overexpress PYY. Although these mice do not differ from wild-type littermates when fed a regular chow diet, transgenic mice appear to be protected against diet-induced obesity. Furthermore, crossing PYY transgenic and leptin deficient mice produced offspring with significantly lower adiposity than leptin deficient controls (Boey et al. 2008). Conditionally transgenic mice in which PYY overexpression is induced in adulthood also do not exhibit an altered phenotype under a regular chow diet. However, these mice exhibit lower food intake during ad libitum conditions and in response to fasting compared with wild-type littermates (Shi et al. 2012). These studies of mutant mice demonstrate a physiological role of PYY in the regulation of energy

homeostasis but the necessity for obesigenic conditions to observe phenotypic changes is unclear.

The PYY response to gastric bypass surgery arguably provides the strongest evidence for a role of this peptide in energy homeostasis. In this regard, the substantial reductions in appetite and successful weight loss achieved by gastric bypass surgery have consistently been associated with marked increases in fasting and postprandial PYY concentrations (Chan et al. 2006b; Korner et al. 2005; le Roux et al. 2006b; Morínigo et al. 2006; Näslund et al. 1997). Furthermore, the blockade of endogenous PYY increased food intake in rats after gastric bypass surgery and exogenous PYY<sub>3-36</sub> infusion reduced food intake in sham operated rats to levels comparable with those subjected to gastric bypass surgery (le Roux et al. 2006b). A recent study by Chandarana et al. (2011) also supported a mediating role of PYY as the decreases in body weight associated with gastric bypass surgery were abolished in PYY knockout mice.

## **2.5 The acute effects of exercise on appetite and food intake**

### **2.5.1 Appetite and energy intake assessment**

Appetite may be defined as the sensory and qualitative aspect of eating behaviour that is responsive to both physiological and environmental influences. Alternatively, although food intake is responsive to the same stimuli, this represents the quantitative aspect of eating behaviour (Blundell et al. 2010). The relationship between appetite and food intake is often imperfect and current recommendations advise that both appetite and food intake are measured within the same experiment (Gregersen et al. 2008; Stubbs et al. 2000). Nevertheless, the measurement of appetite and energy intake in isolation continues to provide useful information as minimising elevations in appetite may provide psychological comfort in response to an energy deficit and the measurement of energy intake alone provides important information regarding energy balance (Blundell et al. 2010).

Appetite perceptions are typically measured using visual analogue scales (VAS). These scales require participants to place a mark on a horizontal line that is typically 100 – 150 mm in length and anchored at each end with descriptive statements (e.g. ‘I am not hungry at all’/ ‘I have never been more hungry’). Although initial questionnaires tended



to focus exclusively on the measurement of hunger, current recommendations suggest that multiple scales should be employed in order to account for the multi-dimensional nature of appetite (Blundell et al. 2010). Such scales typically measure the following aspects of appetite: hunger, satisfaction, fullness, prospective food consumption (Flint et al. 2000).

Acute exercise interventions tend to quantify ad libitum food intake using either a free choice buffet meal or a single course meal of fixed macronutrient composition. Both of these methods can be employed within the laboratory setting, which enables accurate quantification of food intake by weighing food items before and after consumption. Alternatively, some intervention studies allow the participants to leave the laboratory after exercise and monitor food intake using food diaries. Although this increases the external validity of the experiment, self-report measures of food intake are particularly prone to participant bias, which limits the internal validity of this method (Livingstone & Black 2003). It is therefore considered preferable to monitor food intake under laboratory conditions during acute exercise interventions, in order to ensure an accurate quantification of energy intake.

### **2.5.2 Relevance**

The influence of an acute bout of exercise on subsequent appetite and energy intake responses has received considerable interest in the past twenty years and continues to remain a topical issue in the scientific literature. Such continued interest is observed in the following review, which includes 15 journal articles that were published between January 2012 and March 2013, in addition to older articles.

Interest in the appetite and energy intake responses to exercise stems from the acknowledgement that physical activity may enhance weight loss via an increase in energy expenditure (Donnelly et al. 2009). However, the weight loss response to exercise is dependent upon subsequent food intake, as an increase in energy consumption may negate the energy deficit of exercise. Furthermore, any compensatory increases in appetite after exercise are likely to enhance the difficulty of maintaining a negative energy balance and increase psychological discomfort within participants.

The importance of this issue has recently been highlighted in media articles, which have suggested that exercise stimulates compensatory increases in appetite and food

intake that prevent weight loss (Time Magazine 2009) and actually increase body fat (The Daily Telegraph 2009). The conclusions of these articles oppose the majority of findings from the scientific literature, which demonstrate that appetite and energy intake remain largely unchanged in the hours after an acute bout of exercise (Blundell & King 1999; Blundell et al. 2003; Martins et al. 2008). Although this relationship cannot continue indefinitely, the insensitivity of the appetite-regulating system to exercise-induced energy deficits is in stark contrast with the powerful homeostatic responses to food restriction. In this regard, current evidence suggests that food restriction elicits rapid compensatory increases in appetite and food intake, which does not occur in response to an equivalent exercise-induced energy deficit (Hubert et al. 1998; King et al. 2011a). Furthermore, strenuous exercise has been demonstrated to induce an acute paradoxical suppression of appetite (Martins et al. 2008).

### **2.5.3 Exercise-induced anorexia**

Strenuous exercise ( $\geq 60$  % of maximum oxygen uptake ( $\text{VO}_2 \text{ max}$ )) has consistently been found to acutely suppress appetite during and shortly after the exercise bout. This is known as ‘exercise-induced anorexia’ and has been demonstrated during a variety of exercise modes including: running (Broom et al. 2007; Burns et al. 2007; King et al. 2010a), cycling (Becker et al. 2012; Cheng et al. 2009; Evero et al. 2012; Laan et al. 2010; Martins et al. 2007; Thompson et al. 1988; Ueda et al. 2009a), swimming (King et al. 2011b) and resistance exercise (Broom et al. 2009). Although significant changes in appetite have been reported in these studies, values tend to return to control values within 30 min of the cessation of exercise. Such a transient effect is unlikely to influence energy intake in the hours after exercise but may delay the initiation of feeding when food is provided immediately after exercise (King et al. 2013). It therefore remains most important to understand the influence of exercise on resting appetite and energy intake responses in the hours after exercise.

### **2.5.4 Appetite responses in the immediate post-exercise period (0 – 120 min)**

The majority of studies that have investigated the appetite response to exercise have employed an observation period lasting up to 2 h after the exercise bout. The consensus among these studies is that, after the recovery from exercise-induced anorexia, appetite during the post-exercise period does not differ from a resting control trial (Becker et al. 2012; Burns et al. 2007; Erdmann et al. 2007; Evero et al. 2012; Farah et al. 2012;

George & Morganstein 2003; Gonzalez et al. 2013; Hagobian et al. 2013; Kelly et al. 2012; Laan et al. 2010; Larson-Meyer et al. 2012; Martins et al. 2007; Tsofliou et al. 2003; Unick et al. 2010; Ueda et al. 2009a; 2009b). However, although appetite perceptions do not appear to decrease during the post-exercise period, it must be acknowledged that some investigations have reported elevated appetite perceptions after exercise compared with a control trial (Jokisch et al. 2012; Kawano et al. 2013; Verger et al. 1994).

The majority of the studies listed above have investigated the appetite response to exercise in healthy non-obese men and demonstrated no changes in appetite in response to a range of exercise modes including: walking (Farah et al. 2012), cycling (Becker et al. 2012; Evero et al. 2012; Jokisch et al. 2012; Laan et al. 2010; Martins et al. 2007) and running (Burns et al. 2007; Gonzalez et al. 2013; Kelly et al. 2012). The use of male participants in the majority of investigations has led some authors to postulate that sex-based differences may occur in the appetite response to exercise due to the critical relationship between energy balance and reproductive function in females (Wade & Jones 2004). However, current research suggests that females do not exhibit increased appetite perceptions during the 2 h period after exercise (Hagobian et al. 2013; Laan et al. 2010; Larson-Meyer et al. 2012; Martins et al. 2007; Tsofliou et al. 2003; Unick et al. 2010). Furthermore, a recent study by Hagobian et al. (2013) directly compared the appetite response to exercise in male and female participants and concluded that 80 min of cycling at 70 % of  $\text{VO}_2$  max did not stimulate increases in appetite in either sex during the 40 min after exercise.

As obesity is the result of a chronic excess of energy intake over energy expenditure, it is also logical to consider that appetite regulation in response to exercise may differ between lean and obese individuals. However, current research suggests that exercise also fails to stimulate immediate compensatory increases in appetite in overweight and obese populations. In this regard, Unick and colleagues (2010) reported that walking at 70 - 75 % of maximum heart rate to expend  $3 \text{ kcal.kg}^{-1}$  body mass did not stimulate any compensatory increases in appetite during the 60 min after exercise in overweight and obese women. Similarly, Tsofliou et al. (2003) did not observe any increases in appetite during the 60 min after 20 min of brisk walking in ten obese healthy women. Furthermore, in a direct comparison between obese and normal weight young men, Ueda and colleagues (2009b) demonstrated that 60 min of cycling at 50 % of  $\text{VO}_2$  max

did not stimulate any increases in appetite during the 60 min after exercise in either group.

### **2.5.5 Energy intake responses in the immediate post-exercise period (0 – 120 min)**

The majority of studies listed in the previous section also assessed the food intake response to exercise by providing participants with an ad libitum meal  $\leq 2$  h after the exercise bout. In accordance with the appetite responses detailed in the previous section, the majority of these studies demonstrated that energy intake was unaffected by exercise (Balaguera-Cortes et al. 2011; George & Morganstein 2003; Gonzalez et al. 2013; Hagobian et al. 2013; Kelly et al. 2012; King et al. 1994; Maraki et al. 2005; Tsofliou et al. 2003; Unick et al. 2010). However, some studies have demonstrated increases (Laan et al. 2010; Martins et al. 2007; Shorten et al. 2009; Verger et al. 1994) or decreases (Almada et al. 2013; Ueda et al. 2009a; 2009b; Westerterp-Plantenga et al. 1997) in energy intake after exercise.

An early study to investigate the effect of exercise on subsequent food intake responses was performed by King and colleagues in 1994. In this study, cycling at 30 % or 70 % of  $VO_2$  max to expend  $\sim 1460$  kJ did not affect energy intake at an ad libitum buffet meal 15 min after exercise. Furthermore, in a second group of healthy young men, energy intake was unaffected by either 26 or 52 min of cycling at 75 % of  $VO_2$  max. Many of these findings were replicated by Erdmann and colleagues (2007) in a combined sample of normal-weight men and women, as energy intake was unaffected by 30 min of cycling at either 50 or 100 W. However, although energy intake was unchanged in response to 60 min of cycling at a fixed work rate of 50 W, increasing the duration to 120 min stimulated an increase in energy intake. Although these findings suggest that the energy intake response to exercise is dependent upon exercise duration, all trials commenced after a 12 h overnight fast with the ad libitum meal provided 15 min after exercise. Therefore the observed increases in energy intake may have been a result of the extended overnight fast rather than exercise duration *per se*.

Although these studies may be criticised for the provision of a buffet meal so close to the cessation of exercise, other authors have also demonstrated no change in energy intake after exercise when the ad libitum meal was provided up to 1 h after exercise. This relationship has been demonstrated in young men and women (Balaguera-Cortes et al. 2011; Gonzalez et al. 2013; Kelly et al. 2012; Maraki et al. 2005), overweight and

obese women (Tsofliou et al. 2003; Unick et al. 2010) and in response to a variety of exercise modes. Furthermore, Hagobian et al. (2013) reported no change in energy intake in response to exercise in men and women matched for age and  $\text{VO}_2$  max.

The potential influence of exercise mode on the energy intake response to exercise has been demonstrated by Larson-Meyer et al. (2012). In this study, energy intake from an ad libitum meal that was provided two hours after exercise was not affected by 60 min of running at 70 % of  $\text{VO}_2$  max but increased in response to 60 min of walking exercise at the same relative intensity in a separate group of participants. Although this study suggests that exercise mode may influence energy intake responses, it is plausible that the higher percentage body fat and lower  $\text{VO}_2$  max in the walking group may have confounded the results.

In this regard, Finlayson and colleagues (2009) demonstrated that body fat and physical activity levels may influence the energy intake response to exercise in females. This novel study analysed the energy intake response to 50 min of cycling exercise and separated the participants into two groups: compensators and non-compensators. Compensators were defined as the participants that increased energy intake beyond the energy cost of exercise, whereas non-compensators consumed less energy than that expended during exercise. Analysis of between group differences revealed a significantly higher BMI and percentage body fat and a lower habitual exercise frequency in the compensators. Alternatively, recent evidence suggests that this relationship may not occur in men as Jokisch and colleagues (2012) demonstrated that 45 min of cycling at 65 – 75 % of maximum heart rate decreased energy intake in a sample of inactive normal weight young men but not active normal weight young men.

Other studies have also demonstrated a decrease in energy intake but are confounded by the provision of the ad libitum meal within 10 min of exercise completion (Almada et al. 2013; Westerterp-Plantenga et al. 1997), which may have prevented the recovery from exercise-induced anorexia prior to feeding. However, in support of these findings, Ueda and colleagues (2009b) observed a decrease in energy intake at an ad libitum meal that was provided sixty minutes after a 60 min bout of cycling at 50 % of  $\text{VO}_2$  max. Furthermore, this reduction in energy intake occurred in both lean and obese male participants. Other authors have also reported similar energy intake responses in normal

weight and overweight participants (George & Morganstein 2003) but this is not a universal finding (Kissileff et al. 1990).

Some authors have demonstrated an increase in energy intake during the immediate post-exercise period (Laan et al. 2010; Martins et al. 2007; Shorten et al. 2009; Verger et al. 1994). The reasons for such variations in the energy intake response to exercise are unclear but may be influenced by a variety of factors including: participant differences, the composition and timing of the ad libitum meal, variations in exercise mode, and time of day effects. Although measures of absolute energy intake provide important information regarding feeding behaviour, King and colleagues (1994) suggested that it may be more relevant to express the energy intake response to exercise as 'relative energy intake' (REI) after deducting the net energy cost of exercise. Subsequently, all increases in absolute energy intake reported thus far in this review are negated after accounting for the energy expenditure of exercise. Although this approach provides an overview of energy balance, the short monitoring period of the studies described thus far bias the results towards a lower REI during exercise trials as food intake is unlikely to be upregulated sufficiently at a single feeding episode to overturn substantial energy deficits. Therefore, investigations into the energy intake response to multiple ad libitum meals are important and are discussed below.

#### **2.5.6 Appetite responses beyond a single test meal (2 – 9 h)**

Although some authors have suggested that exercise may stimulate appetite in response to a standardised meal in the post-exercise period (Malkova et al. 2008), the majority of studies demonstrate that appetite does not increase above control values during the 2 – 9 hours after exercise. This includes a variety of exercise modes including: running (King et al. 1997; King et al. 2010a; Broom et al. 2009; Wasse et al. 2012; 2013), cycling (Wasse et al. 2013), walking (Borer et al. 2005; 2009; King et al. 2010b; Pomerleau et al. 2004) and resistance exercise (Broom et al. 2009) in both male and female populations.

However, exceptions have been observed within the literature as King et al. (2011b) demonstrated that 60 min of intermittent swimming exercise stimulated an increase in appetite compared with a resting control trial from 1.5 – 6 h post-exercise. This contrasts with previous findings from the same author as 60 min of brisk walking exercise (King et al. 2010b) and 90 min of running exercise (King et al. 2010a) did not

influence resting appetite perceptions during the 7 h and 8.5 h post-exercise period, respectively. These differences occurred despite utilising a similar study protocol and participant population of physically active young men, which suggests a potential influence of exercise mode on subsequent appetite responses. However, subtle changes in meal timing and the recruitment of different participants confound any inferences regarding exercise mode.

The potential confounding influence of these factors is demonstrated by the findings of two studies from Broom and colleagues (2007; 2009), as 60 min of running at 70 % of  $\text{VO}_2$  max in young physically active males stimulated an increase in hunger from 2 – 8 h after exercise in the former but not the latter of the two studies. Such discrepancies highlight the need for within measures study designs when comparing the appetite response to different exercise modes.

### **2.5.7 Energy intake responses beyond a single test meal (2 – 9 h)**

Few studies have monitored food intake in a laboratory setting for more than two hours after an acute exercise bout. However, the available research suggests that exercise does not stimulate any changes in energy intake during the subsequent 22.5 h (King et al. 2010a). This finding is also supported by studies that have used self-report measures of food intake, which have failed to discover any changes in energy intake during the 24 h (Hanlon et al. 2012), 48 h (King et al. 1997) and 72 h (Pomerleau et al. 2004) after exercise.

Studies that have successfully performed prolonged monitoring of food intake in a laboratory setting have typically provided participants with one or two buffet meals during the 3 to 7.5 h after exercise. Studies from our laboratory have reported that energy intake is unchanged by exercise under these laboratory conditions at any of the provided feeding opportunities (King et al. 2010a; 2010b; 2011a; 2011b; Wasse et al. 2012). Furthermore, the provision of an overnight food bag upon leaving the laboratory demonstrated that energy intake continued to remain unchanged for 22.5 h after exercise (King et al. 2010a).

The importance of such prolonged monitoring of energy intake is demonstrated by Pomerleau et al. (2004). In this study, energy intake at an ad libitum buffet meal was significantly higher 1 h after walking at 70 % of  $\text{VO}_2$  max compared with a resting

control. However, after the provision of an additional ad libitum meal 6.5 h after exercise and an overnight snack bag, energy intake did not differ significantly between the trials.

One weakness of the above studies is that meals were provided to participants at pre-defined time points during the trials, which constrains the opportunities for food intake and may hinder the detection of differences in energy intake. This issue was recently addressed by King et al. (2013) who allowed participants unlimited access to a buffet meal during the 6 h after 60 min of running at 70 % of  $\text{VO}_2$  max. In support of previous findings, energy intake remained unchanged after exercise compared with a resting control trial and resulted in a substantially lower REI after exercise.

### **2.5.8 Effect of exercise on macronutrient intake**

The use of ad libitum buffet meals to investigate the food intake response to exercise also enables the quantification of macronutrient intakes. This is an important aspect of studies investigating the food intake response to exercise as the consumption of a high fat meal has been demonstrated to overturn the energy deficit of exercise (Lluch et al. 1998). Although a small number of studies have demonstrated a variety of changes in macronutrient intake in response to exercise (Pomerleau et al. 2004; Thompson et al. 1988; Verger et al. 1994; Wasse et al. 2012; Westerterp-Plantenga et al. 1997), the majority of experiments have reported that macronutrient intake is unchanged in response to exercise (Balaguera-Cortes et al. 2011; George & Morganstein 2003; Hagobian et al. 2013; Imbeault et al. 1997; Jokisch et al. 2012; Kelly et al. 2012; King et al. 1994; King et al. 2010a; 2010b; 2011a; 2011b; Larson-Meyer et al. 2012; Martins et al. 2007; Shorten et al. 2009; Tsofliou et al. 2003).

### **2.5.9 Manipulating exercise protocols to produce the most beneficial responses**

This review has demonstrated a general consensus among the literature that an acute bout of exercise does not stimulate compensatory increases in appetite and energy intake during the hours after exercise. With this knowledge, it may be beneficial for future research to focus on establishing the most effective exercise protocols to reduce appetite and energy balance in the hours after exercise.

Few studies thus far have manipulated exercise protocols using a within measures design. However, Cheng and colleagues (2009) recently compared the appetite



response to moderate intensity cycling exercise when performed either two hours after a high fat breakfast or after a 12 h overnight fast. This study found that exercise induced a more prolonged suppression of hunger when performed after breakfast consumption rather than after an overnight fast. These preliminary findings highlight a potential avenue for future investigations.

More commonly, investigators have manipulated the intensity of exercise when comparing exercise protocols but have thus far yielded equivocal findings. In this regard, Thompson and colleagues (1988) demonstrated greater appetite suppression during cycling exercise at 68 % of  $\text{VO}_2$  max compared with 35 % of  $\text{VO}_2$  max but found no differences in energy intake after exercise. Conversely, Imbeault et al. (1997) found no differences in appetite but reported a decrease in energy intake after running at 75 % of  $\text{VO}_2$  max compared with energy-matched walking at 35 % of  $\text{VO}_2$  max. Alternatively, Ueda and colleagues (2009a) and King et al. (1994) found no differences in the appetite or energy intake responses to cycling at 50 % versus 75 % of  $\text{VO}_2$  max and 30 % versus 70 % of  $\text{VO}_2$  max, respectively. Although equivocal, these findings demonstrate that the appetite and energy intake response to exercise may be influenced by exercise intensity. Furthermore, several recent authors have postulated that HIIE may promote greater weight loss than traditional endurance exercise due to greater reductions in appetite during the post-exercise period (Boutcher 2011; Heydari et al. 2012; Trapp et al. 2008; Tremblay et al. 1994). However, despite such postulations, the appetite and energy balance responses to HIIE have not yet been investigated and warrant future research.

## **2.6 The acute effects of exercise on ghrelin and PYY**

### **2.6.1 Relevance**

Observations of appetite suppression during exercise and the short-term insensitivity of appetite perceptions to exercise-induced energy deficits stimulated significant interest in the mechanisms responsible for these effects. In this regard, appetite-regulating gut hormones have received particular attention as episodic mediators of appetite and food intake. Of these hormones, ghrelin has received explicit attention as the only orexigenic gut peptide and although less research has focussed on the effects of exercise on anorectic gut peptides, an increasing number of studies have measured circulating concentrations of PYY due to its potent appetite suppressive effects. In support of a

mediating role of these peptides in the appetite response to exercise, a recent study by King et al. (2011a) concluded that divergent changes in acylated ghrelin and PYY<sub>3-36</sub> may be responsible for the contrasting appetite and energy intake response to equivalent energy deficits induced via exercise and food restriction. Further literature investigating the effects of exercise on ghrelin and PYY are detailed in the following sections.

### **2.6.2 Ghrelin**

Due to the conspicuous nature of ghrelin as the only orexigenic peptide in the peripheral circulation, many early studies postulated that changes in circulating ghrelin concentrations may be responsible for the acute reductions in hunger observed during exercise (Burns et al. 2007). Further interest was also generated after the discovery that ghrelin concentrations were increased in response to chronic energy deficits (Otto et al. 2001), with authors speculating that increases may also occur in response to acute exercise-induced energy deficits as a stimulus to increase appetite and food intake (Burns et al. 2007; Erdmann et al. 2007).

Research into the effects of exercise on total ghrelin concentrations has yielded equivocal findings with studies showing increases (Borer et al. 2009; Christ et al. 2006; Erdmann et al. 2007; Russel et al. 2009), decreases (Cheng et al. 2009; Toshinai et al. 2007; Vestergaard et al. 2007) and no change (Burns et al. 2007; Dall et al. 2002; Martins et al. 2007) in circulating ghrelin concentrations in response to an acute bout of exercise. Furthermore, these changes in total ghrelin concentrations appeared to oppose the appetite and energy intake responses to exercise in the studies that simultaneously measured these parameters (Borer et al. 2009; Burns et al. 2007; Cheng et al. 2009; Erdmann et al. 2007; Martins et al. 2007).

However, the orexigenic actions of ghrelin are solely mediated by the acylated form of this peptide (Neary et al. 2006) and the measurement of total ghrelin has been shown to mask changes in acylated ghrelin (Hosoda et al. 2004). Therefore, Broom and colleagues (2007) sought to reinvestigate this issue by specifically measuring acylated ghrelin. In this study, 60 min of running at ~ 72 % of VO<sub>2</sub> max induced a significant suppression of acylated ghrelin that was concomitant with a reduction in hunger during and immediately upon completion of exercise. The significant correlation between acylated ghrelin and hunger during the exercise bout suggested that exercise may

differentially affect acylated and total ghrelin concentrations. This was confirmed by Marzullo and colleagues (2008) who demonstrated that acylated ghrelin was suppressed immediately upon completion of an incremental cycle test to exhaustion but that total ghrelin remained unchanged. Furthermore, this divergence occurred in both normal weight and obese participants and was unaffected by the duration of the exercise test.

The simultaneous suppression of appetite and acylated ghrelin during strenuous exercise has been demonstrated in the majority of subsequent investigations (Becker et al. 2012; Broom et al. 2009; Kawano et al. 2013; King et al. 2010a; 2011a; 2011b; Wasse et al. 2012). Such consistent findings contrast those of total ghrelin and suggest that exercise may interfere with the acylation of ghrelin rather than the production or release of ghrelin. A mechanism for this effect is currently unknown but may involve a decrease in blood flow and oxygen delivery to the splanchnic circulation during exercise.

Support for a mediating influence of acylated ghrelin on appetite perceptions during exercise is further strengthened by the findings of several studies that exercise of a mild or moderate intensity does not stimulate any changes in either acylated ghrelin or appetite during exercise (King et al. 2010b; Larson-Meyer et al. 2012; Morris et al. 2010; Ueda et al. 2009b; Unick et al. 2010). However, although the majority of studies demonstrate a concordance between acylated ghrelin and appetite during exercise, this is not true for all studies as Wasse et al. (2013) observed a suppression of acylated ghrelin during strenuous running and cycling exercise but no changes in hunger perceptions.

Additionally, recent studies that have compared the influence of different exercise modes on acylated ghrelin and appetite responses have revealed small divergences between these variables. This was demonstrated in a recent study by Kawano et al. (2013), as acylated ghrelin concentrations were suppressed to a similar extent during skipping and cycling exercise but skipping induced a greater suppression of appetite. Similarly, Broom et al. (2009) reported a greater decrease in hunger during running compared with resistance exercise despite a similar suppression of acylated ghrelin during both exercise bouts. These findings suggest that the comparison of a single exercise trial with a control trial may overestimate the role of acylated ghrelin in mediating exercise-induced changes in appetite. Further research comparing different

exercise protocols is required to elucidate the strength of the relationship between acylated ghrelin and appetite during exercise.

The majority of studies investigating the acylated ghrelin response to exercise have ceased measurement upon provision of an ad libitum meal within two hours of exercise and have demonstrated a rapid recovery of acylated ghrelin to control values after exercise, with values remaining similar thereafter (Hagobian et al. 2013; Kelly et al. 2012; Shorten et al. 2009; Unick et al. 2010). However, discrepant findings have also been reported with studies demonstrating a continued suppressed of acylated ghrelin concentrations at 30 min (Balaguera-Cortes et al. 2011) and 120 min after exercise (Kawano et al. 2013). Alternatively, to the author's knowledge only one study has demonstrated an increase in acylated ghrelin concentrations during the two hour period after exercise (Larson-Meyer et al. 2012). Furthermore, although the majority of these studies have reported a temporal association between acylated ghrelin and appetite during this period (Hagobian et al. 2013; Kelly et al. 2012; Ueda et al. 2009b; Unick et al. 2010), this is not a universal finding (Kawano et al. 2013; Larson-Meyer et al. 2012). The reasons for such discrepancies are not clear as the studies with opposing findings have typically employed similar experimental protocols and participant populations.

In order to better understand the acylated ghrelin response to exercise, several recent studies have measured changes in this peptide over a longer period and in response to feeding after exercise. In accordance with previous findings, the majority of these studies have demonstrated that plasma acylated ghrelin concentrations remain unchanged compared with a resting control trial during the 6 – 8.5 h after exercise (Broom et al. 2009; King et al. 2010a; 2010b; 2011a; 2011b; Wasse et al. 2012). However, other studies have reported a continued suppression of acylated ghrelin for 3 h (Wasse et al. 2013) and 8.5 h after exercise (Broom et al. 2007), despite all experiments being performed by the same research group at Loughborough University with similar exercise protocols and participant characteristics.

These studies are consistent in the finding that acute exercise does not stimulate short-term increases in acylated ghrelin concentrations. This finding was further extended by King et al. (2010a) who reported that an exercise-induced energy deficit of ~ 5000 kJ did not stimulate any increases in acylated ghrelin during a fasted blood sample 22.5 h after exercise when compared with a resting control trial. In contrast, only one study

has reported an increase in acylated ghrelin during a prolonged post-exercise period. In this regard, Morris and colleagues (2010) revealed an increase in circulating acylated ghrelin during the 4 – 10 h after a 60 min bout of cycling at 50 % of  $\text{VO}_2$  peak in healthy males. However, the findings of this study must be interpreted with caution as the experiment was performed during an overnight period with trials starting at 7 pm and ending at 5 am. These contrasting findings may therefore be a due to a time of day effect and may only be applicable to individuals that remain awake during the nocturnal period, such as night shift workers.

The majority of studies that have monitored acylated ghrelin concentrations for a prolonged period after exercise have demonstrated a temporal pattern that is consistent with perceptions of appetite (Broom et al. 2009; King et al. 2010a; 2010b; 2011a; Morris et al. 2010; Wasse et al. 2012). However, this finding is not universal as Wasse et al. (2013) demonstrated a continued suppression of acylated ghrelin for three hours after exercise but hunger perceptions remained unchanged between trials. Furthermore, acylated ghrelin remained suppressed during the eight hours after 60 min of running at 70 % of  $\text{VO}_2$  max in a study by Broom et al. (2007), yet appetite was significantly elevated during this period. Similarly contrasting findings have also been reported by King et al. (2011b) as 60 min of swimming exercise stimulated significant increases in appetite during the 1 – 5 hours after exercise but acylated ghrelin concentrations did not differ between trials.

The reasons for such divergence between acylated ghrelin and appetite in these studies are unclear but this effect questions the causal nature of previously observed correlations between these variables. A greater understanding of the acylated ghrelin response to exercise is certainly required. In this regard, it may be particularly useful to manipulate exercise protocols in order to stimulate greater exercise-related changes in appetite and potentially acylated ghrelin concentrations in order to understand the interaction between these measures.

### **2.6.3 Peptide YY**

The first study to measure circulating PYY concentrations in response to exercise was performed by Martins et al. (2007). In this study, 60 min of cycling at 65 % of maximum heart rate stimulated an increase in plasma PYY concentrations during and upon completion of exercise in healthy young men and women. This increase in PYY

occurred concomitantly with a reduction in hunger and both parameters returned to control values within 30 min of exercise, which suggested a mediating role of this anorectic peptide in exercise-induced anorexia. Such transient increases in circulating total PYY concentrations have since been replicated in response to 60 min of cycling at 50 % of  $\text{VO}_2$  max in normal weight and obese young men (Ueda et al. 2009b) as well as 60 min of running at 70 % of  $\text{VO}_2$  max in healthy young men (Wasse et al. 2012).

Although total PYY concentrations returned to control values within 30 min of exercise cessation in the aforementioned studies, Broom et al. (2009) found PYY to remain elevated for 4.5 h after 60 min of running at 70 % of  $\text{VO}_2$  max. This finding seems counterintuitive to the energy deficit induced via exercise but is supported by more recent findings that total PYY concentrations do not decrease in response to exercise-induced energy deficits (Wasse et al. 2012). Interestingly Broom and colleagues (2009) also demonstrated an elevation in PYY during aerobic but not resistance exercise, which was accompanied by a greater suppression of hunger during the aerobic exercise. Although this strengthens the causal relationship between changes in PYY and appetite perceptions during exercise, appetite during the hours after exercise did not differ between trials despite the continued elevation in PYY after aerobic exercise.

In contrast to the above findings, other authors have found total PYY concentrations to be unaffected by 40 - 45 min of running at 70 % of  $\text{VO}_2$  max (Balaguera-Cortes et al. 2011; Kelly et al. 2012; Shorten et al. 2009). The reasons for such confliction are unclear but exercise duration may play a role as all of these studies employed shorter exercise protocols than those that have demonstrated an increase in PYY with exercise. Furthermore, Balaguera-Cortes et al. (2011) confirmed previous findings that total PYY concentrations do not change in response to intermittent resistance exercise (Broom et al. 2009). Although these findings may suggest that a continuous prolonged exercise protocol is required to increase circulating PYY concentrations, Kawano et al. (2013) recently demonstrated that 30 min of skipping and cycling exercise at ~ 65 % of  $\text{VO}_2$  max induced a transient increase in total PYY upon completion of exercise. In accordance with the anorexigenic properties of PYY, appetite perceptions appear to decrease during exercise when PYY concentrations are increased (Kawano et al. 2013) but remain unaffected when circulating levels of PYY are unchanged (Kelly et al. 2012). However, appetite has not been simultaneously measured with PYY in all studies (Balaguera-Cortes et al. 2011; Shorten et al. 2009) and although post-exercise

PYY responses are in agreement with appetite and energy intake responses in some studies (Balaguera-Cortes et al. 2011; Kelly et al. 2012; Shorten et al. 2009; Wasse et al. 2012), this is not a universal finding (Broom et al. 2009; Kawano et al. 2013; Martins et al. 2007; Ueda et al. 2009b).

The measurement of total PYY in the aforementioned studies includes concentrations of both PYY<sub>1-36</sub> and PYY<sub>3-36</sub>. However, the appetite-suppressing effects of PYY are believed to be mediated specifically by PYY<sub>3-36</sub> (Sloth et al. 2007). Therefore, although a strong correlation between changes in total PYY and PYY<sub>3-36</sub> has been observed (Tsilchorozidou et al. 2008), some studies have sought to specifically measure the response of PYY<sub>3-36</sub> to exercise.

The immediate PYY<sub>3-36</sub> response to exercise appears to be similar to total PYY as transient increases have been observed during and immediately upon completion of 60 min of walking and running exercise at 70 % of VO<sub>2</sub> max (Larson-Meyer et al. 2012), as well as upon completion of prolonged exhaustive running exercise (Russel et al. 2009). Increases have also been observed in response to 30 min of cycling at 50 % and 75 % of VO<sub>2</sub> max (Ueda et al. 2009a). However, immediate increases in PYY<sub>3-36</sub> have not been observed in all studies as Cheng et al. (2009) reported no immediate increases upon completion of 50 min of cycling at 60 % of VO<sub>2</sub> max. Furthermore, Hagobian et al. (2013) reported no changes in PYY<sub>3-36</sub> in response to ~ 80 min of cycling at 70 % of VO<sub>2</sub> max in men despite a transient increase immediately and 15 min upon completion of a similar exercise bout in women. Such confliction between studies supports the findings of the studies that have measured total concentrations of PYY and suggests that previous discrepancies may not have been due to the measurement of total PYY masking changes in PYY<sub>3-36</sub>.

To the author's knowledge only two studies have investigated the PYY<sub>3-36</sub> response to exercise for a prolonged period with the inclusion of test meals (Cheng et al. 2009; King et al. 2011a). In this regard, both studies have demonstrated a prolonged elevation in PYY<sub>3-36</sub> during the 4 – 6.5 h after exercise, despite large differences in exercise protocols. Although these findings may suggest that aerobic exercise induces prolonged increases in PYY<sub>3-36</sub> when performed for 50 – 90 min at 60 – 70 % of VO<sub>2</sub> max, further investigations into this effect are required before conclusions can be made.

The relationship between PYY<sub>3-36</sub> and appetite during exercise also requires further investigation as an inverse temporal pattern has been reported in some (King et al. 2011a; Ueda et al. 2009a) but not all studies (Hagobian et al. 2013; Larson-Meyer et al. 2012). Additionally, although King and colleagues (2011a) recently concluded that PYY<sub>3-36</sub> is a key mediator of the contrasting appetite and energy intake responses to exercise and food restriction, other studies have reported changes in appetite and energy intake in the post-exercise period that appeared to be unrelated to PYY<sub>3-36</sub> concentrations (Cheng et al. 2009; Larson-Meyer et al. 2012).

Further information is clearly required regarding the influence of exercise on plasma PYY<sub>3-36</sub> concentrations and the importance of these changes in determining appetite and energy intake responses.

## **2.7 Summary**

A general consensus among the scientific literature suggests that an acute bout of exercise does not stimulate compensatory increases in appetite and energy intake during the hours after exercise. However, despite such a consensus, several authors have suggested that variations may exist between exercise protocols with some eliciting lower appetite and energy intake responses than others. Such comparisons require a within measures study design in order to remove the bias of participant differences but this issue has thus far received little attention. Furthermore, despite attracting significant interest in recent years, the role of the gut hormones acylated ghrelin and PYY in determining the appetite response to exercise is unclear. The manipulation of exercise protocols also allows further comparisons to be made regarding the relationship between these gut peptides and appetite and feeding responses. This thesis therefore aims to manipulate methods of creating an energy deficit in order to uncover potential strategies for minimising subsequent appetite perceptions and energy intake, as well as further investigating the role of acylated ghrelin and PYY as determinants of these responses.



## CHAPTER III

### General Methods

This chapter describes the experimental methods employed in the studies presented within this thesis. All studies were conducted following the approval of Loughborough University's Ethics Advisory Committee and all volunteers provided written informed consent before participating in these experiments.

#### 3.1 Participants

For the experiments reported in this thesis, participants were recruited from Loughborough University and the local area by word of mouth and email advertising. Volunteers were given a participant information sheet that explained the purpose, protocol and demands of the study as well as any potential risks and discomforts. After a verbal explanation of the study and discussion of any questions, volunteers completed an informed consent form (Appendix A) and a health screen questionnaire (Appendix B) before any experimental procedures began. Participants also completed a questionnaire assessing physical activity levels (Appendix C) and a questionnaire assessing eating habits (Stunkard & Messick 1985) (Appendix D) to screen for confounding eating behaviours. A food preference questionnaire (Appendix E for Chapter 4; Appendix F for Chapter 5; Appendix G for Chapters 6 & 7) was also completed to ensure the acceptability of food items provided during the experiments. Most of the participants were students studying at Loughborough University and were physically active. Prior training was not a prerequisite for participation in these studies but the physical demands of the exercise protocols ensured that all participants were reasonably fit.

The inclusion criteria for participation were as follows:

- male
- non-smoker
- aged 18 – 35 years
- no personal history of cardiovascular disease, metabolic disease or dyslipidaemia
- not dieting
- non-extreme dietary habits

- not taking drugs known to affect digestion or metabolism
- sufficient ability to complete the exercise protocols
- weight stable for the last 3 months, i.e. < 2.3 kg change in body weight (St Jeor et al. 1997)
- tolerance for food items provided during the experiments

### **3.2 Anthropometry**

Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca Ltd, Germany) and body mass was measured to the nearest 0.1 kg using a digital scale (Seca 770, Seca Ltd, Germany). Participants wore light clothing and removed shoes, jewellery and all items from pockets for height and body mass measurements. Body mass index was subsequently calculated as weight in kilograms divided by squared height in metres. Waist circumference was measured with an inelastic polyfibre tape measure (Hokanson, Washington, USA). The measurement was taken at the end of expiration at the narrowest part of the torso between the xiphoid process and the iliac crest (Ross et al. 2008).

Measurements of subcutaneous fat were taken to estimate total body fatness. Skinfold thickness was measured using callipers (Baty International, West Sussex, UK) with the participant standing in a relaxed position. Measurements were taken at the following sites on the right hand side of the body according to ACSM guidelines (2006):

- 1) Triceps - Vertical fold; on the posterior midline of the upper arm, half way between the acromion and olecranon processes, with the arm held freely to the side of the body.
- 2) Biceps - Vertical fold; of the anterior aspect of the arm over the belly of the biceps muscle, 1 cm above the level used to mark the triceps site.
- 3) Subscapular- Diagonal fold at a 45° angle; one centimetre below the inferior angle of the scapula.
- 4) Suprailiac - Diagonal fold; in line with the natural angle of the iliac crest taken in the anterior axillary line immediately superior to the iliac crest.

Each skinfold was lifted by the experimenter's left hand using the thumb and index finger. The skinfold calipers were placed 1 cm away from the thumb and finger,

perpendicular to the skinfold and half way between the crest and base of the fold. The measurement was taken within two seconds of calliper pressure while maintaining the pinch of the skinfold. Each site was measured in triplicate and a fourth measure was taken if previous measurements were not within two millimetres of each other. Measurements were made by rotating through the anatomical sites to allow time for the skin to regain normal texture and thickness. The mean skinfold thickness of each site was calculated and the sum of the sites was entered into the predictive equations of Durnin and Womersley (1974) to calculate body density. Percentage body fat was then estimated using the Siri equation (Siri 1956).

### **3.3 Heart rate measurement**

Heart rate was measured during exercise on preliminary tests and main trials using short-range radio telemetry (Polar T31; Polar Electro, Kempele, Finland).

### **3.4 Ratings of perceived exertion**

The Borg scale was used to ascertain the participant's perceived level of exertion during exercise testing on preliminary sessions and main trials (Borg 1973). This scale ranges from six (no exertion) to 20 (maximal exertion).

### **3.5 Preliminary exercise tests**

#### **3.5.1 Preliminary running tests**

In Chapter 4, participants completed two preliminary exercise tests on a level motorised treadmill (RUNRACE, Technogym, Gambettola, Italy). After familiarisation with the testing equipment, participants completed a 16 min submaximal incremental running test to determine the relationship between running speed and oxygen consumption. The test was designed to exercise participants through a range of intensities from moderate to vigorous, but not maximal. The test was continuous in nature but was divided into four, 4 min stages. The initial running speed was set between 6.5 and 12 km.h<sup>-1</sup> depending on the fitness level of each participant and was increased by 1 - 1.5 km.h<sup>-1</sup> after the completion of each 4 min stage. Samples of expired gas were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, UK) during the final minute of each stage for the determination of oxygen uptake and heart rate and ratings of perceived exertion (RPE) were also monitored at this time. Oxygen consumption was

plotted against running speed for each stage to identify the submaximal running speed-oxygen consumption relationship.

After a 20 – 30 min rest,  $\text{VO}_2$  max was determined using an incremental uphill treadmill running test at constant speed to volitional exhaustion (Jones & Doust 1996). Run speed was set at the speed corresponding to a heart rate of  $\sim 150 \text{ beat}\cdot\text{min}^{-1}$  or an RPE of 12 on the submaximal exercise test. The test commenced on a level treadmill and the incline increased by 1 % every minute until volitional exhaustion, which was reached within 9 – 12 min. Maximum oxygen uptake was determined from an expired gas sample collected during the final minute of the test when participants signalled that they could only continue for an additional 1 min. Heart rate and RPE were monitored throughout the test and verbal encouragement was provided throughout.

The achieved maximum oxygen uptake of each participant was used in combination with individual running speed-oxygen uptake regression equations to determine the running speed corresponding to 70 % of maximum oxygen uptake. Participants began the treadmill exercise at this speed during the main trials (Chapter 4) but the treadmill speed was adjusted to account for cardiovascular drift if necessary.

### **3.5.2 Preliminary cycling test**

In Chapters 5 – 7, participants completed a continuous incremental exercise test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport V2, Groningen, Netherlands) to determine submaximal oxygen consumption and  $\text{VO}_2$  max. Participants began cycling at a work rate of 95 W, with increments of 35 W every 3 min until volitional fatigue. Samples of expired gas were collected into Douglas bags from 1:45 - 2:45 min of each three minute stage to determine submaximal oxygen consumption. Maximum oxygen uptake was determined from an expired gas sample collected during the final minute of the test when participants signalled that they could only continue for an additional 1 min. Heart rate was monitored continuously during the test and RPE was collected during the expired gas collections. Verbal encouragement was provided throughout the test.

Submaximal oxygen consumption was plotted against cycling work rate at each stage to identify the submaximal cycling work rate-oxygen consumption relationship. The determined  $\text{VO}_2$  max was used in combination with the individual cycling work rate-

oxygen uptake regression equation of each participant to determine the work rate that was necessary to elicit the desired percentage of maximum oxygen uptake during exercise in the main trials (65 % of  $\text{VO}_2$  max in Chapters 5 and 7; 60 % of  $\text{VO}_2$  max in Chapter 6). Participants began exercising at this work rate during the main trials but adjustments were made to account for cardiovascular drift if necessary.

### **3.6 Criteria for maximum oxygen uptake**

The criteria for attainment of  $\text{VO}_2$  max was to fulfil  $\geq 2$  of the following criteria: (1) respiratory exchange ratio (RER)  $> 1.1$ , (2) heart rate within 10 beats  $\cdot \text{min}^{-1}$  of age-predicted maximum, and (3) RPE  $> 18$ .

### **3.7 Expired gas analysis**

Expired gas samples were collected into Douglas bags. Oxygen consumption and carbon dioxide production were determined using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser, respectively (Servomex 1440, Crowborough, East Sussex, UK). Prior to sample analysis, the analysers were calibrated with certified reference gases. Expired gas volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, UK) and the expired gas temperature was determined using a thermistor during evacuation (Edale, type 2984, Model C, Cambridge, UK). Barometric pressure was measured using a Fortin barometer (F.D. and company, Watford, UK). Expired gas samples were corrected to standard temperature and pressure (dry).

### **3.8 Calculation of energy expenditure**

For expired gas samples collected at rest and during exercise, oxygen consumption and carbon dioxide production values were used to determine substrate oxidation and energy expenditure using the equation of Frayn (1983).

In Chapter 5, the high levels of nausea experienced by participants during repeated Wingate tests rendered the collection of expired gas samples impractical. Therefore, energy expenditure was estimated using the methods of Medbø et al. (1988). Individual regression equations were developed for each participant from the cycling work rate-energy expenditure relationship during the maximum oxygen uptake test and used to estimate the energy expenditure of the average work rate of the 30 min session, thereby attempting to account for warm up, warm down, sprint and recovery periods. This

method has been validated to provide a reliable relationship between work rate and energy expenditure (Medbø et al. 1988) and as an estimate of the energy cost of supramaximal exercise (Scott et al. 1991). However, it remains plausible that energy expenditure may have been underestimated as this method does not account for potential further increases in energy expenditure as a result of elevations in body temperature, circulation and ventilation during the recovery periods (Børsheim & Bahr 2003) or due to reduced cycling efficiency at supramaximal workloads (Hunter et al. 1998).

### **3.9 Physical activity and dietary control**

For each experimental chapter presented in this thesis (Chapters 4 - 7), all participants completed a weighed food diary in the 24 h before their first main trial and replicated this before each subsequent trial. Participants were instructed to consume identical amounts of food and drink items at identical times during this period to ensure dietary standardisation before each trial. Participants also abstained from alcohol, caffeine and structured sessions of physical activity during the day preceding each main trial. Although the provision of a pre-packaged diet is thought to produce greater dietary standardisation and control prior to experimental trials, this method requires additional time and resources that were not available for the experiments performed throughout this thesis. Furthermore, such an intervention may alter the habitual diet of participants and subsequently influence metabolic variables (Jeacocke & Burke 2010). Therefore, the completion and replication of a weighed food diary was deemed preferable based on the resources available for the experiments contained within this thesis and in order for participants to maintain habitual dietary intakes.

On the morning of the main trials, participants reported to the laboratory having fasted for a minimum of 10 h but with ad libitum water consumption allowed during this fasting period. Participants exerted themselves minimally when travelling to the laboratory and travelled via motorized transport when possible.

### **3.10 Assessment of Appetite**

During each experiment presented in this thesis, perceptions of appetite (hunger, fullness, satisfaction and prospective food consumption (PFC)) were assessed periodically using previously validated visual analogue scales (Flint et al. 2000)

(Appendix H). Each visual analogue scale was 100 mm in length with descriptors anchored at each end describing the extremes (e.g. ‘I am not hungry at all’/ ‘I have never been more hungry’). Participants rated their appetite perceptions by placing a mark across the line corresponding to their feelings. Participants were not able to refer to their previous ratings when completing the appetite scales. The scales were analysed by measuring the horizontal distance from the left hand side of the continuum to the point on the line indicated by the participant. Each visual analogue scale was measured twice to ensure accuracy.

### **3.11 Standardised test meals**

In all of the experimental studies described in this thesis, participants consumed a standardised breakfast test meal. The breakfast meal consisted of toasted white wheatgerm bread, margarine, strawberry jam, banana and orange juice. The macronutrient content of the meal was 72.9 % carbohydrate, 9.5 % protein and 17.6 % fat. In Chapters 6 and 7, a standardised lunch test meal was also provided, which consisted of a tuna and mayonnaise sandwich, salted crisps, chocolate muffin and green apple. The macronutrient content of the meal was 47 % carbohydrate, 17.6 % protein and 35.4 % fat. In all experimental chapters, participants were instructed to consume test meals within 15 min.

During the experimental trials that did not involve food restriction, the breakfast meal provided 30 % of the estimated daily energy needs for each individual for a sedentary day. Similarly, during all experimental trials in Chapters 6 and 7 that did not involve food restriction, the lunch meal provided 35 % of the estimated daily energy needs for each individual for a sedentary day. The energy provided during these meals is detailed below as mean (SD):

- Chapter 4: Breakfast provided 2971 (228) kJ.
- Chapter 5: Breakfast provided 3158 (204) kJ.
- Chapter 6: Breakfast provided 3083 (242) and lunch provided 3597 (282) kJ.
- Chapter 7: Breakfast provided 3074 (221) kJ and lunch provided 3587 (258) kJ.

The energy needs for a sedentary day were calculated using the Mifflin-St Jeor equation and a physical activity factor of 1.4 (Mifflin et al. 1990). This equation was selected because of the inclusion of height, sex, age and body mass, which allows for a more

accurate prediction of resting energy expenditure than body mass alone. In this regard, the Mifflin-St Jeor equation reduces the risk of overestimation compared with the commonly used Harris-Benedict equation (Mifflin et al. 1990). A physical activity factor of 1.4 was selected to represent a sedentary day because participants were confined to the laboratory during all trials (Gibney et al. 2009). The Mifflin equation for males is detailed below:

$$\text{Resting energy expenditure} = (9.99 \times \text{body mass}) + (6.25 \times \text{height}) - (4.92 \times \text{age}) + 5.$$

### **3.12 Ad libitum buffet meals**

During the main trials in Chapters 4 and 5, participants were given access to buffet meals from which they were free to consume food ad libitum. Acceptability of the buffet items was ensured prior to main trials via the completion of a food preference questionnaire (Appendix E for Chapter 4; Appendix F for Chapter 5). The questionnaire required participants to rate preselected food items on a Likert scale ranging from 1 (dislike extremely) to 10 (like extremely). Questionnaires were examined to ensure that food items would be to the taste of each individual. An exclusion criterion was set for any participants rating five or more items as less than five on the food preference questionnaire.

Participants were given 30 min access to two cold buffet meals at distinct time points during main trials in Chapter 4. In Chapter 5, participants were given 30 min access to a cold buffet meal and a hot buffet meal at distinct time points during main trials. The food items presented are listed in Appendix E and Appendix F for Chapters 4 and 5, respectively. Within each experiment, buffet foods were presented identically before each meal and provided diversity in protein, fat and carbohydrate content. At these meals, food was presented in excess of expected consumption and participants were told to eat until “comfortably full” and that additional food was available if desired. Participants consumed meals in isolation so that social influence did not affect food selection. Leftovers were weighed after each buffet meal and food consumption was determined as the weighted difference of buffet items before and after the meal. The energy and macronutrient content of the items consumed was ascertained using manufacturers’ values.



### **3.13 Ad libitum pasta meal**

In Chapters 6 and 7, ad libitum energy intake was assessed at distinct time points using a pasta meal of fixed macronutrient composition. This method attempted to blind the participants to the amount of food eaten after concerns that food intake at the buffet meals in Chapters 4 and 5 was influenced by environmental contingencies.

The ad libitum pasta meal consisted of fusilli pasta (Tesco Fusilli Pasta Twists) mixed with bolognaise sauce (Dolmio Original Bolognaise Sauce). For each meal, 500 g of pasta was cooked in 1.2 L of unsalted water in a microwave at 900 W for 12 min. The pasta was then drained and the cooked weight was recorded 1:30 min after the cessation of cooking. The cooked pasta was then thoroughly mixed with 275 g of bolognaise sauce and placed in a covered saucepan. The macronutrient composition of the meal was 77.5 % carbohydrate, 13.8 % protein and 8.7 % fat.

Participants were provided with a small bowl, which was repeatedly filled with the pasta meal before the participant had emptied it, in an attempt to blind the participant to the amount of food eaten. No time limit was set for eating and participants were instructed to eat until 'comfortably full'. Each participant consumed the meal separately in the presence of a sole experimenter and any discussions about food were avoided. Food intake was determined as the weighted difference in food before and after eating and energy intake was subsequently determined using manufacturers' values.

Previous authors have provided an ad libitum pasta meal with a more balanced macronutrient composition (52 % carbohydrate, 14 % protein and 34 % fat) by using the following ingredients: pasta, tomato sauce, cheddar cheese and olive oil (Gonzalez et al. 2013). However, due to the physical properties of cheese and olive oil, pilot testing for the experiments contained within this thesis revealed that these ingredients did not mix evenly throughout the test meal. Therefore, in order to ensure consistent macronutrient composition throughout the served pasta meal, it was deemed preferable to mix only pasta and bolognaise sauce. The lower proportion of fat in this pasta meal is likely to have produced slightly lower energy intakes as a result of the lower energy density of the meal but this was required in order to ensure accurate quantification of energy intake.

### **3.14 Power**

An effect size of 8 - 10 % is typically considered to be of practical relevance during the assessment of appetite perceptions (Blundell et al. 2010). In order to demonstrate a 10 mm difference in any of the appetite perceptions monitored throughout this thesis (hunger, fullness, satisfaction, PFC), previous research suggests that approximately 8 participants are required in a paired design with a power of 0.8 (Flint et al. 2000). Previous research also suggests that the assessment of ad libitum energy intake requires a sample size of 17 participants to detect a 500 kJ difference in energy intake (Gregersen et al. 2008).

Alternatively, based on the findings of the experimental chapters within this thesis, power calculations determined that a sample size of 12 participants is required to detect a 10 % difference in appetite perceptions during the post-exercise period. Furthermore, although previous research from our laboratory has reported significant changes in energy intake with a sample size of 12 participants using the buffet meals presented in Chapters 4 and 5 of this thesis (King et al. 2011a), a lack of consistent directional effect in response to exercise between participants within this thesis resulted in an estimated required sample size of 128 participants in order to detect a significant change in energy intake. These power calculations were performed using G\*power with an alpha value of 5 % and a power of 80 % (Faul et al. 2007).

Subsequently, a sample size of 12 participants was used for all experiments throughout this thesis in order to provide sufficient power to detect significant changes in appetite perceptions between trials. This also enabled all experiments to be counterbalanced using a Latin square design to prevent any trial-order effects. In order to address the issue of under-powering regarding energy intake, effect sizes (ES) are reported for total ad libitum energy intake in each experimental chapter of this thesis.

### **3.15 Environmental temperature and humidity**

Environmental temperature and humidity were assessed periodically throughout all main experimental trials using a hand-held hygrometer (Omega RH85, Manchester, UK).

### **3.16 Blood sample collection**

Approximately 15 min before commencing all main trials in Chapters 6 and 7, participants rested in a semi-supine position and a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. In Chapter 5, the first 3 blood samples during the sprint exercise trial were collected via venepuncture in order to prevent any arm discomfort caused by an indwelling cannula during sprint exercise. All other blood samples in Chapter 5 were collected from a cannula that was inserted into an antecubital vein at least 15 min prior to use.

Patency of the cannula was maintained by flushing with 10 mL non-heparinised saline (0.9 % (w/v) sodium chloride, Baxter Healthcare Ltd., Norfolk, UK) after each blood sample. To avoid dilution of subsequent samples, residual saline was drawn off immediately prior to collection using a 2 mL syringe. To control for postural changes in plasma volume, participants rested in a semi-supine position for the five min prior to each blood sample and remained in this position during the collection. Exceptions to this occurred when blood samples were collected during cycling exercise in Chapter 5. In this situation, participants remained seated on the bike while the blood sample was collected.

Venous blood samples were collected into pre-chilled 4.9 mL monovettes (Sarstedt, Leicester, UK) for the determination of plasma acylated ghrelin concentrations (Chapters 5 and 7). To prevent the degradation of acylated ghrelin these monovettes contained potassium-ethylenediamine tetra-acetic acid (EDTA) and a 50  $\mu$ L solution containing potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH). Monovettes were promptly spun at  $1165 \times g$  for 10 min in a refrigerated centrifuge at 4 °C (Heraeus Labofuge 400R, Thermo Electron, Osterode, Germany). The plasma supernatant was then dispensed into a plain storage tube and 100  $\mu$ L of 1 M hydrochloric acid was added per millilitre of plasma to preserve acylated ghrelin (Hosoda et al. 2004). Thereafter, samples were spun at  $1165 \times g$  for 5 min in a refrigerated centrifuge prior to storage in 2 mL Eppendorf tubes at -20°C for later analysis.

In Chapter 5, blood samples were also collected into 9 mL EDTA monovettes for the determination of plasma total PYY concentrations. After blood sample collection, these monovettes were promptly spun at  $856 \times g$  for 10 min at 4 °C (Heraeus Labofuge 400R,

Thermo Electron, Osterode, Germany) and the plasma supernatant aliquoted into 2 mL Eppendorf tubes prior to storage at -20°C for later analysis.

For the determination of plasma PYY<sub>3-36</sub> concentrations in Chapters 6 and 7, blood samples were collected into pre-chilled 2 mL syringes containing 10 µL DPP-IV inhibitor (Millipore, Watford, UK) per mL of blood. Syringes were then inverted and the blood dispensed into pre-chilled 2 mL EDTA tubes containing aprotonin (Nordic Pharma, Reading, UK) at a final concentration of 500 KIU per mL of blood. Blood tubes were promptly centrifuged at 1165 × *g* for 10 min at 4 °C. The plasma supernatant was aliquoted into 2 mL Eppendorf tubes prior to storage at -20 °C for later analysis.

At each sampling point, duplicate 20 µL blood samples were collected into micropipettes and triplicate 20 µL blood samples were collected into heparinised microhaematocrit tubes to determine blood haemoglobin and haematocrit concentration, respectively.

### **3.17 Blood sample analysis**

#### **3.17.1 Estimation of changes in plasma volume**

Blood haemoglobin and haematocrit concentrations were used to estimate plasma volume and determine changes over time (Dill & Costill 1974). Haematocrit was determined in triplicate using a microliter-haematocrit centrifuge (MIKRO, 20, Andreas Hettich GmbH and Co.KG, Tuttlingen, Germany). Haemoglobin was determined in duplicate using the cyanmethaemoglobin method with the aid of a spectrophotometer (CECIL CE1011, Cecil Instruments Ltd., Cambridge, UK).

#### **3.17.2 Acylated ghrelin**

Plasma acylated ghrelin concentrations were determined using a commercially available enzyme-linked immunosorbent assay kit (SPI BIO, Montigny le Bretonneux, France) with the aid of a plate reader to measure absorbance (Expert Plus, ASYS Atlantis, Eugendorf, Austria). Precision of analysis was ensured by the quantification of an internal quality control.

### **3.17.3 Total PYY**

Plasma total PYY concentrations were determined using a commercially available enzyme-linked immunosorbent assay kit (Millipore, Watford, UK) with the aid of a plate reader to measure absorbance (Varioskan Flash, Thermo Scientific, Vantaa, Finland). Precision of analysis was ensured by the quantification of internal quality controls exhibiting high and low values.

### **3.17.4 PYY<sub>3-36</sub>**

Plasma PYY<sub>3-36</sub> concentrations were determined using a commercially available radioimmunoassay kit (Millipore, Watford, UK). Precision of analysis was ensured by the quantification of internal quality controls exhibiting high and low values.

### **3.17.5 Precision of analysis**

To eliminate inter-assay variation, samples from each participant were analysed in the same run. The within batch coefficient of variation for each assay is detailed within the methods section of each experimental chapter.

## **3.18 Statistical analysis**

Data was analysed using IBM SPSS statistics version 19 for Windows. All area under the concentration versus time curve (AUC) calculations were performed using the trapezoidal method. Exercise responses between trials were compared using Students paired t-tests (Chapters 4 - 6). One-way repeated measures analysis of variance (ANOVA) was used to examine trial-based differences in baseline and AUC values as well as total ad libitum energy intake (Chapters 4 – 7). Repeated measures, two-factor ANOVA was used to examine differences between trials over time for appetite perceptions (Chapters 4 - 7), energy intake (Chapters 4 and 5), macronutrient intake (Chapters 4 and 5), and circulating concentrations of plasma acylated ghrelin (Chapters 5 and 7), total PYY (Chapter 5) and PYY<sub>3-36</sub> (Chapters 6 and 7). The Pearson product moment correlation coefficient was used to examine relationships between variables. Assumptions of sphericity in the data were checked, and adjustments in the degrees of freedom for the ANOVA were made using the Greenhouse-Geiser method of correction where appropriate. Where significant trial and interaction effects were found, post-hoc analysis was performed using Holm-Bonferroni correction for multiple comparisons.

Statistical significance was accepted at the 5 % level. Results in text and tables are presented as mean (SD). Graphical representations of results are presented as mean (SEM) to avoid distortion of the graphs.

## CHAPTER IV

### **Appetite, energy intake and resting metabolic responses to 60 min treadmill running performed in a fasted versus a postprandial state**

#### **4.1 Introduction**

Obesity is classified as a BMI equal to or greater than  $30 \text{ kg.m}^{-2}$  and represents a major global health problem as it is associated with an increased prevalence of chronic diseases, including type 2 diabetes, hypertension, dyslipidaemia, and cardiovascular disease (National Institute of Health 1998). The prevalence of obesity has increased to such an extent that in 2008 an estimated 9.8 % of adult men and 13.8 % of adult women worldwide were classified as obese (Finucane et al. 2011). Obesity is the result of a chronic positive energy balance achieved via a long term surplus of energy intake over energy expenditure. Therefore methods of maximising energy expenditure and/or minimising energy intake are important in combating obesity.

Exercise is an important component of successful long-term weight control programs and is particularly effective when combined with dietary modifications (Franz et al. 2007). Exercise can induce a negative energy balance not only by increasing energy expenditure but also by modulating energy intake as strenuous exercise has been found to acutely suppress hunger in a phenomenon described as ‘exercise-induced anorexia’ (King & Blundell 1995; King et al. 1994).

The majority of short (1 - 2 d) to medium (7 - 16 d) term intervention studies indicate that acute exercise does not provoke compensatory increases in appetite and food intake or alter macronutrient preferences (Blundell & King 1999; Blundell et al. 2003; Martins et al. 2008). In this regard, recent studies have demonstrated exercise to be more effective than energy restriction in creating an energy deficit without subsequent increases in appetite and energy intake (Hubert et al. 1998; King et al. 2011a). With this knowledge, research must now focus on maximising the effectiveness of exercise as a method of producing a negative energy balance for weight control. One potential avenue for maximisation is to manipulate the timing of exercise in relation to food consumption as both exercise and feeding influence appetite and subsequent energy intake (King et al. 2011a).

A common strategy to facilitate weight and fat loss is to perform aerobic exercise after an overnight fast. Exercise in the fasted state enhances fat oxidation due to decreased glycogen availability and a lipolytic hormonal environment including reduced plasma insulin concentrations and elevated cortisol and epinephrine concentrations (De Bock et al. 2005; Febbraio et al. 2000; Maughan et al. 2010). However, recent evidence suggests that postprandial exercise may be more beneficial for weight control than fasted exercise as a result of more favourable effects on appetite regulation and resting metabolism. In this regard, Cheng et al. (2009) recently demonstrated that 50 min of cycling at 60 % of  $\text{VO}_2$  max resulted in more prolonged hunger suppression when performed 2 h after a high fat breakfast (70 % fat, 26 % carbohydrate, 4 % protein) rather than after a 12 h overnight fast. Furthermore, previous evidence suggests that post-meal decreases in appetite are attenuated after fasted compared with postprandial exercise (Borer et al. 2005). Such findings indicate that energy intake may be lower after postprandial exercise than fasted exercise. However, ad libitum energy intake was not assessed in these studies and it is therefore unknown if such appetite responses would influence energy intake and subsequent energy balance.

Postprandial exercise may also promote metabolic changes that are more beneficial to weight loss than those induced by fasted exercise. Paoli et al. (2011) recently demonstrated that 36 min of treadmill exercise stimulated a greater increase in resting energy expenditure in the 24 h after exercise when performed 40 min after a 673 kcal Mediterranean breakfast (53 % fat, 22 % carbohydrate, 25 % protein) compared with when exercise was completed immediately before breakfast after a 12 h overnight fast. Furthermore, in comparison with exercise in the fasted state, postprandial exercise resulted in a higher proportional fat metabolism in the 24 hours after exercise, which has been shown to be predictive of fat loss (Barwell et al. 2009).

As well as substrate metabolism being influenced by exercise and energy intake, there has recently been renewed interest in the theory that substrate metabolism may itself be a biological determinant of feeding behaviour (Hopkins et al. 2011; King et al. 2012). Evidence from pharmacological studies suggests that fatty acid oxidation may inhibit food intake via the prolongation of post-meal satiety (Gatta et al. 2009; Scharrer & Langhans 1986). Furthermore, the glycogenostatic theory proposed by Flatt in 1987 suggests that short term feeding behaviour is designed to replenish and maintain glycogen stores (Flatt 1987). Similarly, higher proportional carbohydrate oxidation



during exercise has been suggested to increase subsequent energy intake (Almeras et al. 1995; Kissileff et al. 1990). However, this finding is inconsistent (Imbeault et al. 1997; Klausen et al. 1999) and requires further investigation.

The ability of exercise to induce a negative energy balance depends on the energy cost of exercise as well as its ability to modify appetite, energy intake and post-exercise metabolic responses (Tremblay & Therrien 2006). This study seeks to compare these variables in response to fasted and postprandial exercise in order to elucidate the most beneficial timing of exercise as a method of inducing a negative energy balance. Therefore the primary purpose of the present study was to investigate the effect of 60 min of treadmill running on prolonged appetite, energy intake and resting metabolic responses when performed 1.5 h before or 2.5 h after a high carbohydrate breakfast. It was hypothesised that postprandial exercise would stimulate greater increases in resting energy expenditure and induce a more prolonged appetite suppression than fasted exercise, resulting in lower energy intake and a greater negative 24 h energy balance.

## **4.2 Methods**

### **4.2.1 Participants**

Twelve healthy men matching the inclusion criteria listed in Chapter 3.1, volunteered to participate in this study. The physical characteristics of the participants (mean (SD)) were as follows: age 23 (3) years, BMI 22.9 (2.1)  $\text{kg}\cdot\text{m}^{-2}$ , body mass 70.3 (8.8) kg, body fat 13.8 (4.1) %, waist circumference 78.4 (6.5) cm,  $\text{VO}_2$  max 57.5 (9.7)  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ .

### **4.2.2 Preliminary trial**

Prior to main trials participants visited the laboratory to undergo screening, and preliminary anthropometric measurements as detailed in Chapters 3.1 and 3.2. After familiarisation with the testing equipment, participants also completed a submaximal and maximal treadmill running test in order to determine the relationship between running speed and oxygen consumption and to determine  $\text{VO}_2$  max, respectively. Details of these tests are described in Chapter 3.5.1.

### **4.2.3 Experimental protocol**

Participants performed three experimental trials (control, fasted exercise and postprandial exercise) separated by at least one week in a counterbalanced Latin Square design. Each trial lasted 10 h and commenced at 8 am after an overnight fast of at least 10 h. Participants standardised their food intake and physical activity in the 24 h preceding each main trial and exerted themselves minimally when travelling to the laboratory, as described in Chapter 3.9.

During each trial, appetite perceptions were assessed at baseline and every 30 min throughout using 100 mm visual analogue scales (see Chapter 3.10). Resting expired gas samples were collected into Douglas bags for 5 min after 5 min rest in a seated position at: -5 min, 2.25, 3.25, 6.25, 7.25, 8.25, 9.25, and 10 h. Energy expenditure and substrate oxidation were subsequently calculated using the methods described in Chapter 3.8.

The fasted exercise trial (FAST) commenced with a 60 min run on a level treadmill at a speed predicted to elicit 70 % of  $\text{VO}_2$  max. Samples of expired gas were collected every 15 min during exercise to estimate energy expenditure and to monitor the intensity of

the run, with adjustments made to the treadmill speed if necessary. Heart rate and RPE were also assessed at these times. After the run, participants rested for 9 h in the laboratory (sitting reading, working at a desk or watching television). Identical procedures were completed during the postprandial exercise trial (FED) except the run was completed from 4 – 5 h rather than 0 – 1 h. The control trial (CON) was identical to the two exercise trials except that no exercise was performed. In order to estimate net energy expenditure of the run (gross energy expenditure of run minus resting energy expenditure), samples of expired gas were collected when resting during the control trial at time points when exercise was taking place in FAST and FED to determine resting energy expenditure. Figure 4.1 provides an overview of the protocol for the experimental trials.

#### **4.2.4 Standardised breakfast and ad libitum food intake**

Participants were provided with a standardised breakfast (see Chapter 3.11) at 1.5 h and were given 30 min access to ad libitum buffet meals (see Chapter 3.12) at 5.5 and 9.5 h. Upon leaving the laboratory, overnight food consumption was monitored via a weighed food diary. Water was available ad libitum throughout each trial.

#### **4.2.5 Statistical Analysis**

Data was analysed using the methods described in Chapter 3.18.

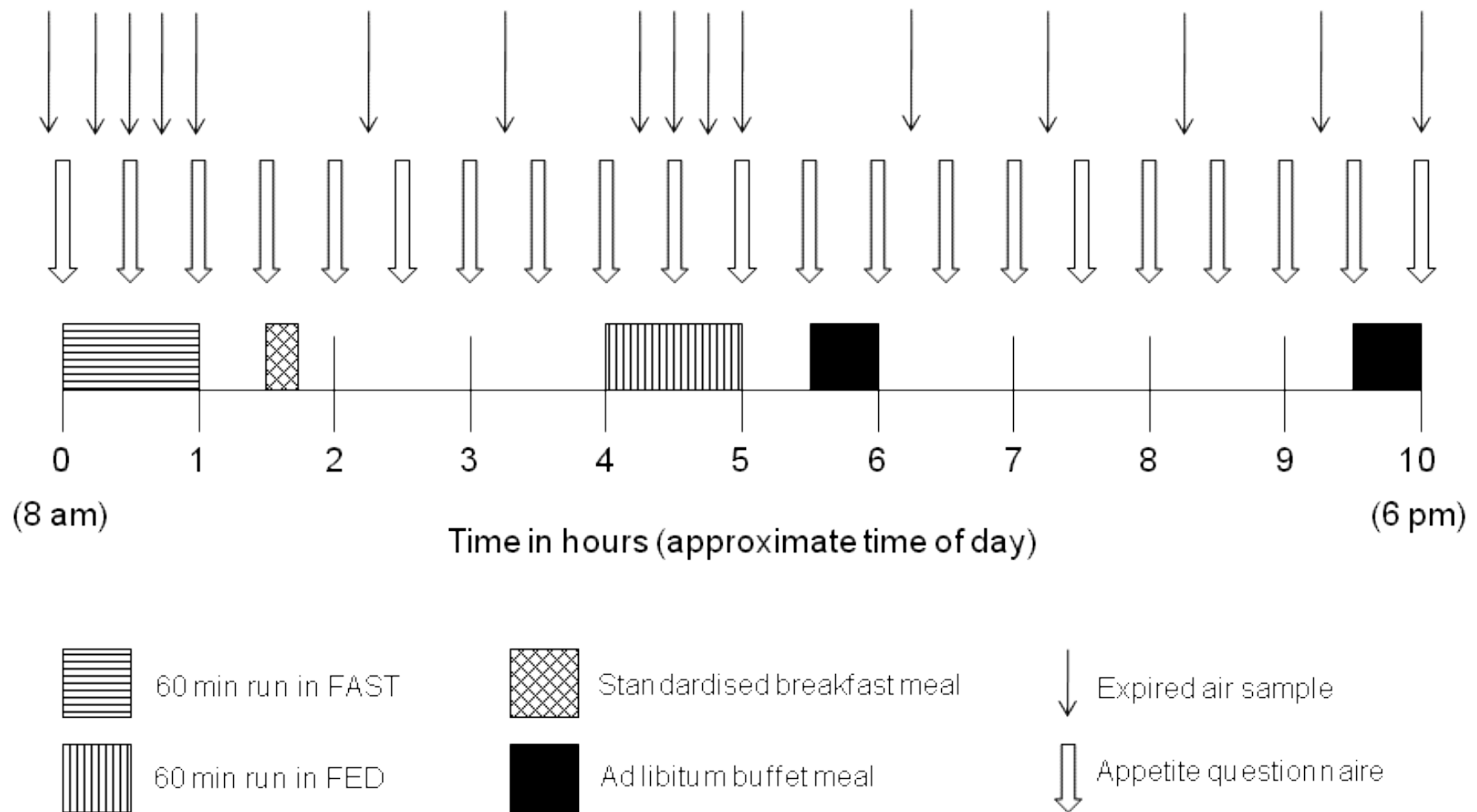


Figure 4.1. Schematic representation of the protocol for the experimental trials.

## **4.3 Results**

### **4.3.1 Exercise responses**

Participants completed the 60 min run at 10.2 (2.5) km.h<sup>-1</sup> during both FAST and FED. This elicited a mean oxygen consumption equivalent to 71.1 (2.1) and 71.9 (2.7) % of VO<sub>2</sub> max and generated a mean heart rate of 162 (12) and 165 (14) beats.min<sup>-1</sup> in FAST and FED, respectively. The net energy expenditure of the run was 3247 (423) kJ in FAST and 3234 (435) kJ in FED. None of these values differed significantly between exercise trials.

The mean RER during exercise was significantly lower in FAST than FED (0.91 (0.03) vs. 0.93 (0.03); P = 0.008), reflecting a higher proportional contribution of fat and lower contribution of carbohydrate to energy provision during fasted exercise (FAST: 31.2 (10.6) % fat, 68.8 (10.6) % carbohydrate; FED: 22.3 (11.6) % fat; 77.7 (11.6) % carbohydrate).

Mean RPE did not differ between trials (13 (1) and 12 (1) in FAST and FED respectively) and indicated that participants perceived the intensity of the run to be 'fairly hard'.

### **4.3.2 Baseline parameters**

No between trial differences existed at baseline for appetite perceptions, energy expenditure or RER (Table 4.1).

Table 4.1. Baseline appetite, resting energy expenditure and resting respiratory exchange ratio (RER) values in the control, fasted and postprandial trials.

		Control	Fasted	Postprandial	P
Hunger	0-100	63 (16)	62 (18)	61 (18)	0.891
Satisfaction	0-100	27 (13)	24 (17)	25 (16)	0.897
Fullness	0-100	23 (15)	16 (11)	16 (8)	0.168
Prospective food consumption	0-100	73 (10)	67 (16)	71 (14)	0.235
Resting energy expenditure	$\text{kJ}\cdot\text{min}^{-1}$	4.85 (0.88)	5.06 (0.63)	4.77 (0.96)	0.488
Resting RER		0.83 (0.08)	0.83 (0.06)	0.82 (0.05)	0.723

Values are mean (SD), N = 12. No significant differences between trials.

### 4.3.3 Appetite

Two-factor ANOVA revealed a main effect of time (all  $P < 0.0005$ ) and a trial  $\times$  time interaction (all  $P < 0.03$ ) for each appetite perception assessed (hunger, fullness, satisfaction and PFC) indicating that responses differed over time between the trials (Figure 4.2). Analysis also revealed a main effect of trial for satisfaction ( $P = 0.037$ ) demonstrating elevated perceptions during FED compared with FAST ( $P = 0.01$ ).

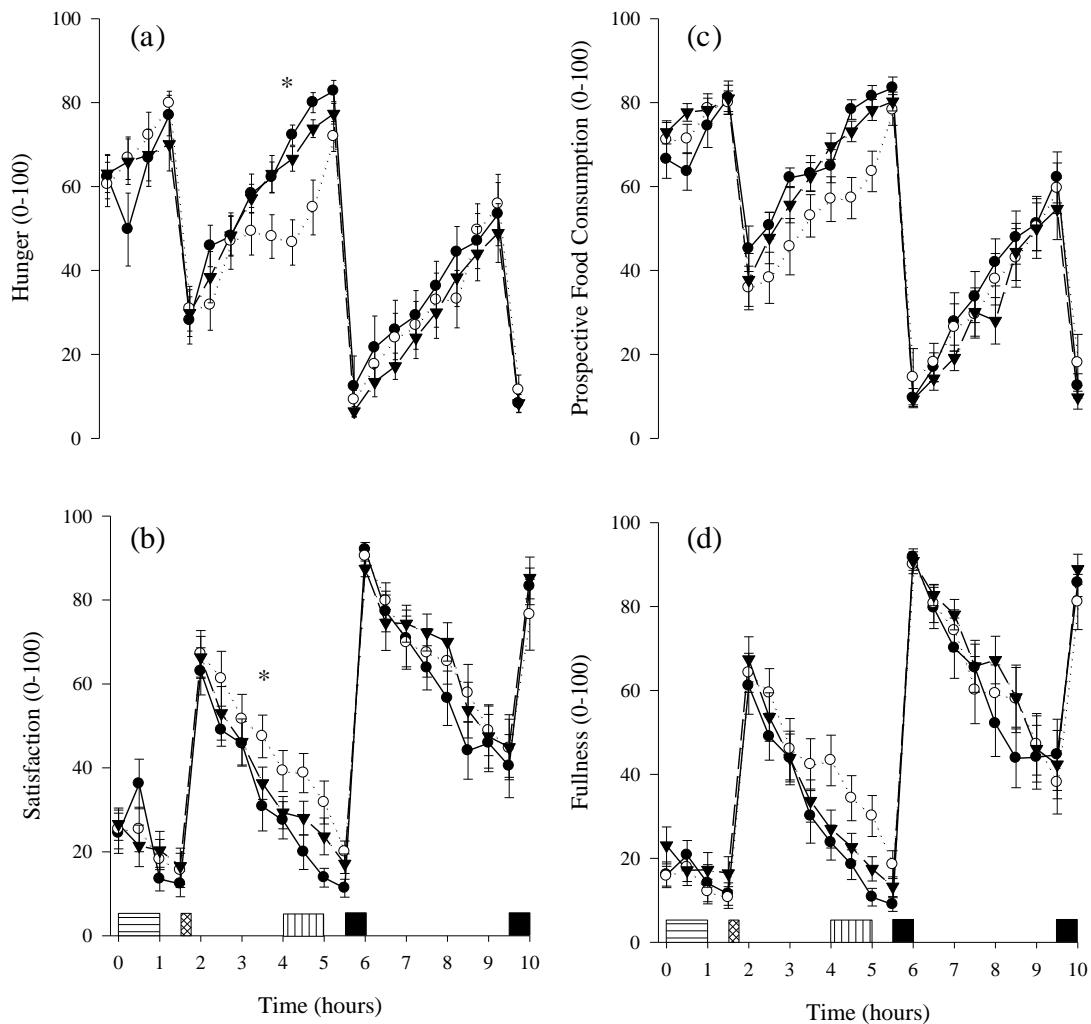


Figure 4.2. Perceptions of hunger (a), satisfaction (b), prospective food consumption (c) and fullness (d) in CON ( $\blacktriangledown$ ), FAST ( $\bullet$ ) and FED ( $\circ$ ) trials. Values are mean (SEM),  $N = 12$ . Dashed line indicates CON, solid line indicates FAST, dotted line indicates FED. Horizontally shaded rectangle indicates fasted exercise, vertically shaded rectangle indicates postprandial exercise, hatched shaded rectangle indicates standardised breakfast meal, black rectangles indicate ad libitum buffet meals. \*Significant difference between FAST and FED (both  $P < 0.0005$ ).

Post-hoc analysis of trial x time interactions revealed a decrease in PFC and an increase in satisfaction during exercise in FAST compared with CON at 0.5 h. Differences in ratings of satisfaction and fullness were apparent at 3.5 h as well as differences in PFC from 2.5 - 3.5 h, demonstrating greater appetite in FAST than FED shortly after breakfast. Each appetite perception differed in FED compared with CON and FAST from 4 – 5.5 h, indicating suppressed appetite during and shortly after exercise in FED (all  $P < 0.05$ ). However, after Holm-Bonferroni adjustment only higher satisfaction at 3.5 h and lower hunger at 4.5 h in FED compared with FAST remained significant ( $P < 0.0005$ ).

Between trial differences in appetite ratings were also evaluated using AUC values calculated from baseline to breakfast (0 – 1.5 h), breakfast to the first buffet meal (2 – 5.5 h), for the 4 h after the first buffet meal (6 – 10 h) and for the entire 10 h trial. One-way ANOVA revealed a main effect of trial for all appetite perceptions from 2.5 – 5.5 h (all  $P < 0.05$ ), indicating lower appetite in FED than FAST. Total 10 h AUC for satisfaction was also significantly higher in FED than FAST ( $P = 0.034$ ). However, no other differences existed between trials.

#### **4.3.4 Energy and macronutrient intake**

Two-factor ANOVA showed a significant difference in the amount of energy consumed at the separate meals during the course of the trials (main effect of time,  $P < 0.0005$ ). However, this differential was not influenced by the trials (Table 4.2). Effect sizes for total ad libitum energy intake were as follows: CON vs. FAST:  $d = 0.07$ ; CON vs. FED:  $d = 0.17$ ; FAST vs. FED:  $d = 0.25$ ). For fat, protein and carbohydrate intake, two-factor ANOVA revealed a main effect of time ( $P < 0.0005$ ) indicating that the intake of these macronutrients varied across the meals within each trial. There was no difference in macronutrient intake between trials (Table 4.3).



Table 4.2. Ad libitum energy intake during the control (CON), fasted (FAST) and postprandial (FED) trials.

		CON	FAST	FED
Buffet 1 (5.5 – 6 h)	kJ	7205 (1707)	7540 (1515)	6820 (2238)
Buffet 2 (9.5 – 10 h)	kJ	4448 (1703)	4548 (1774)	4732 (2013)
Overnight (10 – 24 h)	kJ	1803 (908)	1565 (1197)	1377 (1000)
Total trial (0 – 24 h)	kJ	13,452 (2682)	13,652 (2385)	12,929 (2933)

Values are mean (SD), N = 12. No significant differences between trials.

Table 4.3. Macronutrient intake in the control (upper panel), fasted (middle panel) and postprandial (lower panel) trials.

		Fat	Carbohydrate	Protein
<b>Control</b>				
Buffet 1	g	61.1 (25.0)	240.2 (57.1)	52.8 (17.4)
Buffet 2	g	40.2 (17.0)	142.7 (57.6)	32.5 (16.3)
Overnight	g	14.8 (10.1)	62.2 (30.1)	12.2 (9.4)
Total	g	116.1 (36.9)	445.1 (84.0)	97.5 (27.3)
<b>Fasted</b>				
Buffet 1	g	65.8 (24.4)	245.3 (58.1)	57.1 (12.4)
Buffet 2	g	37.7 (18.0)	155.8 (59.9)	31.1 (16.6)
Overnight	g	15.8 (14.6)	47.7 (36.1)	10.4 (9.0)
Total	g	119.3 (33.0)	448.7 (94.5)	98.6 (21.5)
<b>Postprandial</b>				
Buffet 1	g	53.5 (28.5)	236.9 (74.4)	50.2 (14.6)
Buffet 2	g	44.4 (23.3)	147.8 (61.9)	35.2 (20.9)
Overnight	g	12.2 (12.3)	48.3 (35.1)	6.6 (4.7)
Total	g	110.1 (42.6)	433.0 (86.7)	92.0 (25.5)

Values are mean (SD), N = 12. No significant differences between trials.

### 4.3.5 Energy expenditure and substrate utilisation

Two-factor ANOVA revealed a significant trial, time and trial x time interaction for energy expenditure throughout the trials (all  $P < 0.0005$ ; Figure 4.3). Energy expenditure was higher during the exercise trials than control ( $P < 0.0005$ ). At individual time points post hoc analysis indicated elevated energy expenditure in FAST than CON at 0.25, 0.5, 0.75, 1, 4.25, 4.5, 6.25, 7.25 and 8.25 h, as well as higher energy expenditure in FAST than FED at 0.25, 0.5, 0.75, 1, 2.25 and 9.25 h (all  $P < 0.05$ ). Energy expenditure was higher in FED than FAST and CON at 4.25, 4.5, 4.75 and 5 h. However, after Holm-Bonferroni adjustment only higher energy expenditure in FAST than FED and CON at 0.25, 0.5, 0.75 and 1 h (i.e. during exercise in FAST), and higher energy expenditure in FED than FAST and CON at 4.25, 4.5, 4.75, and 5 h (i.e. during exercise in FED) remained significant (all  $P < 0.0005$ ).

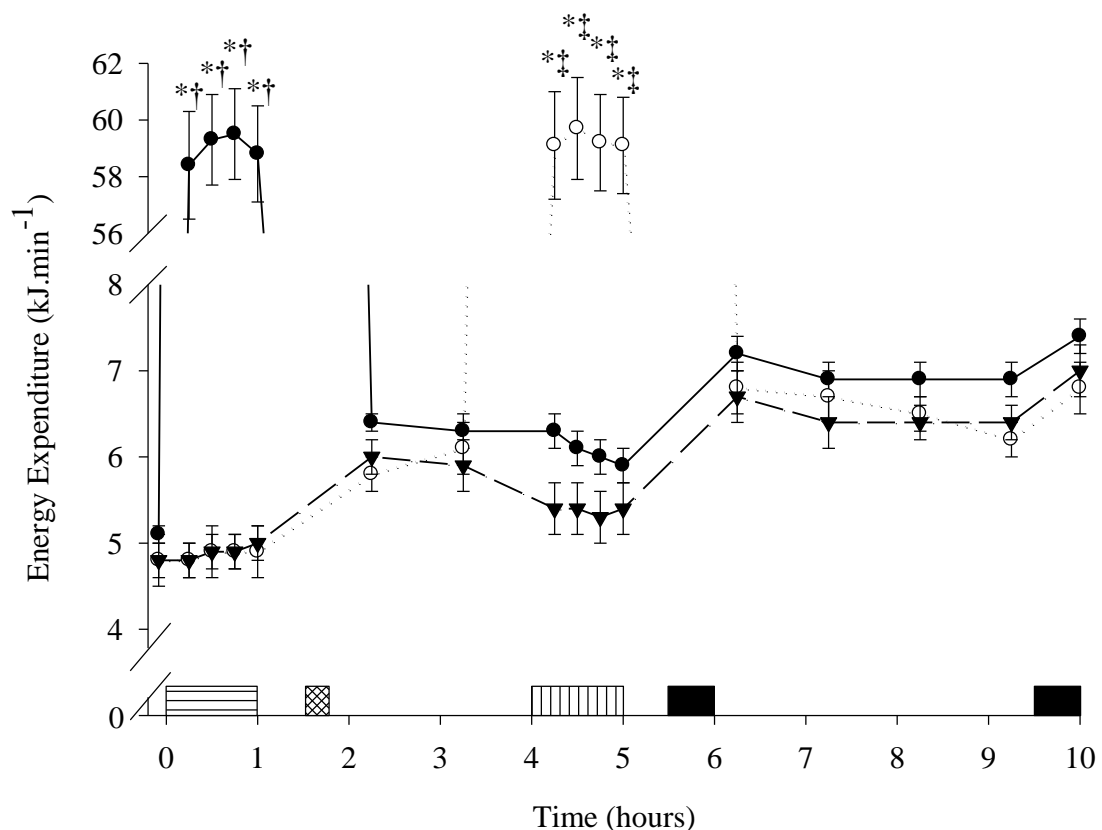


Figure 4.3. Energy expenditure in CON (▼), FAST (●) and FED (○) trials. Values are mean (SEM),  $N = 12$ . Dashed line indicates CON, solid line indicates FAST, dotted line indicates FED. Horizontally shaded rectangle indicates fasted exercise, vertically shaded rectangle indicates postprandial exercise, hatched shaded rectangle indicates standardised breakfast meal, black rectangles indicate ad libitum buffet meals. \*Significant difference between FAST and FED, †FAST different from CON, ‡FED different from CON (all  $P < 0.0005$ ).

Two-factor ANOVA for RER revealed a significant time and trial x time interaction (both  $P < 0.0005$ ) but no main effect of trial ( $P = 0.273$ ; Figure 4.4). Post-hoc analysis indicated higher RER in FAST than FED and CON at 0.25, 0.5 and 1 h, as well as higher RER in FED than FAST and CON at 4.25, 4.5, 4.75 and 5 h. At rest, RER was higher in CON than FAST at 3.25, 4.75, 5 and 6.25 h and in CON than FED at 6.25 and 7.25 h (all  $P < 0.05$ ). However, after Holm-Bonferroni adjustment only elevated RER in FAST than FED and CON at 0.25, 0.5 and 1 h (i.e. during exercise in FAST), and higher RER in FED than FAST and CON at 4.25, 4.5 and 4.75, and in FED than FAST at 5 h (i.e. during exercise in FED) remained significant ( $P \leq 0.001$ ).

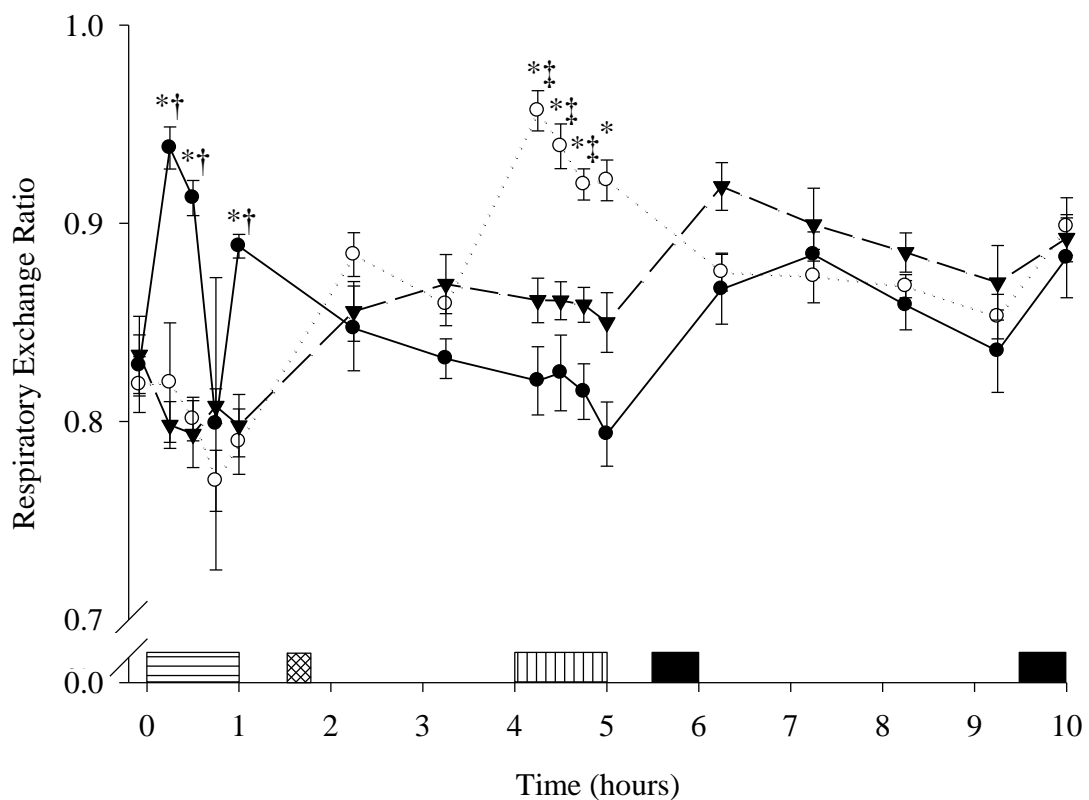


Figure 4.4. Respiratory exchange ratio values in CON (▼), FAST (●) and FED (○) trials. Values are mean (SEM),  $N = 12$ . Dashed line indicates CON, solid line indicates FAST, dotted line indicates FED. Horizontally shaded rectangle indicates fasted exercise, vertically shaded rectangle indicates postprandial exercise, hatched shaded rectangle indicates standardised breakfast meal, black rectangles indicate ad libitum buffet meals. \* Significant difference between FAST and FED, † FAST different from CON, ‡ FED different from CON (all  $P < 0.0005$ ).

In order to calculate area under the curve values exclusively for *resting* energy expenditure and RER, data during exercise was substituted for control data from equivalent time points. One-way ANOVA revealed a significant main effect of trial for area under the resting energy expenditure verses time curve from 0 – 5 h, 5 – 10 h and for the total 10 h trial (all  $P < 0.015$ ). Post-hoc analysis indicated higher resting energy expenditure in FAST than CON and FED for each time period (all  $P < 0.05$ ) but these differences were no longer significant after Holm-Bonferroni adjustment.

One-way ANOVA revealed a significant main effect of trial for area under the RER verses time curve from 5 – 10 h and for the total 10 h trial (all  $P < 0.025$ ). Post-hoc analysis indicated lower RER in FAST and FED than CON from 5 - 10 h and in FAST than CON for the total trial (all  $P < 0.05$ ). After Holm-Bonferroni adjustment, RER during FAST remained lower than CON for 5 – 10 h and the total 10 h trial (all  $P < 0.01$ ).

#### **4.3.6 Relative energy intake**

After accounting for the energy expenditure induced by exercise, participants remained in energy deficit in both FAST and FED compared with CON (both  $P < 0.0005$ ) but no differences existed between exercise trials (CON 13,451 (2682) kJ); FAST 10,406 (2289) kJ; FED 9699 (2866) kJ). Relative energy intake was 22.6 and 27.9 % lower than CON in FAST and FED after accounting for the energy cost of exercise. Similar findings existed after energy intake was adjusted for estimated energy expenditure for the entire 10 h trial based on exercising and resting energy expenditure (CON 9774 (2694) kJ; FAST 6481 (2318) kJ; FED 6017 (3050) kJ; both  $P < 0.0005$ ). Relative energy intake was 33.7 and 38.4 % lower than CON in FAST and FED after accounting for the estimated energy expenditure for the 10 h trial. Closer inspection of the data revealed that 7 of 12 participants exhibited a higher REI in FAST than FED, while 5 of the 12 participants demonstrating a higher REI in FED than FAST. All participants exhibited lower REI in both exercise trials compared with control.

#### **4.4 Discussion**

The purpose of this investigation was to examine the appetite, energy intake and resting metabolic responses to a prolonged bout of treadmill running when performed under fasted or postprandial conditions. The primary findings arising from this study were that postprandial exercise invoked a more substantial and prolonged suppression of appetite than fasted exercise. However, this did not result in any differences in energy intake between trials and elicited a negative energy balance relative to control in both exercise trials.

Fasted exercise reduced sensations of prospective food consumption and increased feelings of satisfaction during the exercise bout, which returned to normal levels upon completion. This indicates a suppression of appetite during exercise and is consistent with the phenomenon of 'exercise-induced anorexia' (King & Blundell 1995; King et al. 1994). Exercise in the postprandial period inhibited post-meal increases in hunger and PFC and decreases in satisfaction and fullness during and immediately after exercise. This is again consistent with exercise-induced anorexia and supports the findings of Cheng et al. (2009) that postprandial exercise has greater appetite suppressive effects than fasted exercise. The mechanisms underlying appetite suppression during exercise are still unclear but recent evidence suggests that exercise-induced changes in episodic appetite regulating gut peptides such as peptide YY, glucagon-like-peptide-1, pancreatic polypeptide and acylated ghrelin may be implicated (Broom et al. 2009; King et al. 2011a; Martins et al. 2007; Ueda et al. 2009a).

In addition to exercise-induced changes in appetite, there was also a tendency for increased perceptions of PFC and decreased feelings of satisfaction and fullness in FAST than FED immediately after breakfast, prior to the postprandial exercise bout. This supports the findings of Borer et al. (2005) that the post-meal decline in appetite is attenuated when fasted exercise is performed prior to breakfast. Although this suggests that a small compensatory rise in appetite may occur after fasted exercise, it is also plausible that appetite perceptions were lower in FED after breakfast due to the anticipation of forthcoming exercise. This finding coupled with the greater and more prolonged appetite suppression during FED exercise, resulted in higher satisfaction during the FED trial than FAST and indicates greater appetite suppression when exercise is performed after, rather than immediately before, breakfast.

However, such differences in appetite did not influence ad libitum energy or macronutrient intake during the remainder of the day. This may be explained by the transient nature of exercise-induced anorexia as appetite was suppressed almost exclusively during and immediately after exercise. This suggests that appetite suppression during exercise has little influence on subsequent energy intake and supports previous evidence that exercise does not provoke short term compensatory changes in food and macronutrient intake (Blundell & King 1999; Blundell et al. 2003; Martins et al. 2008).

Consistent with a longstanding body of literature, resting energy expenditure and proportional fat oxidation tended to be higher after both exercise sessions compared with the control trial (Poehlman 1989; Votruba et al. 2002). However, the tendency for higher resting energy expenditure after fasted exercise than postprandial exercise and no difference in resting RER between exercise trials contrasts with recent findings by Paoli et al. (2011). Such differences are likely to be a result of variations in study protocol as exercise was performed 2.25 h into the postprandial period in the present study but only 40 min after breakfast in that of Paoli et al. (2011). The postprandial increase in energy expenditure in the present study began to subside before the commencement of exercise, whereas exercise in the study conducted by Paoli et al. (2011) corresponded with a substantial postprandial increase in energy expenditure, which was concluded to be the stimulus for an elevation in resting 24 h energy expenditure.

Reductions in resting RER after exercise have been suggested to promote the replenishment of muscle glycogen stores (Hopkins et al. 2011) and a positive relationship has been demonstrated between muscle glycogen utilisation during exercise and post-exercise increases in fat oxidation (Henderson et al. 2007). Despite elevated carbohydrate oxidation during postprandial exercise compared with fasted exercise, it is plausible that muscle glycogen utilisation did not differ between exercise bouts in the present study. Evidence suggests that increases in muscle glycogen utilisation only occur during postprandial exercise when rebound hypoglycaemia occurs (Febbraio & Stewart 1996; Hargreaves et al. 1985). Such rebound hypoglycaemia is unlikely to have occurred during postprandial exercise in the present study due to the substantial time period between breakfast and postprandial exercise. Such similar levels of glycogen depletion during the exercise bouts may explain similar RER values

observed at rest during the afternoon of the exercise trials. However, this remains speculative as blood glucose and muscle glycogen content were not measured in the present study.

Despite renewed interest in the idea that substrate metabolism may act as a biological determinant of feeding behaviour (Hopkins et al. 2011; King et al. 2012), there was no relationship between substrate oxidation and energy/macronutrient intake in the present study. This adds to the equivocal evidence regarding this theory and suggests that other physiological mediators of appetite and energy intake may affect these variables. It is also plausible that previously recognised relationships between glycogen availability and energy intake may be coincidental with other factors (e.g. hormones) acting to regulate energy intake while also mediating substrate metabolism (Hopkins et al. 2011). This is supported by evidence that peripheral ghrelin infusion increases carbohydrate oxidation as well as increasing appetite and energy intake (Tschöp et al. 2000). Such relationships may be implicated in previous findings that increased carbohydrate oxidation is related to increased energy intake.

The substantial energy deficits incurred during both the fasted and postprandial exercise bouts did not elicit any compensatory increases in energy intake during the remainder of the day, which resulted in a negative 24 h energy balance in both exercise trials compared with control. Although the main determinants of energy balance are behavioural (i.e. exercise energy expenditure and energy intake) (King et al. 2007), the stimulation of resting energy expenditure after exercise further increased the energy gap between control and exercise trials, resulting in energy deficits of 33.7 and 38.4 % in the fasted and postprandial trials relative to control. It has previously been demonstrated that individual differences occur in the energy intake responses to exercise-induced energy deficits (Finlayson et al. 2009) and are implicated in the success of weight loss programs (King et al. 2008; 2009). However, all participants in the present study incurred a negative energy balance in both exercise trials compared with control. It is plausible that compensatory increases in energy intake may occur in the longer term and a longer observation period is needed to elucidate such responses. However, this study substantiates evidence that appetite and energy intake responses to exercise are certainly not as immediate or powerful as those to diet-induced energy deficits (Hubert et al. 1998; King et al. 2011a).



This study is limited by a lack of mechanistic investigation as circulating concentrations of appetite-regulating gut hormones were not measured but have previously been suggested to mediate the appetite response to exercise (Broom et al. 2009). This issue is addressed in Chapters 5 – 7, as blood samples are regularly collected throughout all trials for the determination of plasma gut hormone concentrations. A further limitation of this study is that energy intake may have been influenced by the different time periods between the cessation of exercise and the ad libitum meals in the fasted and postprandial trials. However, this is unavoidable when controlling for feeding state and manipulating the timing of exercise in relation to breakfast and represents an ecologically valid scenario. In this regard, it was deemed preferable to investigate the effect of exercising either before or after breakfast, rather than the effect of exercise after either breakfast consumption or omission.

In conclusion, this is the first study to directly compare the ad libitum energy intake response to fasted and postprandial exercise and demonstrates that enhanced appetite suppression during postprandial exercise does not transpire into altered energy intake. Subsequently both fasted and postprandial exercise induced a negative daily energy balance relative to the control trial but there appears to be no additional benefit of exercising before or after breakfast. It may be beneficial for future experiments to manipulate the exercise session, rather than the timing of exercise, as a method of minimising the energy intake response to exercise. In this regard, several authors have suggested that HIIE may induce a greater reduction in food intake than traditional endurance exercise as a result of greater appetite suppression during the post-exercise period (Boutcher 2011; Heydari et al. 2012; Trapp et al. 2008; Tremblay et al. 1994). However, despite such postulations, the appetite and energy balance responses to HIIE have not yet been examined and will be investigated in the following two chapters of this thesis. In this regard, the appetite and energy intake response to continuous endurance exercise is compared with a recently popularised ‘sprint interval exercise’ protocol in Chapter 5 and a high volume HIIE protocol in Chapter 6.

## CHAPTER V

### **Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise**

#### **5.1 Introduction**

Within the last decade, an increasing number of studies have investigated the health and fitness implications of low volume, supramaximal sprint interval exercise. Despite substantially lower total work and energy expenditure, sprint interval exercise appears to elicit improvements in  $\text{VO}_2$  max, insulin sensitivity, postprandial lipaemia, and endothelial function that are comparable to those induced by traditional high volume prolonged endurance exercise (Babraj et al. 2009; Burgomaster et al. 2008; Freese et al. 2011; Rakobowchuk et al. 2008; Richards et al. 2010).

In the context of current society, where a ‘lack of time’ is commonly cited as a barrier to exercise (Troost et al. 2002), sprint interval exercise may offer a time-efficient method of inducing exercise-related health benefits. However, the weight loss response to low volume, sprint interval training remains equivocal (Babraj et al. 2009; Richards et al. 2010; Whyte et al. 2010) and is likely to be influenced by the extent of compensatory changes in appetite and energy intake after exercise (King et al. 2008).

Appetite and food intake are regulated at the physiological level by the neuroendocrine system, of which appetite-regulating gut hormones play a role as episodic mediators of hunger and satiety (Murphy & Bloom 2006). Ghrelin is the only known orexigenic gut peptide and is produced primarily by cells in the oxyntic glands of the stomach. Ghrelin is acylated via n-octanoylation of the serine 3 residue and although only ~10 % of ghrelin circulates in the acylated form, this is believed to be the form of ghrelin solely responsible for appetite stimulation (Ghigo et al. 2005).

Conversely, PYY is an anorexigenic gut peptide, which is synthesised and released from L-cells predominantly located in the distal GI tract. Peptide YY circulates in the blood in two forms: the intact 36 amino acid peptide  $\text{PYY}_{1-36}$ , and the truncated 34 amino acid peptide  $\text{PYY}_{3-36}$ . Although both forms are present in the blood,  $\text{PYY}_{3-36}$  is more abundant and exerts more potent anorexigenic effects (Karra & Batterham 2010).

A consistent body of evidence indicates that plasma acylated ghrelin concentrations are acutely suppressed and PYY concentrations elevated, during and immediately after strenuous endurance exercise (Broom et al. 2009; King et al. 2011a; Ueda et al. 2009a). Such hormonal changes occur concomitantly with a decrease in appetite during exercise and have been postulated as a potential mechanism underlying exercise-induced appetite suppression (Broom et al. 2009). Furthermore, in contrast to energy restriction, energy deficits induced by exercise do not appear to elicit compensatory changes in appetite, energy intake, or circulating concentrations of acylated ghrelin and PYY<sub>3-36</sub> in the hours after exercise (King et al. 2011a).

The influence of exercise intensity on appetite and energy intake responses has received little attention and only a small range of submaximal intensities (35 to 75 % of VO<sub>2</sub> max) have been investigated, yielding equivocal findings. Thompson and colleagues (1988) demonstrated greater appetite suppression during exercise as intensity increased but no difference in energy intake after exercise, whereas Imbeault et al. (1997) found no differences in appetite but decreased energy intake as exercise intensity increased. Alternatively, Ueda and colleagues (2009a) found no difference in the appetite or energy intake responses to different intensity exercise bouts. The effect of supramaximal exercise has not yet been investigated and may help to elucidate the importance of exercise intensity in determining the appetite, hormonal and energy intake responses to exercise.

The purpose of this study was to investigate the appetite, acylated ghrelin, total PYY and 24 h energy intake responses to 30 min of low volume sprint interval exercise compared with 60 min of traditional endurance exercise. It was hypothesised that appetite and acylated ghrelin would be suppressed, and total PYY elevated, to a greater extent during sprint interval exercise than during endurance exercise, resulting in lower energy intake in the hours after sprint exercise.

## **5.2 Methods**

### **5.2.1 Participants**

Twelve healthy men matching the inclusion criteria listed in Chapter 3.1, volunteered to participate in this study. The physical characteristics of the participants (mean (SD)) were as follows: age 23 (3) years, BMI 24.2 (2.9) kg.m<sup>-2</sup>, body mass 78.1 (9.6) kg, body fat 14.4 (4.7) %, waist circumference 81.6 (7.6) cm, VO<sub>2</sub> max 46.3 (10.2) mL.kg<sup>-1</sup>.min<sup>-1</sup>.

### **5.2.2 Preliminary trial**

Prior to main trials participants visited the laboratory to undergo screening and preliminary anthropometric measurements as detailed in Chapters 3.1 and 3.2. After familiarisation with the testing equipment, participants also completed a continuous incremental exercise test to exhaustion on an electromagnetically braked cycle ergometer to determine submaximal oxygen consumption and VO<sub>2</sub> max. Details of this test are described in Chapter 3.5.2.

### **5.2.3 Experimental protocol**

Participants performed three experimental trials (control, continuous endurance exercise and sprint interval exercise) separated by one week in a counterbalanced Latin Square design. In the 24 h preceding each main trial, participants standardised their food intake and physical activity, as described in Chapter 3.9. Participants arrived at the laboratory at 8.45 am after an overnight fast of at least 10 h and exerted themselves minimally when travelling to the laboratory.

Upon arrival to the laboratory, participants completed a baseline appetite questionnaire and then consumed a standardised breakfast meal within 15 min. The 8 h trial commenced upon completion of the meal. Sixty min of continuous endurance exercise was performed in the endurance exercise trial (END) from 1.75 – 2.75 h, whereas 30 min of supramaximal sprint interval exercise was performed in the sprint interval exercise trial (SIE) from 2.25 – 2.75 h. No exercise was performed in the control trial (CON). Participants rested within the laboratory for the remainder of the day in all trials (sitting reading, working at a desk or watching television).

During each trial, appetite perceptions were assessed at baseline, 0.25, 1.75, 2.25, 2.5, 2.75, 3 h and every 30 min thereafter using 100 mm visual analogue scales (see Chapter 3.10). Resting expired gas samples were collected into Douglas bags for 5 min after 5 min rest in a semi-supine position at: 4.5, 5.5, 7 and 8 h for determination of resting energy expenditure and substrate oxidation (see Chapter 3.8). Water was available ad libitum throughout each trial. Figure 5.1 provides an overview of the protocol for the experimental trials.

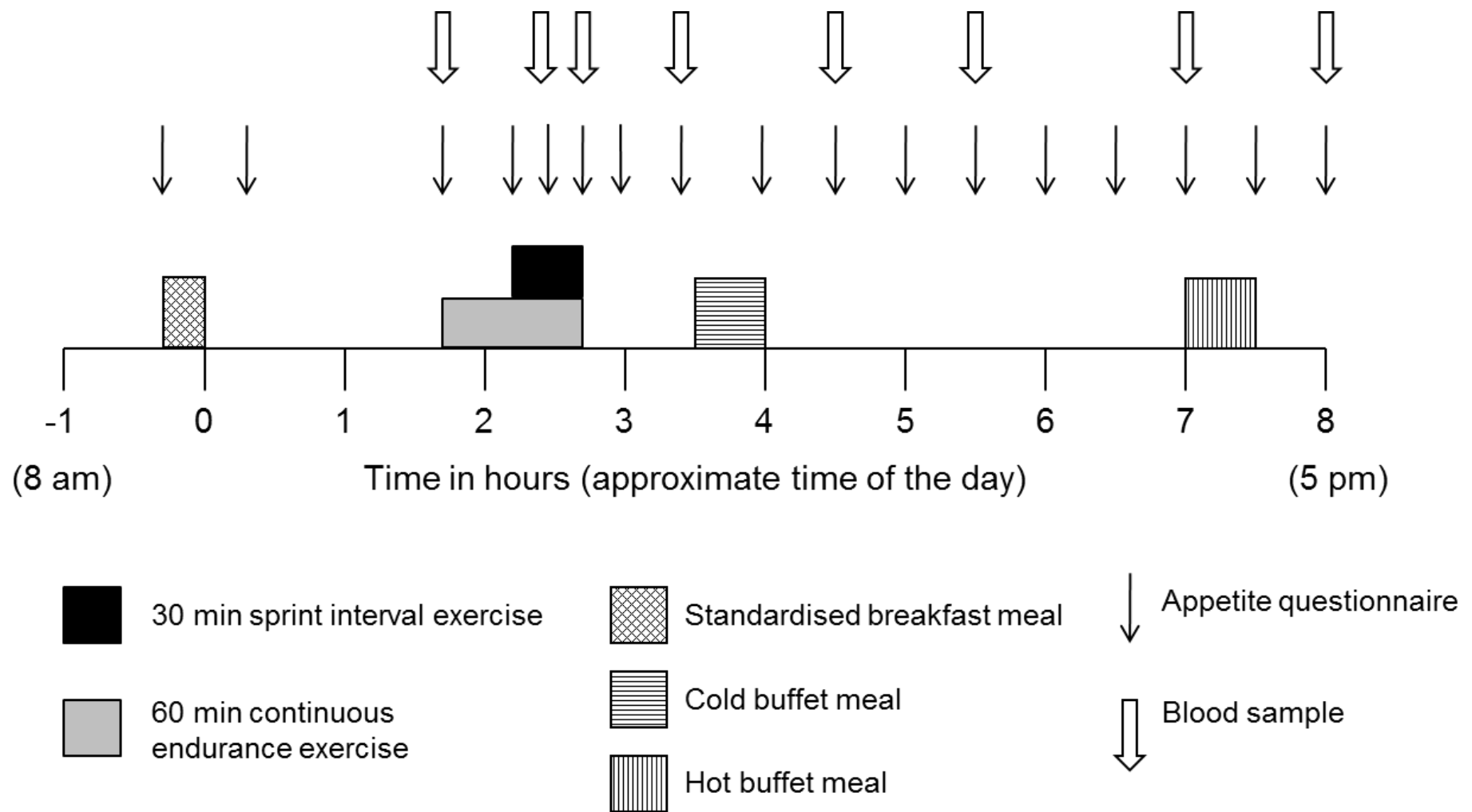


Figure 5.1. Schematic representation of the protocol for the experimental trials.

#### **5.2.4 Endurance exercise session**

Participants performed 60 min of continuous cycling exercise on an electromagnetically braked cycle ergometer (Lode Excalibur Sport V2, Groningen, Netherlands) at a work rate predicted to elicit 65 % of  $\text{VO}_2$  max. Samples of expired gas were collected at 10, 20, 30, 45 and 60 min during exercise to estimate energy expenditure and to monitor the intensity of the cycle, with adjustments made to the work rate if necessary. Heart rate and RPE were also assessed at these times.

#### **5.2.5 Sprint interval exercise session**

The sprint interval exercise session was modelled on recent studies conducted by Burgomaster and colleagues (2007; 2008). After a 3.5 min warm up at a fixed power output of 30 W, participants performed six 30 s sprints on an electronically braked cycle ergometer (Lode Excalibur Sport 925900, Groningen, Netherlands) against a resistance equivalent to 7.5 % of body mass. Participants were instructed to begin pedalling as fast as possible ~2 s before the appropriate load was applied by a computer interfaced with the ergometer and loaded with the appropriate software (Lode Ergometry Manager Wingate Test Plus). Participants were instructed to reach maximum fatigue by the end of the session and were verbally encouraged throughout. Peak power, mean power and fatigue index were subsequently determined using an online data acquisition system. Sprints were separated by 4 min recovery periods during which participants remained on the bike and cycled at a power output of 30 W. A 3.5 min warm-down at a fixed power output of 30 W concluded the session. Collection of expired gas during exercise was rendered impractical due to the high levels of nausea experienced by participants. Therefore, energy expenditure was estimated using the methods of Medbø et al. (1988) (see Chapter 3.8). Heart rate and RPE were recorded upon completion of each sprint.

#### **5.2.6 Net energy expenditure of exercise**

To estimate the net energy expenditure of exercise (gross energy expenditure of exercise minus energy expenditure during the control trial), expired gas was collected into Douglas bags for 5 min every 15 min between 1.75 and 2.75 h during the control trial and energy expenditure subsequently calculated using the equation of Frayn (1983).

### **5.2.7 Standardised breakfast, ad libitum buffet meals and overnight food bag**

Participants were provided with a standardised breakfast (see Chapter 3.11) upon their arrival to the laboratory and were given 30 min ad libitum access to a cold buffet meal and hot buffet meal (see Chapter 3.12) at 3.5 h and 7 h, respectively. At the end of each trial, participants were free to select any of the items presented at the buffet meals to be included in an overnight food bag. Leftovers were returned the next day to determine the weighted difference in food items and subsequently energy intake.

### **5.2.8 Blood sampling**

During CON and END all venous blood was collected via a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden), which was inserted into an antecubital vein at 1 h. To minimise arm discomfort during the sprint exercise, the first 3 blood samples were collected via venepuncture and a cannula was then inserted at 3.25 h, from which the remaining blood samples were collected. Venous blood samples were collected into pre-chilled 9-mL and 4.9-mL EDTA monovettes at 1.75, 2.47, 2.75, 3.5, 4.5, 5.5, 7 and 8 h for the measurement of plasma total PYY and acylated ghrelin, respectively. Details of the blood collection procedures are described in Chapter 3.16.

### **5.2.9 Biochemical analysis**

Commercially available enzyme immunoassays were used to determine plasma concentrations of acylated ghrelin and total PYY, as described in Chapters 3.17.2 and 3.17.3. The within batch coefficient of variation for the assays were 5.7 % and 4.2 % for acylated ghrelin and total PYY, respectively.

### **5.2.10 Statistical analysis**

Data was analysed using the methods described in Chapter 3.18.



## 5.3 Results

### 5.3.1 Exercise responses

During END, participants completed the 60 min cycle at 154 (34) W. This elicited a mean oxygen consumption equivalent to 68.1 (4.3) % of  $\text{VO}_2$  max and a mean RER of 0.97 (0.04), which reflected a proportional contribution to energy provision of 91 (16) % carbohydrate and 9 (16) % fat. During SIE, participants completed the six sprint intervals at a work rate of 537 (66) W, which resulted in a mean work rate of 79 (7) W for the 30 min session including warm up, warm down and recovery periods.

Average heart rate, peak heart rate and RPE were significantly higher during sprint exercise than endurance exercise (average heart rate 167 (10) vs. 153 (13)  $\text{beats}\cdot\text{min}^{-1}$ ; peak heart rate 172 (5) vs. 160 (11)  $\text{beats}\cdot\text{min}^{-1}$ ; RPE 18 (1) vs. 14 (1) for SIE and END, respectively; both  $P < 0.01$ ). External work completed and net energy expenditure of exercise were significantly higher in END than SIE (external work 556 (121) vs. 143 (13) kJ; net energy expenditure 2640 (418) vs. 594 (50) kJ; both  $P < 0.0005$ ).

### 5.3.2 Appetite

Fasting appetite perceptions did not differ between trials at baseline (hunger ( $P = 0.465$ ), satisfaction ( $P = 0.558$ ), fullness ( $P = 0.520$ ) and PFC ( $P = 0.567$ )). Two-factor ANOVA revealed a main effect of time (all  $P < 0.0005$ ) and a trial x time interaction (all  $P < 0.0005$ ) for each appetite perception (Figure 5.2). Post-hoc analysis of trial x time interactions demonstrated suppressed appetite immediately upon cessation of the sprint exercise at 2.75 h but elevated appetite during the afternoon of the sprint exercise trial at 5, 5.5 and 6.5 h (see Figure 5.2). There was also a trend towards a main effect of trial for hunger ( $P = 0.084$ ) indicating higher hunger perceptions in SIE than END.

Table 5.1 shows the AUC values for each appetite perception. One-way ANOVA revealed trends for a main effect of trial for hunger ( $P = 0.089$ ) and PFC ( $P = 0.052$ ) from 3.5 – 8 h, indicating greater appetite during the afternoon of SIE than CON and END. A trend towards a main effect of trial was also present for total 8 h hunger AUC ( $P = 0.079$ ) indicating elevated hunger perceptions in SIE compared with END.

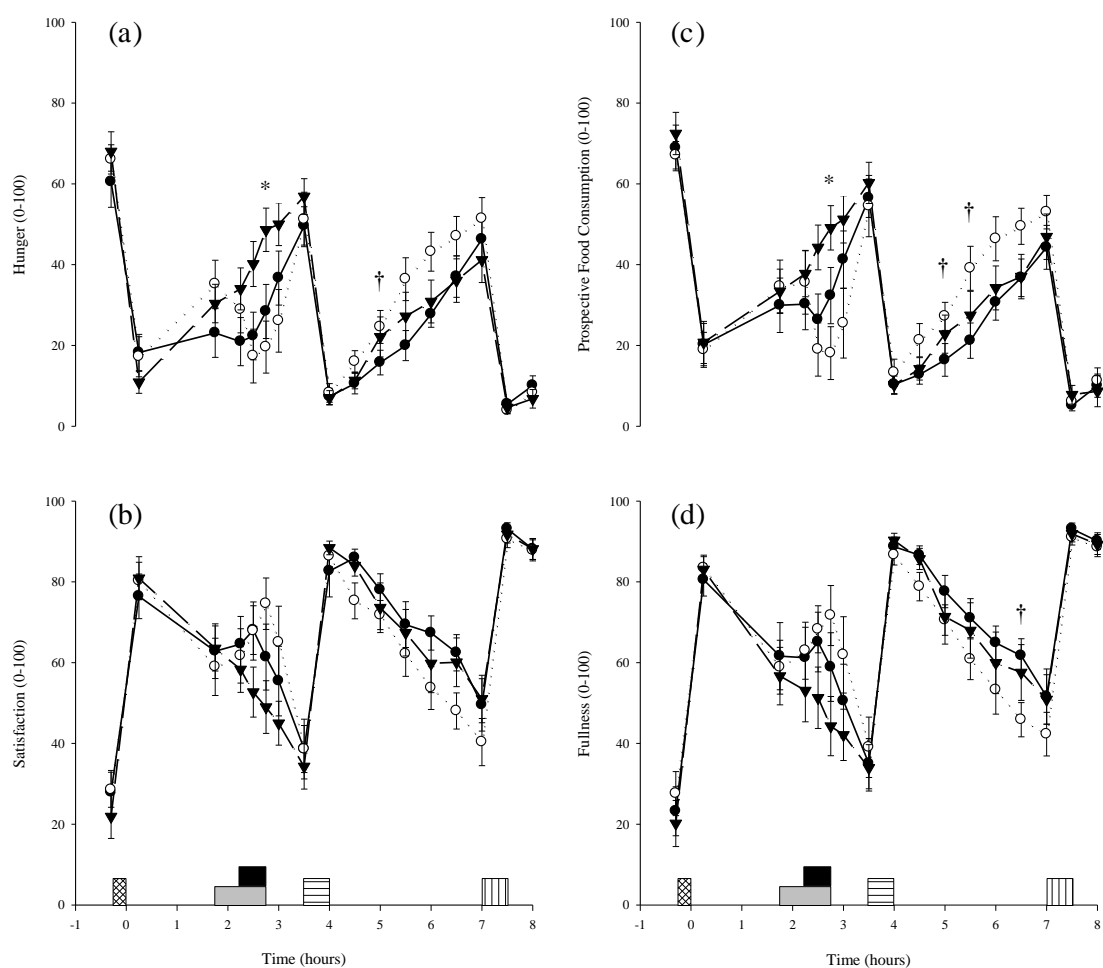


Figure 5.2. Perceptions of hunger (a), satisfaction (b), prospective food consumption (c) and fullness (d) in CON ( $\blacktriangledown$ ), END ( $\bullet$ ) and SIE ( $\circ$ ) trials. Values are mean (SEM),  $N = 12$ . Lightly shaded rectangle indicates endurance exercise, black rectangle indicates sprint interval exercise, hatched shaded rectangle indicates standardised breakfast meal, horizontally shaded rectangle indicates cold buffet meal, vertically shaded rectangle indicates hot buffet meal. \* Different between SIE and CON, † Different between SIE and END ( $P < 0.05$ ).

Table 5.1. Time-averaged area under the curve values for appetite perceptions in the control, endurance and sprint trials.

	<b>Morning (0-3.5 h)</b>	<b>Afternoon (3.5-8 h)</b>	<b>Total trial (0-8 h)</b>
<b>Hunger</b>			
Control	33 (13)	24 (12)	28 (9)
Endurance	27 (16)	22 (8)	24 (10)
Sprint	29 (17)	29 (10)	29 (11)
<b>Satisfaction</b>			
Control	60 (17)	71 (13)	66 (11)
Endurance	63 (18)	72 (12)	68 (12)
Sprint	65 (17)	66 (11)	65 (12)
<b>Fullness</b>			
Control	57 (18)	71 (15)	65 (13)
Endurance	62 (19)	73 (10)	68 (12)
Sprint	65 (18)	66 (10)	65 (12)
<b>PFC</b>			
Control	38 (17)	26 (12)	31 (11)
Endurance	32 (18)	23 (9)	27 (11)
Sprint	30 (18)	32 (11)	31 (13)

Values are mean (SD), N = 12. PFC = prospective food consumption. Afternoon AUC for hunger and PFC tended to be highest in the sprint trial (One-way ANOVA: P = 0.089 and P = 0.052, respectively). Total 8 h AUC for hunger tended to be highest in the sprint trial (One-way ANOVA: P = 0.079).

### 5.3.3 Energy and macronutrient intake

Two-factor ANOVA revealed a main effect of time for energy intake (Table 5.2) and macronutrient intake (Table 5.3; both P < 0.0005) but no trial or interaction effects. Effect sizes for total ad libitum energy intake were as follows: CON vs. END:  $d = 0.19$ ; CON vs. SIE:  $d = 0.01$ ; END vs. SIE:  $d = 0.20$ ).

Table 5.2. Ad libitum energy intake during the control (CON), endurance (END) and sprint (SIE) trials.

		<b>CON</b>		<b>END</b>		<b>SIE</b>	
<b>Buffet 1 (cold)</b>	kJ	5715	(1866)	6121	(2000)	5460	(1611)
<b>Buffet 2 (hot)</b>	kJ	4632	(1075)	5201	(1180)	4749	(1146)
<b>Overnight</b>	kJ	2594	(1172)	2222	(1054)	2711	(1372)
<b>Total trial</b>	kJ	12,941	(3113)	13,548	(3205)	12,920	(2983)

Values are mean (SD), N = 12. No significant differences between trials.

Table 5.3. Macronutrient intake in the control (upper panel), endurance (middle panel) and sprint (lower panel) trials.

		<b>Fat</b>	<b>Carbohydrate</b>	<b>Protein</b>
<b>Control</b>				
Buffet 1	g	57.7 (23.8)	163.8 (52.5)	48.0 (16.2)
Buffet 2	g	21.4 (6.7)	165.0 (38.2)	63.4 (22.7)
Overnight	g	20.3 (13.7)	89.8 (33.6)	19.6 (12.7)
Total	g	99.4 (33.2)	418.6 (95.7)	131.0 (34.7)
<b>Endurance</b>				
Buffet 1	g	59.5 (28.4)	182.6 (54.9)	49.3 (11.7)
Buffet 2	g	25.6 (6.9)	182.2 (48.8)	71.0 (20.0)
Overnight	g	16.7 (11.3)	81.2 (32.6)	14.1 (12.6)
Total	g	101.8 (37.6)	446.0 (92.0)	134.4 (33.2)
<b>Sprint</b>				
Buffet 1	g	50.7 (20.9)	164.7 (41.6)	47.4 (20.8)
Buffet 2	g	24.2 (8.8)	163.7 (40.4)	65.6 (17.7)
Overnight	g	18.7 (12.1)	101.3 (47.7)	18.7 (13.4)
Total	g	93.5 (28.8)	429.8 (98.0)	131.7 (31.0)

Values are mean (SD), N = 12. No significant differences between trials.

### **5.3.4 Relative energy intake**

One-way ANOVA revealed a significant main effect of trial for relative energy intake (energy intake minus the net energy expenditure of exercise), demonstrating lower REI in END than CON (15.7%;  $P = 0.006$ ) and a tendency towards lower REI in END than SIE (11.5%;  $P = 0.082$ ) but no difference in REI between SIE and CON ( $P = 0.221$ ; Con 12,941 (3113); END 10,908 (3238); SIE 12,326 (2987) kJ). Closer inspection of the data revealed that 11 out of 12 participants had a lower REI in END than CON but only 6 out of 12 participants had a lower REI in SIE than CON. Nine out of 12 participants had a lower REI in END than SIE.

### **5.3.5 Plasma acylated ghrelin and total PYY concentrations**

Initial plasma acylated ghrelin concentrations did not differ between trials ( $P = 0.569$ ). Two-factor ANOVA revealed a significant trial ( $P < 0.0005$ ), time ( $P = 0.001$ ) and trial x time interaction effect ( $P < 0.0005$ ) for absolute plasma acylated ghrelin concentrations (Figure 5.3a). Post-hoc analysis of between trial differences revealed higher plasma acylated ghrelin concentrations in CON than END ( $P = 0.024$ ) and SIE ( $P < 0.0005$ ). Post-hoc analysis of trial x time interactions demonstrated suppressed acylated ghrelin immediately and 0.75 h after exercise in SIE relative to CON (both  $P < 0.002$ ). Analysis of delta acylated ghrelin concentrations revealed the same trial ( $P = 0.047$ ), time ( $P = 0.001$ ) and trial x time interaction effects ( $P < 0.0005$ ; Figure 5.3b).

Area under the acylated ghrelin versus time curve was significantly lower in END and SIE than CON from 1.75 – 3.5 h and 1.75 – 8 h (all  $P < 0.05$ ). Values were also lower in SIE than CON from 3.5 – 8 h ( $P < 0.0005$ ). Analysis of delta AUC values revealed significantly lower values in SIE than CON from 1.75 – 3.5 h ( $P = 0.003$ ) but no between trial differences for 3.5 – 8 h ( $P = 0.355$ ) and 1.75 – 8 h ( $P = 0.09$ ).

Initial plasma total PYY concentrations did not differ between trials ( $P = 0.255$ ). Two-factor ANOVA revealed a significant time ( $P < 0.0005$ ) and trial x time interaction effect ( $P = 0.001$ ) for plasma total PYY concentrations (Figure 5.4a). Post-hoc analysis of trial x time interactions indicated elevated total PYY immediately after exercise in END and SIE relative to CON (both  $P < 0.01$ ). However, after Holm-Bonferroni adjustment only END remained significantly higher than CON ( $P < 0.05$ ), due to greater individual variability in the total PYY response to SIE. Analysis of delta total

PYY concentrations also failed to reveal a main effect of trial ( $P = 0.334$ ) but demonstrated a significant time ( $P < 0.0005$ ) and trial x time interaction effect ( $P = 0.001$ ). In accordance with the analysis of absolute PYY concentrations, post-hoc analysis indicated elevated delta values immediately after exercise in END and SIE relative to CON (both  $P < 0.012$ ) but this did not reach statistical significance after Holm-Bonferroni adjustment (Figure 5.4b).

One-way ANOVA revealed a tendency for higher absolute total PYY AUC in END and SIE than CON from 1.75 – 3.5 h ( $P = 0.068$ ) but there was no difference between trials for 3.5 – 8 h ( $P = 0.369$ ) and 1.75 – 8 h ( $P = 0.201$ ). Analysis of delta AUC values replicated these findings (1.75-3.5 h:  $P = 0.057$ ; 3.5-8 h:  $P = 0.304$ ; 1.75-8 h:  $P = 0.416$ ).

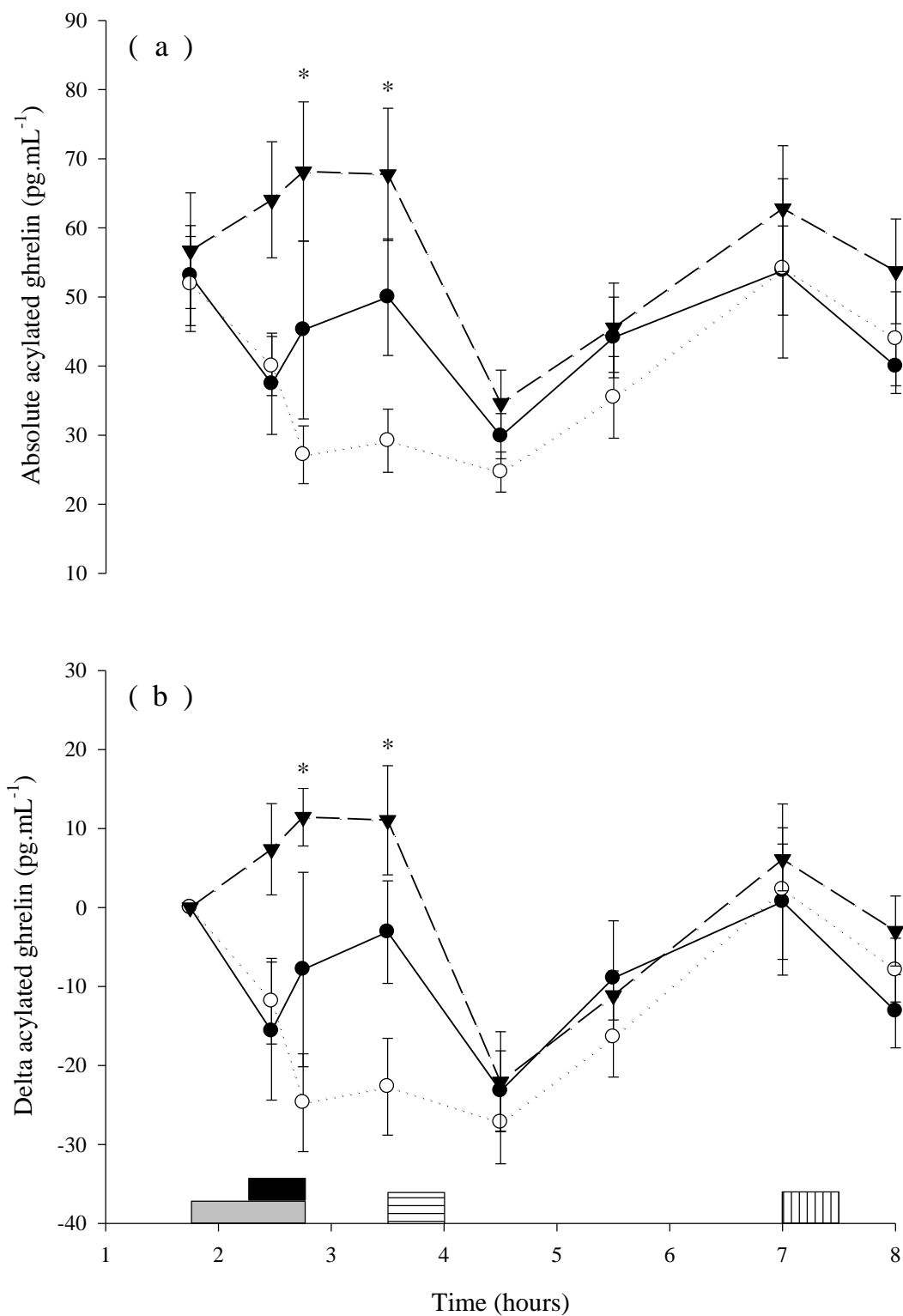


Figure 5.3. Absolute (a) and delta (b) acylated ghrelin concentrations in CON (▼), END (●) and SIE (○) trials. Values are mean (SEM), N = 12. Lightly shaded rectangle indicates endurance exercise, black rectangle indicates sprint interval exercise, horizontally shaded rectangle indicates cold buffet meal, vertically shaded rectangle indicates hot buffet meal. \* Different between SIE and CON (P < 0.05).

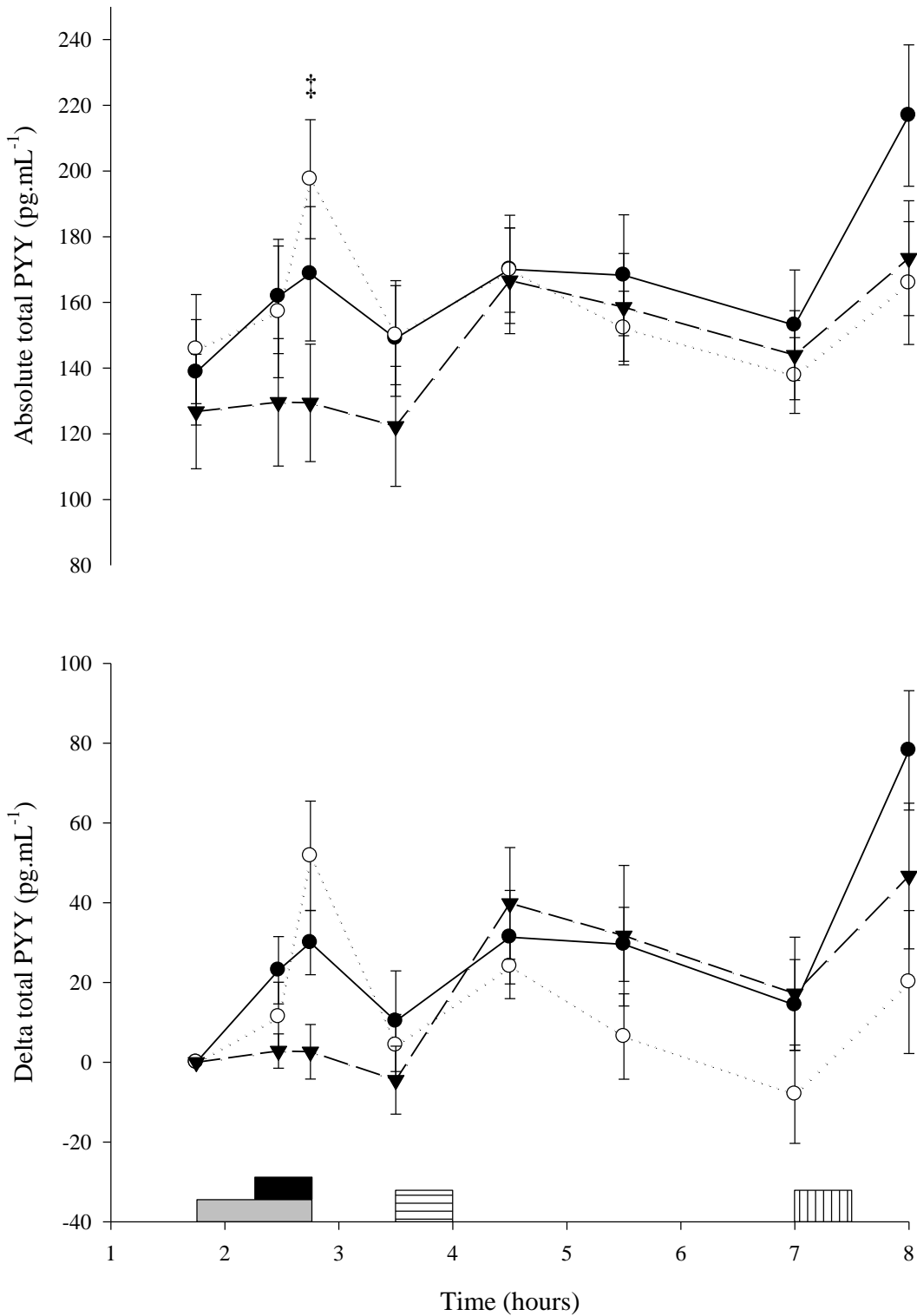


Figure 5.4. Absolute (a) and delta (b) total PYY concentrations in CON (▼), END (●) and SIE (○) trials. Values are mean (SEM), N = 12. Lightly shaded rectangle indicates endurance exercise, black rectangle indicates sprint interval exercise, horizontally shaded rectangle indicates cold buffet meal, vertically shaded rectangle indicates hot buffet meal. ‡Different between END and CON (P < 0.05).



### 5.3.6 Resting energy expenditure and substrate utilisation

Two-factor ANOVA revealed a main effect of time for resting energy expenditure and respiratory exchange ratio during the afternoon of the trials (both  $P < 0.0005$ ) but no trial or interaction effects (Figure 5.5).

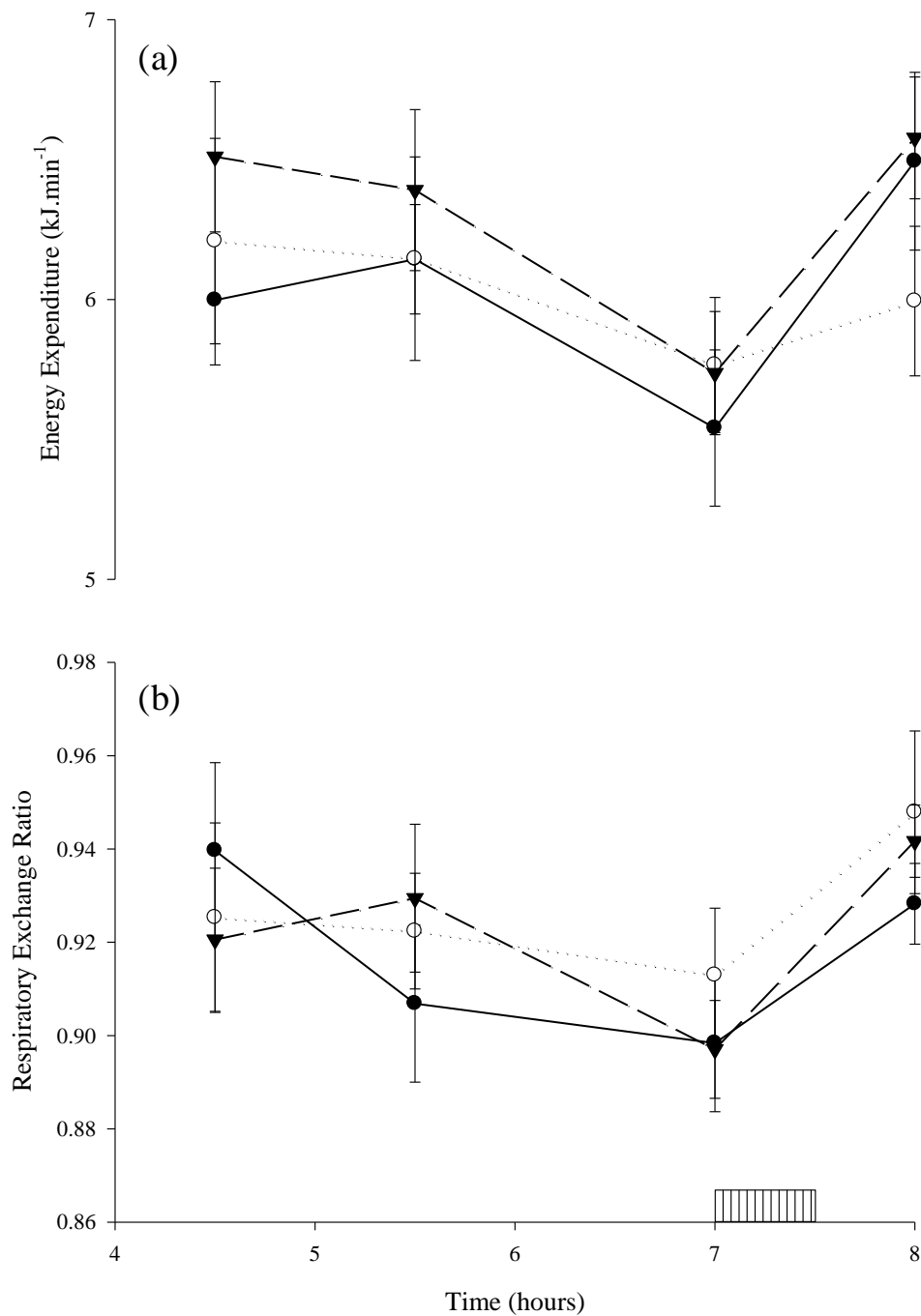


Figure 5.5. Resting energy expenditure (a) and respiratory exchange ratio (b) in CON (▼), END (●) and SIE (○) trials. Values are mean (SEM),  $N = 12$ . Vertically shaded rectangle indicates hot buffet meal. No significant differences between trials.

### **5.3.7 Correlations**

Plasma acylated ghrelin concentration was positively related to appetite at 5.5 h on END but negatively related to appetite at 3.5 h on SIE ( $P < 0.05$ ). There was no correlation between plasma acylated ghrelin concentration immediately prior to ad libitum meals and subsequent energy consumption. Similarly, the percentage change in plasma acylated ghrelin after a meal was not related to energy intake at that meal.

Plasma total PYY concentration was negatively correlated with appetite at 1.75 h during CON and at 2.47, 2.75 and 3.5 h during END (all  $P < 0.05$ ). The percentage increase in total PYY after ad libitum buffet meals was weakly but significantly positively correlated with energy intake during the meals ( $r = 0.279$ ,  $P = 0.017$ ).

## 5.4 Discussion

The purpose of this investigation was to compare the effect of low volume, supramaximal sprint interval exercise and continuous endurance exercise on appetite, acylated ghrelin, total PYY and energy intake responses. The primary findings are that sprint interval exercise induced a greater suppression of appetite and plasma acylated ghrelin concentrations during exercise but resulted in compensatory increases in appetite (though not food intake) in the hours after exercise. Furthermore, continuous endurance exercise induced a substantial 24 h energy deficit relative to the control and sprint trials, due to the high level of energy expenditure during the exercise bout.

In accordance with previous research, there was a marked decrease in appetite during both exercise bouts, which coincided with a decrease in plasma acylated ghrelin and an increase in plasma PYY concentrations (Broom et al. 2009; King et al. 2011a; Ueda et al. 2009a). The decreases in appetite and acylated ghrelin both occurred to a greater extent during sprint exercise, which strengthens the association between exercise-induced suppressions of these variables. However, despite greater appetite suppression during sprint exercise, endurance exercise stimulated more consistent increases in plasma total PYY concentrations. Such confliction would suggest that decreases in appetite during exercise are not dependent on changes in circulating concentrations of total PYY and that such changes are likely to be influenced by exercise mode and duration as well as intensity.

Despite simultaneous appetite and acylated ghrelin suppression during exercise, appetite perceptions returned to similar values between all trials immediately prior to the first buffet meal, yet acylated ghrelin remained significantly suppressed in the sprint trial, resulting in a negative correlation between these variables. Additionally, appetite was highest and acylated ghrelin concentrations lowest in the afternoon of the sprint trial. Such divergence between appetite and acylated ghrelin concentrations may question the role of exercise-induced decreases in acylated ghrelin as a determinant of appetite, despite simultaneous suppression during the exercise bout.

Although increases in circulating ghrelin concentrations have been shown to increase appetite and energy intake in humans (Ghigo et al. 2005), the influence of physiological decreases in circulating ghrelin are less clear. Callahan and colleagues (2004) previously demonstrated that plasma ghrelin concentrations were depressed to a greater

extent after higher caloric preloads but that the recovery of plasma ghrelin concentrations did not predict subsequent meal initiation. Furthermore, circulating ghrelin concentrations have been found to be inversely related to BMI and depressed in obese individuals (Tschöp et al. 2001). However, such changes in ghrelin do not appear to reduce appetite or aid weight loss in the obese (Cummings et al. 2002). It must be noted that ghrelin is only one component of the appetite-regulating neuroendocrine system and that appetite is likely to reflect many hormonal and psychological factors (King et al. 2007; Murphy & Bloom 2006). Nevertheless, the present study adds to the debate surrounding the importance of physiological decreases in acylated ghrelin as a determinant of appetite. This issue is likely to be clarified only when specific ghrelin antagonists become available for use in clinical investigations.

Despite substantial changes in appetite and acylated ghrelin and total PYY concentrations, energy and macronutrient intake did not differ between trials at any of the three feeding opportunities. This supports previous evidence that exercise does not provoke short-term compensatory increases in energy intake and suggests that the behavioural act of food intake may largely be governed by environmental contingencies and short-term postingestive physiological responses (Blundell & King 1999). Similar absolute food intake between trials resulted in a substantial negative energy balance in the endurance trial compared with the sprint and control trials due to the high level of energy expenditure during the endurance exercise bout (~2640 kJ). It is unknown whether compensatory increases in energy intake occur in the longer term but it appears that prolonged endurance exercise is more beneficial for inducing a negative 24 h energy balance than low volume, sprint interval exercise. The practicalities of recommending multiple Wingate tests as a physical activity initiative for the general population must also be questioned as sprint interval exercise in the present study stimulated feelings of nausea in 11 out of 12 participants and induced vomiting in 3 participants.

One limitation of the present study is that energy expenditure of the sprint exercise was estimated rather than measured directly. Although it is possible that this may have underestimated the energy cost of exercise, numerous studies have acknowledged that sprint interval exercise expends substantially lower amounts of energy than prolonged endurance exercise (Babraj et al. 2009; Freese et al. 2011; Richards et al. 2010; Whyte et al. 2010). Furthermore, the estimated *net* energy cost of the sprint interval exercise in

this study was slightly higher than previous estimates of *gross* energy expenditure (Babraj et al. 2009; Whyte et al. 2010). The methods employed in the present study attempted to account for the oxygen deficit of the sprints (Medbø et al. 1988; Scott et al. 1991), which is thought to be the primary determinant of elevations in energy expenditure during the recovery periods (Hagberg et al. 1980). However, it remains plausible that energy expenditure may have been underestimated as other factors were not accounted for, such as elevated body temperature, circulation and ventilation during recovery periods (Børsheim & Bahr 2003) or due to less efficient cycling at supramaximal workloads (Hunter et al. 1998). Such factors are likely to be trivial in comparison to the vast differences in exercise volume and work completed between exercise protocols but nonetheless must be acknowledged.

Boutcher (2011) recently speculated that high intensity intermittent exercise may be more beneficial for weight loss than continuous moderate intensity exercise due to greater post-exercise decreases in appetite and increases in resting energy expenditure and fat oxidation. However, the present study provides evidence that appetite is higher in the hours after sprint interval exercise than continuous endurance exercise, despite greater appetite suppression during the sprint interval exercise. Furthermore, resting energy expenditure and substrate oxidation did not differ between trials during the 1.75 – 5.25 h after exercise. Although it would have been preferable to collect such data using the ventilated hood system, the Douglas bag method employed in this study was sufficiently sensitive to detect meal-related changes in resting energy expenditure of  $\sim 0.5 \text{ kJ}\cdot\text{min}^{-1}$  and respiratory exchange ratio values of  $\sim 0.03$ . This suggests that any meaningful changes in resting metabolism as a result of exercise would also have been detected using this method.

To the authors' knowledge only two studies have previously compared the resting metabolic responses to supramaximal interval exercise and moderately-strenuous endurance exercise (Bahr 1992; Laforgia et al. 1997). Of these studies, only one demonstrated a significant difference in resting metabolism between protocols but deemed that the 135 kJ higher energy expenditure in the 9 h after supramaximal exercise was of 'negligible physiological significance' for energy balance (Laforgia et al. 1997). Such findings have led some recent authors to conclude that to induce a negative energy balance, exercisers should focus on maximising energy expenditure during the exercise bout itself (Laforgia et al. 2006; Warren et al. 2009). This

contention is supported by the finding of the present study that energy intake is unresponsive to substantial differences in exercise volume and intensity. Longer duration high intensity intermittent exercise may represent a more successful approach to inducing energy deficits than supramaximal sprint interval exercise as a result of higher exercise volume, external work and energy expenditure. However, this has yet to be examined and will be investigated in Chapter 6.

In conclusion, this is the first study to investigate appetite and energy intake responses to the recently popularised 'sprint interval training' protocol. Energy intake was unaffected by exercise, which resulted in a negative 24 h energy balance after traditional endurance exercise compared with sprint interval exercise, due to differences in energy expenditure. Furthermore, sprint exercise stimulated compensatory increases in appetite in the hours after exercise despite lower circulating acylated ghrelin concentrations.

## CHAPTER VI

### **Appetite, energy intake and PYY<sub>3-36</sub> responses to energy-matched continuous exercise and submaximal high intensity exercise.**

#### **6.1 Introduction**

High intensity intermittent exercise appears to induce many similar health and performance-related physiological adaptations as traditional continuous steady state exercise (SSE) (Gibala et al. 2012). However, the effect of HIIE on body composition remains a contentious issue, with studies showing greater (Irving et al. 2008; Trapp et al. 2008; Tremblay et al. 1994), lesser (Helgerud et al. 2007; Nybo et al. 2010) or no difference (Moholdt et al. 2009; Rognmo et al. 2004; Tjønnna et al. 2008; Warburton et al. 2005; Wisløff et al. 2007) in the weight and fat loss responses to HIIE compared with SSE.

Despite such conflicting findings, it has been repeatedly postulated that HIIE has the potential to facilitate greater fat loss than SSE due to a greater reduction in appetite during the post-exercise period (Boutcher 2011; Heydari et al. 2012; Trapp et al. 2008; Tremblay et al. 1994). However, thus far, the experiment conducted in Chapter 5 is the only published study to have investigated the appetite response to a HIIE protocol and demonstrated that appetite perceptions and daily energy balance were higher after six 30 s Wingate tests than after 60 min of continuous cycling at ~68 % of  $\text{VO}_2$  max. The mechanisms underlying this increase in appetite are unknown but occurred despite a lower exercise energy expenditure and greater appetite suppression during the sprint exercise.

The practical application of such supramaximal exercise has recently been questioned due to the high levels of nausea previously experienced by participants and the increased risk of acute cardiovascular events during such intense exercise (Chapter 5; Gibala et al. 2012; Richards et al. 2010; Whyte et al. 2010). Conversely, high volume HIIE involving repeated 4 min exercise intervals at ~90 % of  $\text{VO}_2$  max appears to be well-tolerated, even in clinical populations (Moholdt et al. 2009; Rognmo et al. 2004; Tjønnna et al. 2008; Wisløff et al. 2007).

High volume HIIE represents an intermediate between SSE and supramaximal interval exercise (Talanian et al. 2007). It is therefore unclear whether this exercise mode will

stimulate compensatory increases in appetite similar to supramaximal interval exercise (see Chapter 5), or whether the appetite response will be more akin to continuous submaximal exercise, which does not seem to elicit any compensatory increases in appetite (King et al. 2010a; 2011a). Understanding the appetite response to popular exercise protocols is important in determining the most effective method of inducing a negative energy balance without stimulating compensatory increases in appetite, which are inversely associated with exercise-induced weight loss (King et al. 2008). Furthermore, high volume HIIE allows comparisons with SSE to be matched for duration, total work and energy expenditure. Therefore, investigations into this exercise protocol may help to elucidate the influence of exercise mode on appetite and energy intake responses.

The purpose of this investigation was to examine the appetite and energy intake responses to ten 4 min cycling bouts at 85-90 % of  $\text{VO}_2$  max compared with 60 min of continuous cycling at 60 % of  $\text{VO}_2$  max. Circulating concentrations of PYY<sub>3-36</sub> were also measured, as previous research suggests that this anorexigenic gut hormone may be implicated in the appetite response to exercise (King et al. 2011a; Ueda et al. 2009a). It was hypothesised that high intensity intermittent cycling would stimulate compensatory increases in appetite during the hours after exercise but that this would not occur in response to continuous steady state cycling.



## **6.2 Methods**

### **6.2.1 Participants**

Twelve healthy men matching the inclusion criteria listed in Chapter 3.1, volunteered to participate in this study. The physical characteristics of the participants (mean (SD)) were as follows: age 22 (3) years, BMI 23.7 (3.0) kg.m<sup>-2</sup>, body mass 75.0 (12.0) kg, body fat 13.7 (3.9) %, waist circumference 78.6 (6.4) cm, VO<sub>2</sub> max 52.4 (7.1) mL.kg<sup>-1</sup>.min<sup>-1</sup>.

### **6.2.2 Preliminary trial**

Prior to main trials participants visited the laboratory to undergo screening, and preliminary anthropometric measurements as detailed in Chapters 3.1 and 3.2. After familiarisation with the testing equipment, participants also completed a continuous incremental exercise test to exhaustion on an electromagnetically braked cycle ergometer to determine submaximal oxygen consumption and VO<sub>2</sub> max. Details of this test are described in Chapter 3.5.2.

### **6.2.3 Experimental protocol**

Participants performed three experimental trials (control, steady state exercise and high intensity intermittent exercise) separated by one week in a counterbalanced Latin Square design. All trials were identical except that 60 min of exercise was performed in the steady state exercise (SSE) and high intensity intermittent exercise (HIIE) trials. Exercise commenced at 2 h and finished at 3 h. No exercise was performed in the control trial (CON). Participants rested within the laboratory for the remainder of the day in all trials (sitting reading, working at a desk or watching television).

In the 24 h preceding each main trial, participants standardised their food intake and physical activity, as described in Chapter 3.9. Participants arrived at the laboratory at 8.30 am after an overnight fast of at least 10 h and exerted themselves minimally when travelling to the laboratory.

During each trial, appetite perceptions (hunger, satisfaction, fullness and PFC) were assessed at baseline, 0.25, 2 h and every 30 min thereafter using 100 mm visual analogue scales (see Chapter 3.10). An overall appetite score was calculated as the

mean value of the four appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al. 2000). Water was available ad libitum throughout each trial.

Figure 6.1 provides an overview of the protocol for the experimental trials.

#### **6.2.4 Steady state exercise session**

Participants performed 60 min of continuous cycling exercise on an electromagnetically braked cycle ergometer (Lode Excalibur Sport V2, Groningen, Netherlands) at a work rate predicted to elicit 60 % of  $\text{VO}_2$  max. Samples of expired gas were collected at 10, 20, 30, 45 and 60 min during exercise to estimate energy expenditure and to monitor the intensity of the exercise, with adjustments made to the work rate if necessary. Heart rate and RPE were also assessed at these times.

#### **6.2.5 High intensity intermittent exercise session**

The high intensity exercise session consisted of ten 4 min cycling bouts at a work rate predicted to elicit 85 – 90 % of  $\text{VO}_2$  max separated by 2 min of rest. Samples of expired gas were collected during the last minute of repetition number 1, 3, 5, 8 and 10 to monitor the intensity of the exercise and to calculate the energy expenditure of the session. Adjustments were made to the work rate based on the intensity and the participants' tolerance to the exercise. Heart rate and RPE were monitored during the final minute of each repetition.

#### **6.2.6 Net energy expenditure of exercise**

To estimate the net energy expenditure of exercise (gross energy expenditure of exercise minus energy expenditure during the control trial), expired gas was collected into Douglas bags for 5 min every 15 min from 2 – 3 h during the control trial and energy expenditure was subsequently calculated using the equation of Frayn (1983). Data from previous experiments suggested that the net energy expenditure of exercise should be comparable between the high intensity and steady state exercise sessions (Leggate et al. 2010; Warren et al. 2009).

#### **6.2.7 Standardised test meals and ad libitum meal**

After the collection of baseline measures, participants were provided with a standardised breakfast (see Chapter 3.11). Participants were instructed to consume the

breakfast within 15 min and the 8 h trial commenced upon completion of the meal. A standardised lunch was provided at 3.75 h (see Chapter 3.11), which was also consumed within 15 min.

At 7 h an ad libitum meal was provided (see Chapter 3.13). In contrast to the buffet meals used in Chapters 4 and 5, this ad libitum meal was of fixed macronutrient composition and the participants were blinded to the amount of food consumed. This was deemed necessary after concerns that food intake at buffet meals in the previous two chapters was influenced by environmental contingencies.

### **6.2.8 Blood sampling**

Upon arrival to the laboratory, participants rested in a semi-supine position and a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. Blood samples were collected at: baseline, 2, 3, 3.75, 5, 6, 7 and 8 h for the determination of PYY<sub>3-36</sub> concentrations, as described in Chapter 3.16.

### **6.2.9 Biochemical analysis**

Plasma concentrations of PYY<sub>3-36</sub> were determined using a commercially available radioimmunoassay (see Chapter 3.17.4). The within batch coefficient of variation for the assay was 7.2 %.

### **6.2.10 Statistical analysis**

Data was analysed using the methods described in Chapter 3.18.

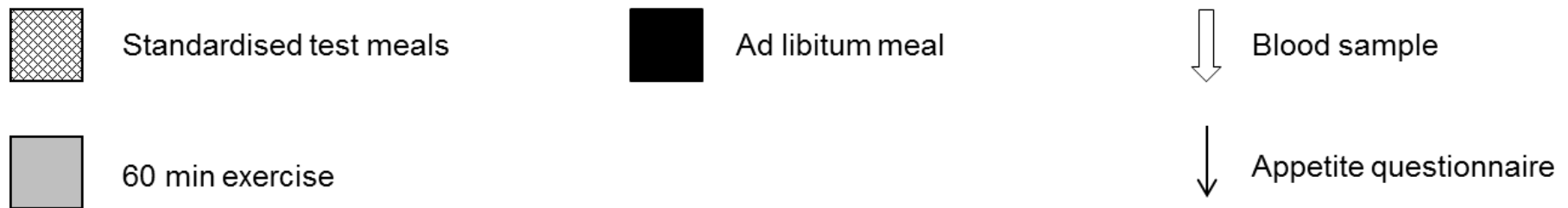
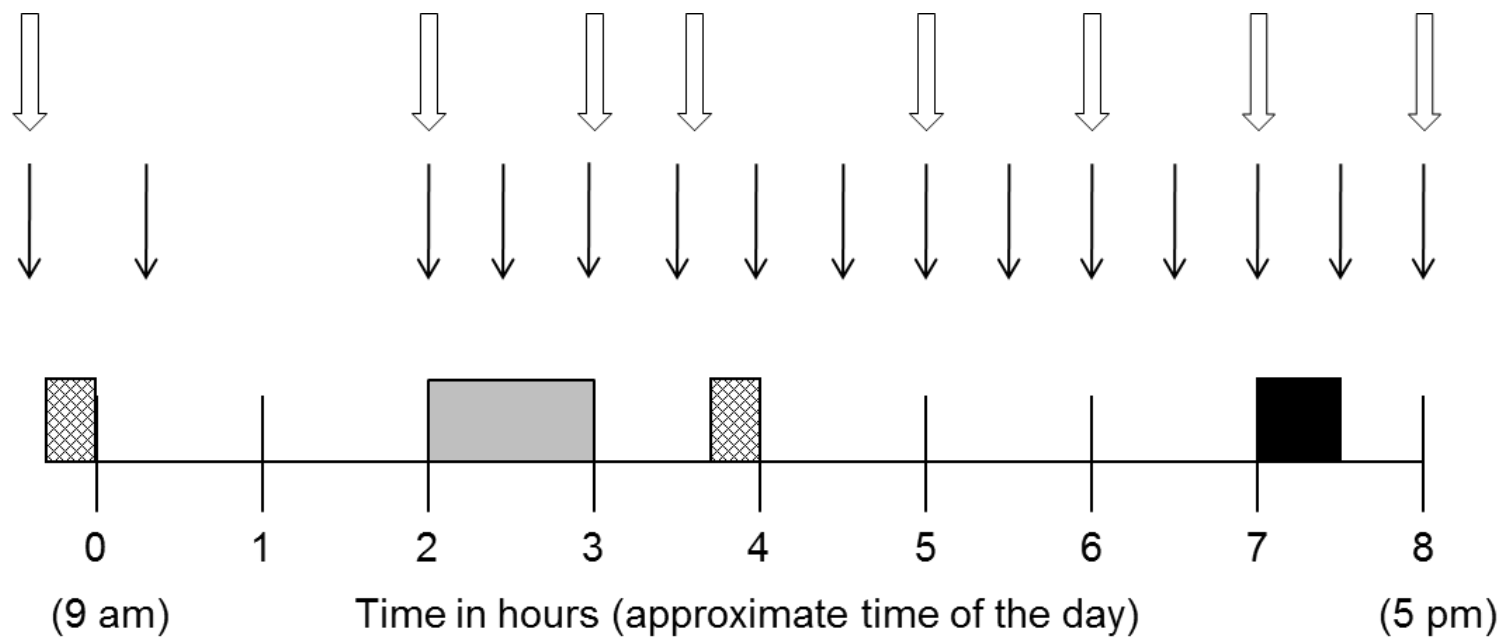


Figure 6.1. Schematic representation of the protocol for the experimental trials.

## 6.3 Results

### 6.3.1 Exercise responses

The exercise responses for SSE and HIIE are detailed in Table 6.1. Work rate, oxygen consumption, heart rate, RPE and RER were significantly higher during HIIE than SSE (all  $P < 0.0005$ ). However, due to the rest periods during HIIE, the net energy expenditure of exercise and external work performed did not differ between protocols (both  $P > 0.64$ ).

Table 6.1. Exercise responses during steady state exercise (SSE) and high intensity intermittent exercise (HIIE).

		SSE	HIIE	P
Exercise intensity	% of VO <sub>2</sub> max	59.5 (1.6)	85.8 (4.0)	<0.0005*
Heart rate	beats.min <sup>-1</sup>	143 (8)	171 (10)	<0.0005*
RPE	6 - 20	13 (1)	17 (1)	<0.0005*
RER		0.93 (0.04)	1.00 (0.03)	<0.0005*
Power output	W	146 (16)	222 (24)	<0.0005*
External work	kJ	527 (58)	525 (65)	0.877
Net energy expenditure	kJ	2451 (208)	2429 (266)	0.649

Values are mean (SD), N = 12. \*Different between SSE and HIIE ( $P < 0.05$ ). RPE = rating of perceived exertion, RER = respiratory exchange ratio.

### 6.3.2 Appetite

Overall appetite scores did not differ significantly between trials at baseline ( $P = 0.345$ ). Two-factor ANOVA revealed a main effect of time and a trial x time interaction for appetite perceptions (both  $P < 0.0005$ ) but no main effect of trial (Figure 6.2). Post-hoc analysis of trial x time interactions revealed suppressed appetite during and upon completion of exercise in HIIE compared with CON at 2.5 and 3 h ( $P < 0.05$ ). One-way

ANOVA revealed a trend towards a main effect of trial for appetite AUC from 0 – 3.5 h, indicating lower appetite in HIIE than CON ( $P = 0.06$ ). There were no between trial differences in appetite AUC for 3.5 – 8 h and 0 – 8 h (Table 6.2).

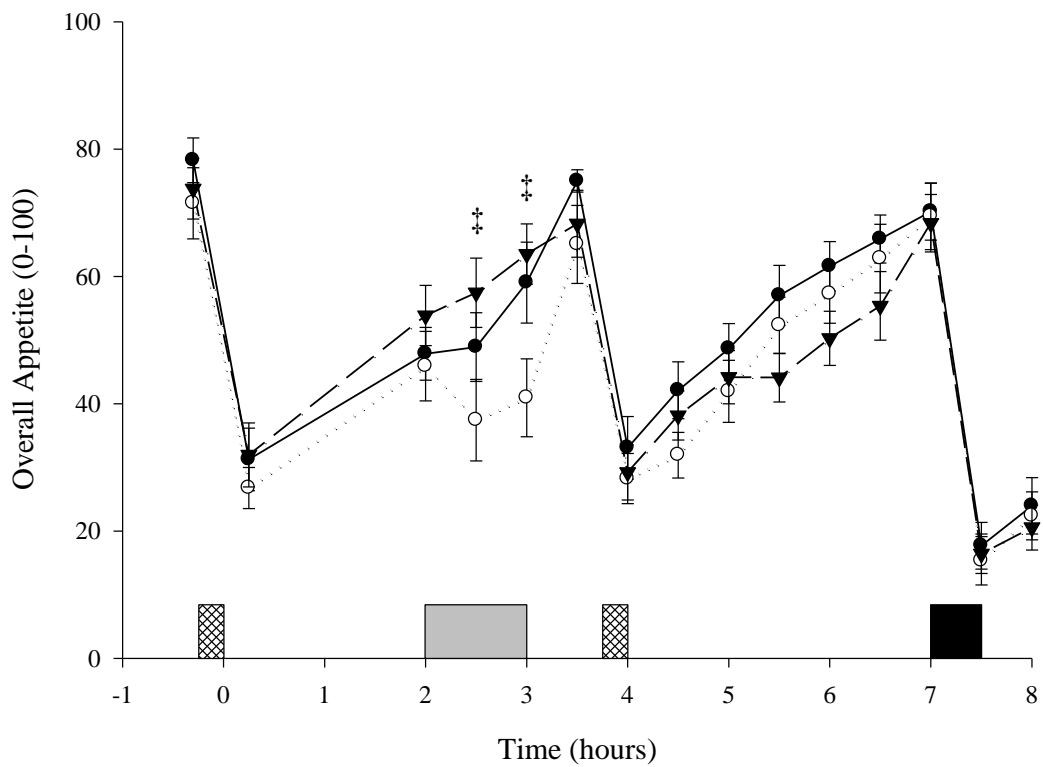


Figure 6.2. Overall appetite perceptions in CON (▼), SSE (●) and HIIE (○). Values are mean (SEM),  $N = 12$ . Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal. ‡HIIE different from CON ( $P < 0.05$ ).

Table 6.2. Time-averaged area under the curve values for overall appetite perceptions in the control (CON), steady state exercise (SSE) and high intensity intermittent exercise (HIIE) trials.

	<b>Morning (0–3.5 h)</b>	<b>Afternoon (3.5–8 h)</b>	<b>Total Trial (0–8 h)</b>
<b>Overall Appetite (0 – 100)</b>			
CON	51 (15)	43 (10)	47 (11)
SSE	48 (13)	50 (10)	49 (10)
HIIE	41 (14)	45 (11)	43 (11)
P	0.060	0.228	0.256

Values are mean (SD), N = 12.

### 6.3.3 Energy Intake

Absolute energy intake at the ad libitum meal was not significantly different between trials ( $P = 0.833$ ; CON 4759 (1268); SSE 4813 (1316); HIIE 4952 (1351) kJ). Effect sizes for total ad libitum energy intake were as follows: CON vs. SSE:  $d = 0.04$ ; CON vs. HIIE:  $d = 0.14$ ; SSE vs. HIIE:  $d = 0.10$ ). One-way ANOVA revealed a significant main effect of trial for relative energy intake (energy intake minus the net energy expenditure of exercise), demonstrating lower REI in SSE than CON (50.3%;  $P < 0.0005$ ) and HIIE than CON (44.7%;  $P < 0.0005$ ) but no difference between SSE and HIIE ( $P = 0.625$ ; CON 4759 (1268); SSE 2362 (1224); HIIE 2523 (1402) kJ). Closer inspection of the data revealed that all 12 participants had the highest REI during CON. Seven participants had a higher REI in HIIE than SSE, while 5 participants had a higher REI in SSE than HIIE.

### 6.3.4 Plasma PYY<sub>3-36</sub> concentrations

Due to problems with venous cannulation, blood sampling was not possible for one participant. Therefore, data for PYY<sub>3-36</sub> are presented from 11 participants. Fasting PYY<sub>3-36</sub> concentrations did not differ significantly between trials ( $P = 0.356$ ; CON 76.5 (13.4); SSE 72.5 (13.7); HIIE 71.9 (9.5) pg.mL<sup>-1</sup>). Two-factor ANOVA revealed a main

effect of time ( $P < 0.0005$ ) but no trial or interaction effects for absolute PYY<sub>3-36</sub> concentrations (Figure 6.3a). Alternatively, analysis of delta PYY<sub>3-36</sub> concentrations revealed a significant trial ( $P = 0.019$ ), time ( $P < 0.0005$ ) and trial x time interaction effect ( $P = 0.025$ ) (Figure 6.3b). Post-hoc analysis of between trial differences revealed higher plasma PYY<sub>3-36</sub> concentrations in HIIE than CON ( $P = 0.015$ ). Post-hoc analysis of trial x time interactions demonstrated elevated PYY<sub>3-36</sub> concentrations upon completion of exercise in SSE compared with CON ( $P = 0.002$ ).

One-way ANOVA did not demonstrate any between trial differences in AUC values for absolute PYY<sub>3-36</sub> concentrations for 0 – 3.75 h, 3.75 – 8 h and for the total 8 h trial (Table 6.3). However, analysis of AUC values for delta PYY<sub>3-36</sub> concentrations revealed a significant main effect of trial for all three time periods (Table 6.3). Peptide YY<sub>3-36</sub> responses to exercise were highly variable between participants with delta AUC values for the 8 h trial being highest in HIIE for 6 participants and SSE for 5 participants. Values were lowest in the control trial for 8 of 11 participants.



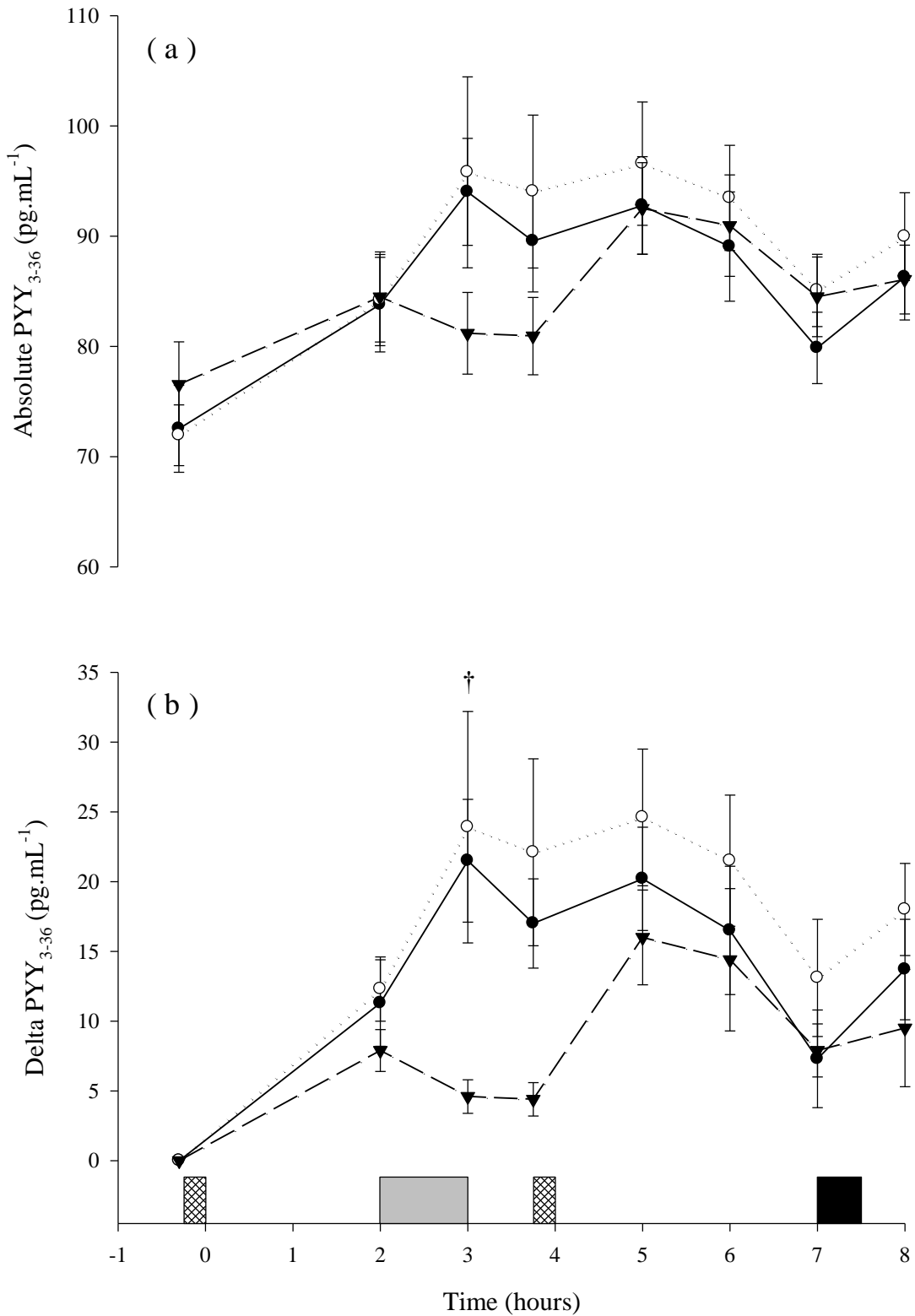


Figure 6.3. Absolute (a) and delta (b) PYY<sub>3-36</sub> concentrations in CON (▼), SSE (●) and HIIE (○). Values are mean (SEM), N = 11. Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal. †SSE different from CON (P < 0.05).

Table 6.3. Time-averaged area under the curve values for absolute and delta PYY<sub>3-36</sub> concentrations in the control (CON), steady state exercise (SSE) and high intensity intermittent exercise (HIIE) trials.

	<b>Morning (0 – 3.75 h)</b>	<b>Afternoon (3.75 – 8 h)</b>	<b>Total Trial (0 – 8 h)</b>
<b>Absolute PYY<sub>3-36</sub> (pg.mL<sup>-1</sup>)</b>			
CON	81.2 (13.2)	87.8 (11.4)	84.7 (11.5)
SSE	83.7 (13.7)	87.6 (13.2)	85.8 (12.8)
HIIE	84.6 (14.8)	92.0 (14.2)	88.5 (14.0)
P	0.731	0.474	0.619
<b>Delta PYY<sub>3-36</sub> (pg.mL<sup>-1</sup>)</b>			
CON	4.7 (2.9) <sup>†</sup>	11.2 (9.6) <sup>‡</sup>	8.2 (5.9) <sup>‡</sup>
SSE	11.2 (8.4)	15.1 (11.4)	13.3 (9.3)
HIIE	12.7 (10.9)	20.1 (13.5)	16.6 (11.9)
P	0.027	0.050	0.026

Values are mean (SD), N = 11. <sup>†</sup>SSE different from CON, <sup>‡</sup>HIIE different from CON (P ≤ 0.05).

## 6.4 Discussion

The primary finding of this investigation is that an acute bout of SSE and high volume HIIE did not stimulate any compensatory increases in appetite or energy intake, which resulted in a substantial negative daily energy balance in both exercise trials relative to control. Plasma PYY<sub>3-36</sub> concentrations were increased in the hours after exercise but to a greater extent after HIIE.

It is well established that appetite is acutely suppressed during exercise  $\geq 60$  % of VO<sub>2</sub> max (Blundell et al. 2003). However, the comparative appetite responses to exercise intensities above this threshold remain unclear (Chapter 5; Imbeault et al. 1997; Thompson et al. 1988; Ueda et al. 2009a). In the present study, appetite was suppressed during both exercise bouts but to a greater extent during HIIE. This supports previous findings from supramaximal interval exercise (see Chapter 5) and suggests that modern day HIIE protocols above  $\sim 85$  % of VO<sub>2</sub> max elicit greater appetite suppression during exercise than moderate intensity exercise at 60 – 65 % of VO<sub>2</sub> max. The lack of clarity among previous findings is likely to be a result of the lower exercise intensities employed (Imbeault et al. 1997; Thompson et al. 1988; Ueda et al. 2009a).

The HIIE protocol employed in this study did not stimulate any compensatory increases in appetite during the hours after exercise, which suggests that the observed increases in appetite after supramaximal interval exercise in Chapter 5 are likely to be a result of the extreme intensity rather than the intermittent nature of exercise. It remains plausible that a threshold exercise intensity may exist for the stimulation of appetite during the post-exercise period but this requires further investigation.

In accordance with previous research, circulating concentrations of PYY<sub>3-36</sub> increased upon completion of exercise (Broom et al. 2009; King et al. 2011a; Ueda et al. 2009a). However, this response was highly variable between participants and a statistically significant effect was only found upon completion of SSE, despite appetite-suppression being greatest upon completion of HIIE. Although surprising, this supports the total PYY response to exercise in Chapter 5 and suggests that appetite suppression during exercise is not solely dependent on increases in PYY<sub>3-36</sub>. Furthermore, plasma PYY<sub>3-36</sub> concentrations were highest in the hours after HIIE but appetite did not differ between trials. These findings highlight the complex nature of appetite regulation, which involves the integration of a wide range of neuroendocrine and psychological factors

(Evero et al. 2012; Morton et al. 2006) and is therefore unlikely to be explained by the measurement of a single appetite-regulating hormone. Nevertheless, increases in circulating PYY<sub>3-36</sub> in response to exercise represent a physiological adjustment to promote satiety, which contrasts the decreases observed with food restriction (King et al. 2011a).

The mechanisms underlying such increases in PYY<sub>3-36</sub> in response to exercise are unknown. However, it seems reasonable to postulate that such changes may be related to an increase in sympathetic nervous system activity, which occurs during exercise (Hagberg et al. 1979) and has been shown to stimulate PYY secretion (Brechet et al. 2001; Zhang et al. 1993). Sympathetic activity increases with exercise intensity (Perini et al. 1989), which may explain the more-prolonged increase in PYY<sub>3-36</sub> after HIIE. However, this theory does not explain the significant increase in PYY<sub>3-36</sub> concentrations immediately upon completion of SSE but not HIIE. This confliction may be a result of intestinal blood flow, which decreases with exercise intensity (Clausen 1977; Gil et al. 1998) and may have therefore reduced the transport of PYY<sub>3-36</sub> into the peripheral circulation immediately after exercise in HIIE (Mailman 1982). These are the first postulations to be made regarding the mechanisms of exercise-induced increases in PYY and although this would explain the findings of the present study, it must be noted that this is speculation and requires future investigation.

Energy intake at the ad libitum meal did not differ between trials. In combination with the appetite data, this would suggest that the exercise protocols utilised in this study did not stimulate a physiological or conscious drive to eat. Furthermore, despite differences in PYY<sub>3-36</sub> immediately prior to the standardised lunch meal, the lack of difference in appetite perceptions between trials at this point suggests that differences in energy intake would also have been unlikely if an ad libitum lunch meal was provided. This suggestion is further supported by the findings from Chapter 5 as ad libitum energy intake and appetite perceptions did not differ between trials 45 min after exercise despite differences in gut hormone concentrations. The use of a standardised meal was therefore deemed to be preferable in the present study in order to better understand changes in PYY<sub>3-36</sub> during the hours after exercise without the confounding influence of prior differences in food intake.

The absence of any compensatory increases in energy intake resulted in a substantial negative daily energy balance in both exercise trials compared with control. However, for the same energy expenditure of exercise, HIIE was significantly more strenuous than SSE, as reflected by higher heart rates and perceived exertion. Therefore, although a single bout of HIIE has been demonstrated to be more enjoyable than SSE (Bartlett et al. 2011), exercise practitioners must also be aware that HIIE elicits greater physical stress than energy-matched SSE. Research suggests that SSE and high volume HIIE also induce similar improvements in  $VO_2$  max, maximal mitochondrial enzyme activity and fat oxidation during submaximal exercise (Perry et al. 2008; Talanian et al. 2007; 2010). Therefore a beneficial and sustainable approach to exercise training may be to include a combination of SSE and HIIE based on the preferences of individual participants.

Some authors have postulated that HIIE may be more beneficial for fat loss than SSE due to greater post-exercise decreases in appetite and increases in resting energy expenditure (Boutcher 2011; Heydari et al. 2012; Trapp et al. 2008; Tremblay et al. 1994). However, despite greater appetite suppression during HIIE, the findings of the present and previous chapter of this thesis suggest that post-exercise appetite perceptions are not reduced compared with SSE. The HIIE protocols investigated thus far represent both extremes of the HIIE spectrum, i.e. high volume, submaximal interval exercise in the present study and very low volume, supramaximal interval exercise in Chapter 5. Such findings convincingly demonstrate that HIIE does not elicit lower appetite perceptions in the hours after exercise compared with SSE. Additionally, although not measured in the present study, previous research indicates that energy-matched SSE and HIIE induce comparable changes in resting metabolism during the recovery from exercise (McGarvey et al. 2005; Warren et al. 2009). These findings suggest that any additional fat loss benefits of HIIE are mediated by other unknown mechanisms.

This study also confirms the finding from Chapters 4 and 5 that 60 min of prolonged endurance exercise does not stimulate any compensatory increases in appetite or energy intake during the hours after exercise. However, such exercise is physically demanding and would be difficult to maintain as part of a weight loss program. Furthermore, evidence that exercise elicits more beneficial appetite responses than food restriction has only been demonstrated when such large energy deficits and abrupt methods of

food restriction have been employed (Hubert et al. 1998; King et al. 2011a). These issues are investigated in Chapter 7 as the appetite, energy intake and gut hormones responses to a government recommended dose of physical activity are compared with an equivalent energy deficit induced via food restriction.

## CHAPTER VII

### **Appetite, gut hormone and energy intake responses to moderate energy deficits induced via exercise and food restriction.**

#### **7.1 Introduction**

Obesity is characterised by an excess accumulation of body fat and is associated with an increased prevalence of chronic diseases including type 2 diabetes, osteoarthritis, cardiovascular disease and some forms of cancer (Bray 2004). Consequently, overweight and obesity has recently been classified as one of the top five global risk factors for mortality and one of the top ten risk factors for morbidity (World Health Organisation 2009). However, weight loss as little as 3 % has been associated with favourable changes in chronic disease risk factors and therefore represents a major public health priority (Donnelly et al. 2009).

For weight loss to occur, a sustained negative energy balance is required and is typically achieved by decreasing energy intake (i.e. dieting) or increasing energy expenditure (i.e. exercising). Although both interventions may induce a negative energy balance, current research suggests that exercise and food restriction elicit contrasting homeostatic responses. In this regard, acute food restriction appears to stimulate rapid compensatory increases in appetite and energy intake that do not occur in response to equivalent energy deficits induced by exercise (Hubert et al. 1998; King et al. 2011a). Furthermore, King et al. (2011a) reported immediate decreases in circulating concentrations of the anorexigenic gut hormone PYY<sub>3-36</sub> and increases in the orexigenic gut hormone acylated ghrelin in response to food restriction but no compensatory changes in response to exercise. Such findings suggest that these appetite-regulating gut hormones have a mediating role in the immediate appetite and energy intake responses to acute energy deficits but this requires further investigation.

Although these studies have provided interesting information regarding energy homeostasis and the regulation of appetite, large and abrupt methods of energy restriction have been employed as food intake was reduced by ~1820 kJ at a single meal (Hubert et al. 1998) and ~4820 kJ across two meals (King et al. 2011a). Such substantial decreases in energy intake at individual meals increases the likelihood that

compensatory increases in appetite will occur and does not represent a practical strategy for energy restriction.

The current UK government and ACSM guidelines recommend a minimum of 150 min.wk<sup>-1</sup> of moderate intensity physical activity, spread over most days of the week (British Heart Foundation 2010; Donnelly et al. 2009). This may be interpreted as five 30 min exercise bouts performed on separate days of the week and is considered to be sufficient to reduce chronic disease risk, prevent significant weight gain, and elicit modest weight loss in overweight and obese populations (Donnelly et al. 2009). The appetite and energy intake response to such a practical energy deficit achieved via exercise and food restriction is unknown. This requires further investigation as compensatory increases in appetite contribute to the difficulty of maintaining an energy deficit in current society where energy dense, highly palatable foods are abundant and easily accessible. Furthermore, increases in appetite are commonly cited as a reason for unsuccessful dieting (Ikeda et al. 2004) and are inversely related to exercise-induced weight loss (King et al. 2008).

The purpose of this study was to investigate the appetite, acylated ghrelin, PYY<sub>3-36</sub> and energy intake responses to a 30 min bout of moderate intensity cycling compared with an equivalent energy deficit achieved via food restriction. The findings of this study may contribute to further understanding the most effective method of inducing an energy deficit while minimising compensatory increases in appetite. This study also enables further investigation into the sensitivity of the appetite-regulating system and the role of acylated ghrelin and PYY<sub>3-36</sub> in energy homeostasis via the utilisation of small, yet practical, energy deficits. It was hypothesised that appetite and acylated ghrelin would increase, and that PYY<sub>3-36</sub> would decrease in response to food restriction but that these variables would remain unaffected by exercise, resulting in a higher energy intake in the food restriction trial.



## **7.2 Methods**

### **7.2.1 Participants**

Twelve healthy men matching the inclusion criteria listed in Chapter 3.1, volunteered to participate in this study. The physical characteristics of the participants (mean (SD)) were as follows: age 24 (5) years, BMI 23.8 (2.7) kg.m<sup>-2</sup>, body mass 75.3 (10.3) kg, body fat 14.2 (4.0) %, waist circumference 80.3 (6.6) cm, VO<sub>2</sub> max 55.4 (9.1) mL.kg<sup>-1</sup>.min<sup>-1</sup>.

### **7.2.2 Preliminary trials**

Prior to main trials participants visited the laboratory for two preliminary trials. During the first visit, participants underwent screening and preliminary anthropometric measurements as detailed in Chapters 3.1 and 3.2. After familiarisation with the testing equipment, participants also completed a continuous incremental exercise test to exhaustion on an electromagnetically braked cycle ergometer to determine submaximal oxygen consumption and VO<sub>2</sub> max. Details of this test are described in Chapter 3.5.2.

Participants visited the laboratory on a second occasion for a familiarisation trial. Participants performed 30 min of continuous cycling exercise on an electromagnetically braked cycle ergometer (Lode Excalibur Sport V2, Groningen, Netherlands) at a work rate predicted to elicit 65 % of VO<sub>2</sub> max. Samples of expired gas were collected for 60 s at 6, 18 and 30 min during exercise to monitor the intensity of the cycle, with adjustments made to the work rate if necessary. Heart rate and RPE were also measured at these times. Energy expenditure of exercise was calculated using the equation of Frayn (1983) (see Chapter 3.8), for the determination of energy provision during the main trials.

### **7.2.3 Experimental protocol**

Participants performed three, 8 h experimental trials (control, exercise-induced energy deficit and diet-induced energy deficit) separated by one week in a counterbalanced Latin Square design. In the 24 h preceding each main trial, participants standardised their food intake and physical activity, as described in Chapter 3.9. Participants arrived at the laboratory at 8.00 am after an overnight fast of at least 10 h and exerted themselves minimally when travelling to the laboratory.

During each trial, appetite perceptions (hunger, satisfaction, fullness and PFC) were assessed at baseline, 0.25, 0.5 h and every 30 min thereafter using 100 mm visual analogue scales (see Chapter 3.10). An overall appetite score was calculated as the mean value of the four appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al. 2000). Water was available ad libitum throughout each trial. Figure 7.1 provides an overview of the protocol for the experimental trials.

#### **7.2.4 Energy deficits**

Participants rested within the laboratory throughout all trials (sitting reading, working at a desk or watching television), except from 0 – 0.5 h during the exercise-induced energy deficit (Ex-Def) trial where participants replicated the exercise bout performed during the familiarisation trial. To calculate the net energy expenditure of exercise (gross energy expenditure of exercise minus resting energy expenditure), expired gas was collected into Douglas bags for 5 min every 10 min between 0 and 0.5 h during the control (Con) and diet-induced energy deficit (Food-Def) trials in order to estimate resting energy expenditure (Frayn 1983).

Participants were provided with a standardised breakfast at 1 h and a standardised lunch at 4 h (see Chapter 3.11). The energy content of the test meals was identical in Con and Ex-Def. The breakfast meal provided 30 % and the lunch meal provided 35 % of the estimated daily energy needs of each individual for a sedentary day, which was calculated using the Mifflin-St Jeor equation and a physical activity factor of 1.4 (Mifflin et al. 1990), as described in Chapter 3.11. In Food-Def, the energy content of the test meals was reduced by deducting the net energy expenditure of exercise from the energy provided at the test meals during Con and Ex-Def. The total amount deducted was divided proportionally between the breakfast and lunch meals. Therefore, equivalent energy deficits were induced in Ex-Def and Food-Def relative to Con.

#### **7.2.5 Ad libitum meal**

At 7 h an ad libitum meal of fixed macronutrient composition was provided, as described in Chapter 3.13.

### **7.2.6 Blood sampling**

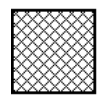
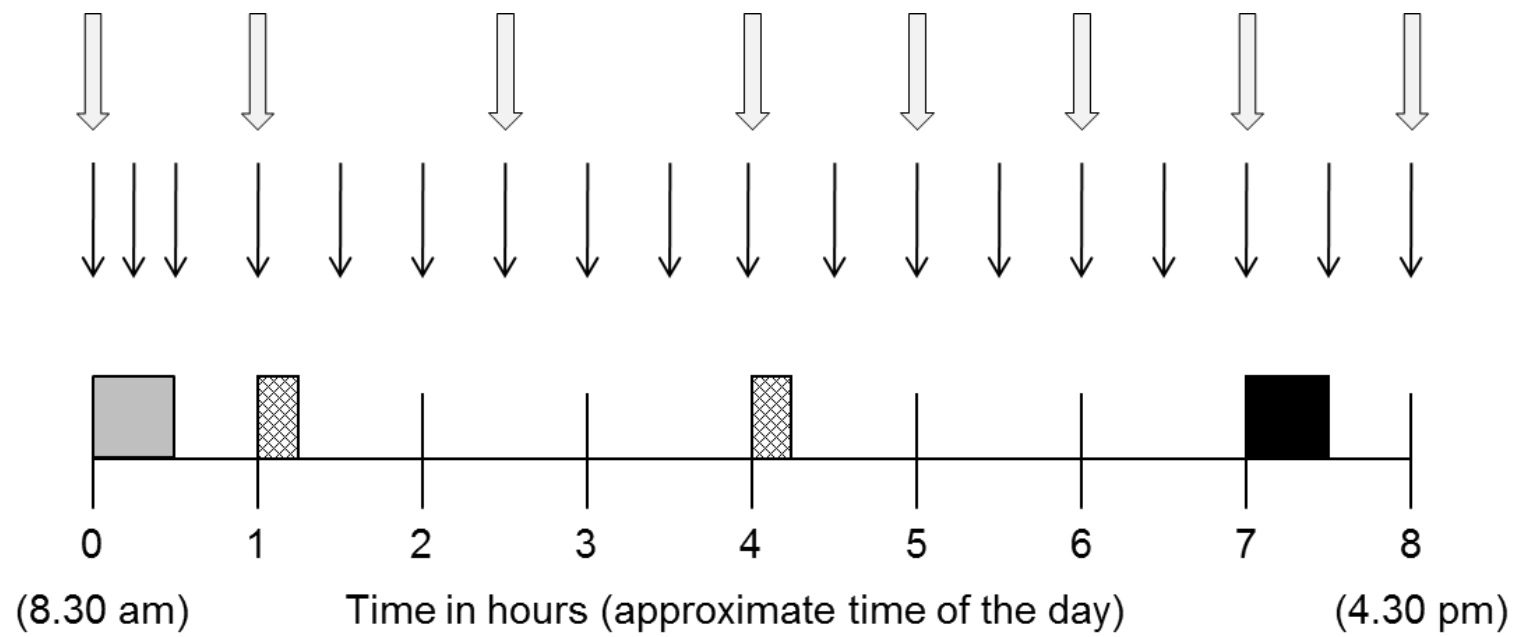
Upon arrival to the laboratory, participants rested in a semi-supine position and a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. Blood samples were collected at baseline, 1, 2.5, 4, 5, 6, 7 and 8 h for the determination of plasma acylated ghrelin and PYY<sub>3-36</sub> concentrations, as described in Chapter 3.16.

### **7.2.7 Biochemical analysis**

A commercially available enzyme immunoassay was used to determine plasma concentrations of acylated ghrelin (see Chapter 3.17.2). Plasma concentrations of PYY<sub>3-36</sub> were determined using a commercially available radioimmunoassay, as described in Chapter 3.17.4. The within batch coefficient of variation for the assays was 6.8 and 7.2 % for acylated ghrelin and PYY<sub>3-36</sub>, respectively.

### **7.2.8 Statistical analysis**

Data was analysed using the methods described in Chapter 3.18.



Standardised test meals



Ad libitum meal



Blood sample



30 min cycling at 65% of  $VO_2$  max



Appetite questionnaire

Figure 7.1. Schematic representation of the protocol for the experimental trials.

## 7.3 Results

### 7.3.1 Exercise responses

Participants completed the 30 min cycle at 186 (38) W. This elicited an oxygen consumption equivalent to 64.5 (3.2) % of  $\text{VO}_2$  max and a net energy expenditure of 1469 (256) kJ. The non-protein RER was 0.93 (0.04), which reflected a proportional contribution to energy provision of 78 (13) % carbohydrate and 22 (13) % fat. Heart rate and RPE were 156 (16)  $\text{beats}\cdot\text{min}^{-1}$  and 13 (1), respectively.

### 7.3.2 Appetite

Overall appetite ratings did not differ between trials at baseline (Con 74 (14); Food-Def 74 (14); Ex-Def 77 (10);  $P = 0.735$ ). Two-factor ANOVA revealed a significant main effect of time ( $P < 0.0005$ ) and a trend towards a main effect of trial ( $P = 0.085$ ) and trial x time interaction (0.079) for appetite perceptions (Figure 7.2). Table 7.1 shows the AUC values for overall appetite perceptions. One-way ANOVA revealed a main effect of trial for appetite AUC from 4 – 8 h ( $P = 0.021$ ). Subsequent post-hoc analysis demonstrated significantly higher appetite in Food-Def than Ex-Def ( $P = 0.033$ ). Appetite AUC did not differ between trials for 0 – 1 h and 1 – 4 h but tended to be higher in Food-Def than Ex-Def across the entire 8 h trial ( $P = 0.059$ ).

### 7.3.3 Energy intake

The combined energy intake of the breakfast and lunch test meals was 6661 (479) kJ in Con and Ex-Def and 5183 (378) kJ in Food-Def. Consequently, the energy deficit induced by food restriction was 1478 (275) kJ. This was comparable with the energy deficit induced through exercise (1469 (256) kJ; Paired samples t-test,  $P = 0.60$ ).

One-way ANOVA revealed no between trial differences in energy intake at the ad libitum meal ( $P = 0.634$ ; Con 4376 (1634); Food-Def 4481 (1846); Ex-Def 4217 (1850) kJ). Effect sizes for total ad libitum energy intake were as follows: Control vs. Ex-Def:  $d = 0.09$ ; Control vs. Food-Def:  $d = 0.06$ ; Ex-Def vs. Food-Def:  $d = 0.14$ ). Subsequently, energy balance was 1628 (915) kJ and 1373 (1047) kJ lower in Ex-Def and Food-Def compared with Con (both  $P \leq 0.001$ ).

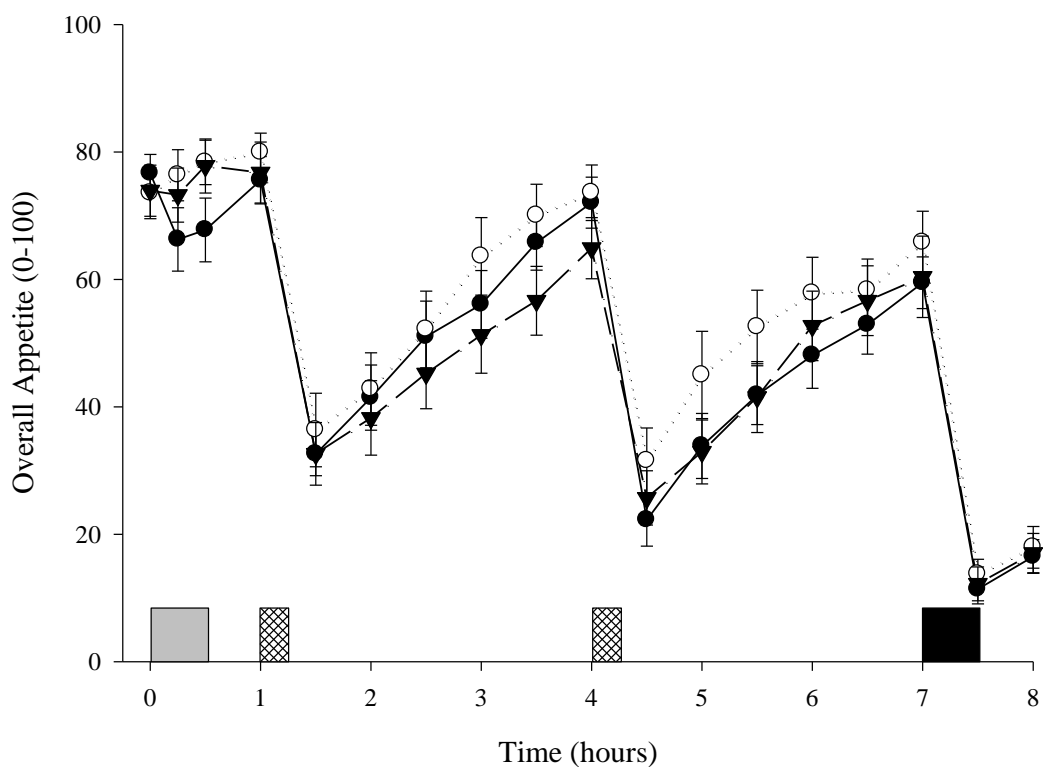


Figure 7.2. Overall appetite perceptions in Con (▼), Ex-Def (●) and Food-Def (○). Values are mean (SEM), N = 12. Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.

Table 7.1. Time-averaged area under the curve values for overall appetite perceptions in the Control, Ex-Def and Food-Def trials.

	<b>Preprandial (0-1 h)</b>	<b>Morning (1-4 h)</b>	<b>Afternoon (4-8 h)</b>	<b>Total trial (0-8 h)</b>
<b>Overall Appetite (0 - 100)</b>				
Control	76 (14)	49 (16)	40 (13)	48 (13)
Ex-Def	70 (14)	53 (13)	39 (11)	48 (11)
Food-Def	78 (12)	57 (15)	46 (14)	54 (13)
P	0.386	0.120	0.021*	0.059

Values are mean (SD), N = 12. \* Different between Ex-Def and Food-Def (P < 0.05).

### 7.3.4 Plasma acylated ghrelin concentrations

Fasting plasma acylated ghrelin concentrations did not differ significantly between trials at baseline (Con 189 (262); Ex-Def 242 (386); Food-Def 268 (427) pg.mL<sup>-1</sup>; P = 0.174). Two-factor ANOVA demonstrated that there was no trial (P = 0.198), time (P = 0.173) or trial x time interaction effect (P = 0.412) for absolute acylated ghrelin concentrations (Figure 7.3a). Analysis of delta values replicated these findings (main effect of trial: P = 0.178; main effect of time: P = 0.173; trial x time interaction: P = 0.412; Figure 7.3b).

Area under the curve values for absolute acylated ghrelin concentrations revealed no between trial differences for any time period (0-1 h: P = 0.342; 1-4 h: P = 0.254; 4-8 h: P = 0.312; 0-8 h: P = 0.349). Analysis of delta AUC values revealed a tendency for higher acylated ghrelin concentrations from 0-1 h in Con than Ex-Def (P = 0.081) but no between trial differences for any other time period (1-4 h: P = 0.116; 4-8 h: P = 0.217; 0-8 h: P = 0.160).

Subsequent boxplot analysis of absolute and delta acylated ghrelin AUC values revealed three consistently outlying participants within the data set. These participants exhibited fasted acylated ghrelin concentrations that were between 6 and 39 standard deviations higher than the mean fasting value of the remaining nine participants. In accordance with previous research, these three participants were removed from the data set for subsequent analysis (Broom et al. 2007; Hansen et al. 2002; King et al. 2011b). After the removal of these participants from the data, two-factor ANOVA revealed a significant trial (P = 0.011), time (P < 0.0005) and trial x time interaction (P < 0.0005) for absolute acylated ghrelin concentrations (Figure 7.4a). Post-hoc analysis of between trial differences demonstrated lower acylated ghrelin concentrations in Ex-Def than Con (P = 0.033) and Food-Def (P = 0.042). Trial x time interactions were not significant at any time point after Holm-Bonferroni adjustment. Analysis of delta values after the removal of outliers also revealed a significant main effect of time (P < 0.0005) and a trial x time interaction (P < 0.0005) but only a trend for a main effect of trial (P = 0.078) (Figure 7.4b). Removal of the outliers did not affect the interpretation of the appetite or PYY<sub>3-36</sub> findings.

The absolute and delta plasma acylated ghrelin concentrations for one outlying participant are displayed in Figure 7.5 in order to highlight the variation in acylated ghrelin profiles.

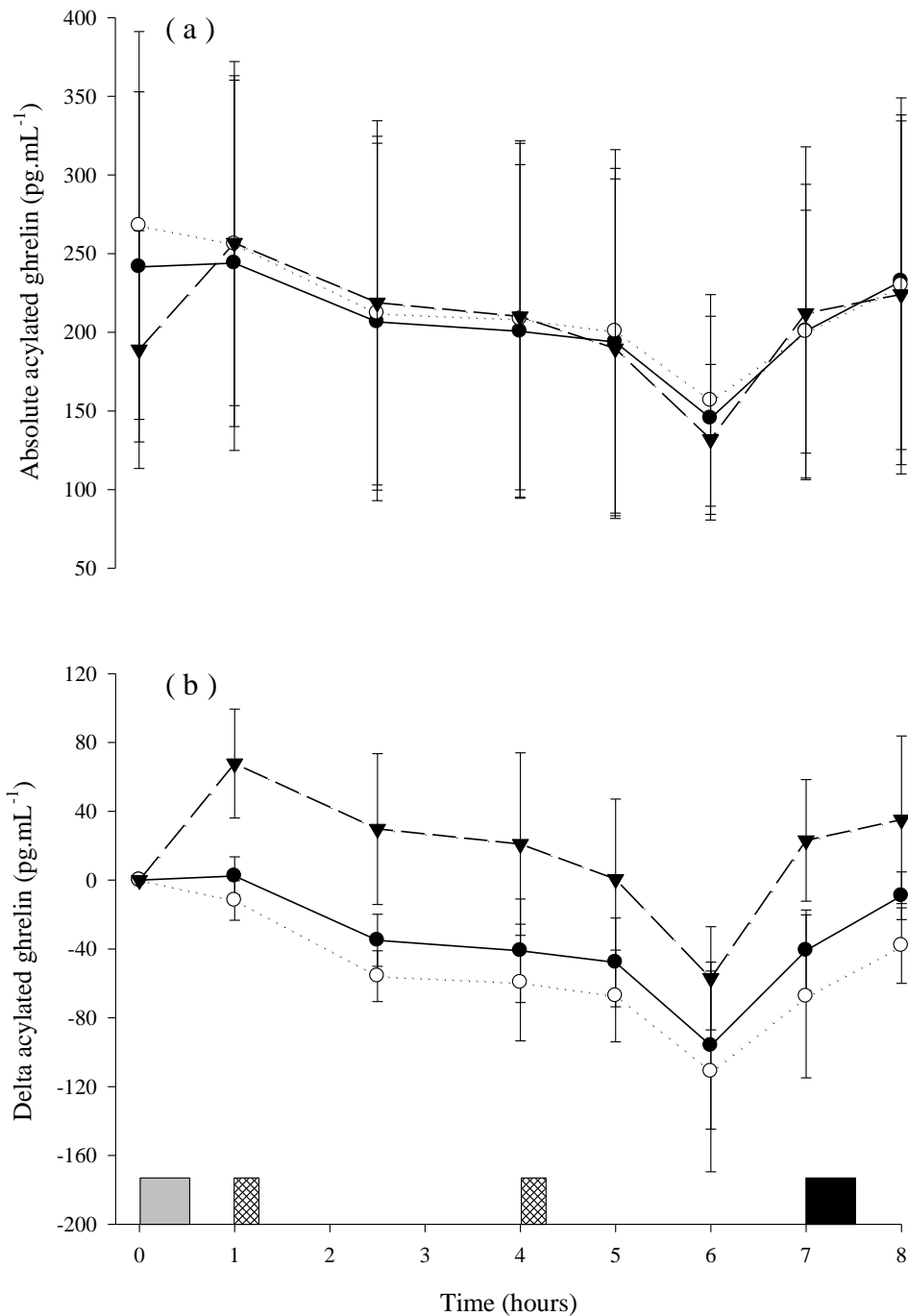


Figure 7.3. Absolute (a) and delta (b) acylated ghrelin concentrations including all participants in Con (▼), Ex-Def (●) and Food-Def (○). Values are mean (SEM), N = 12. Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.



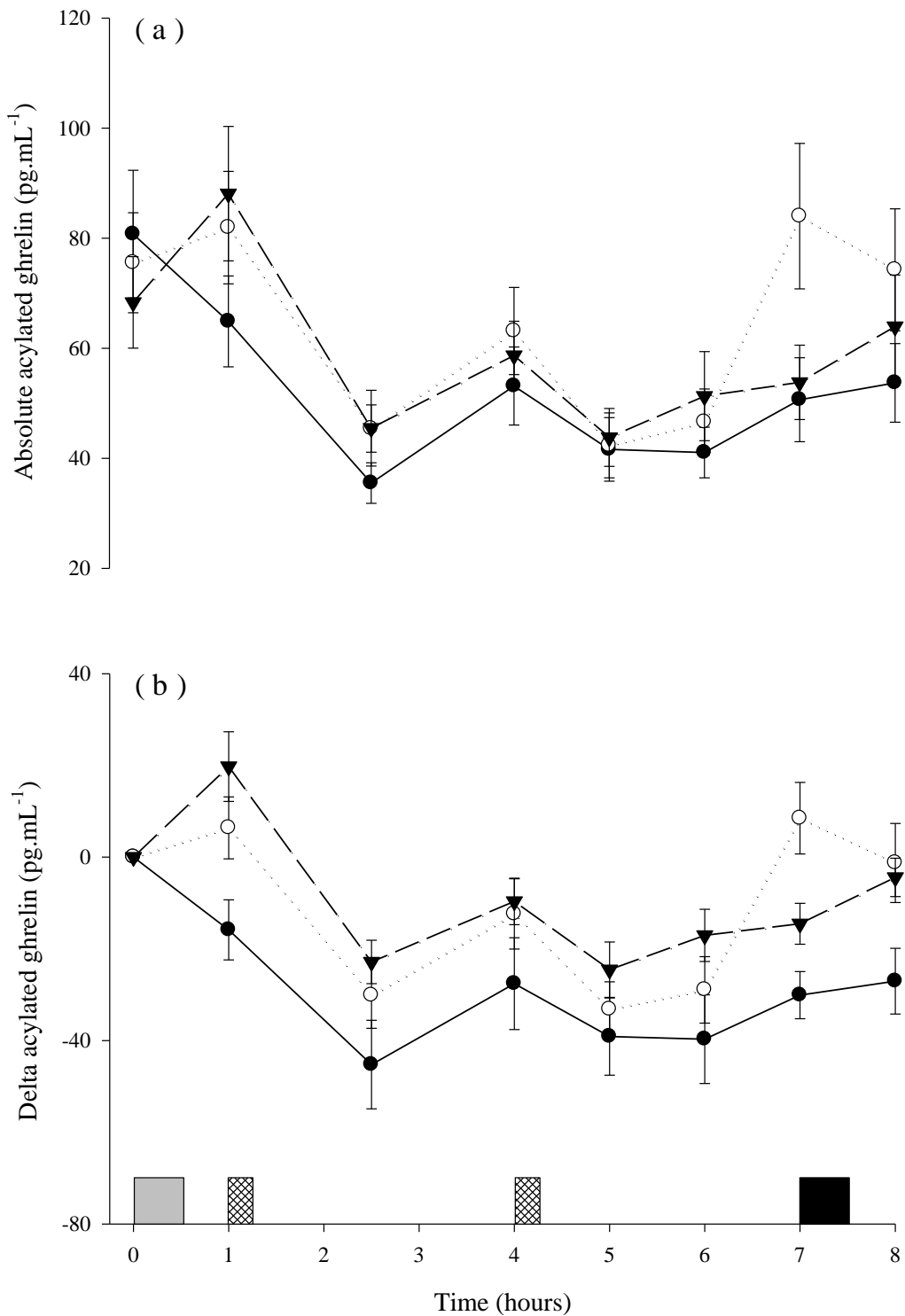


Figure 7.4. Absolute (a) and delta (b) acylated ghrelin concentrations after the removal of outliers in Con (▼), Ex-Def (●) and Food-Def (○). Values are mean (SEM), N = 9. Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.

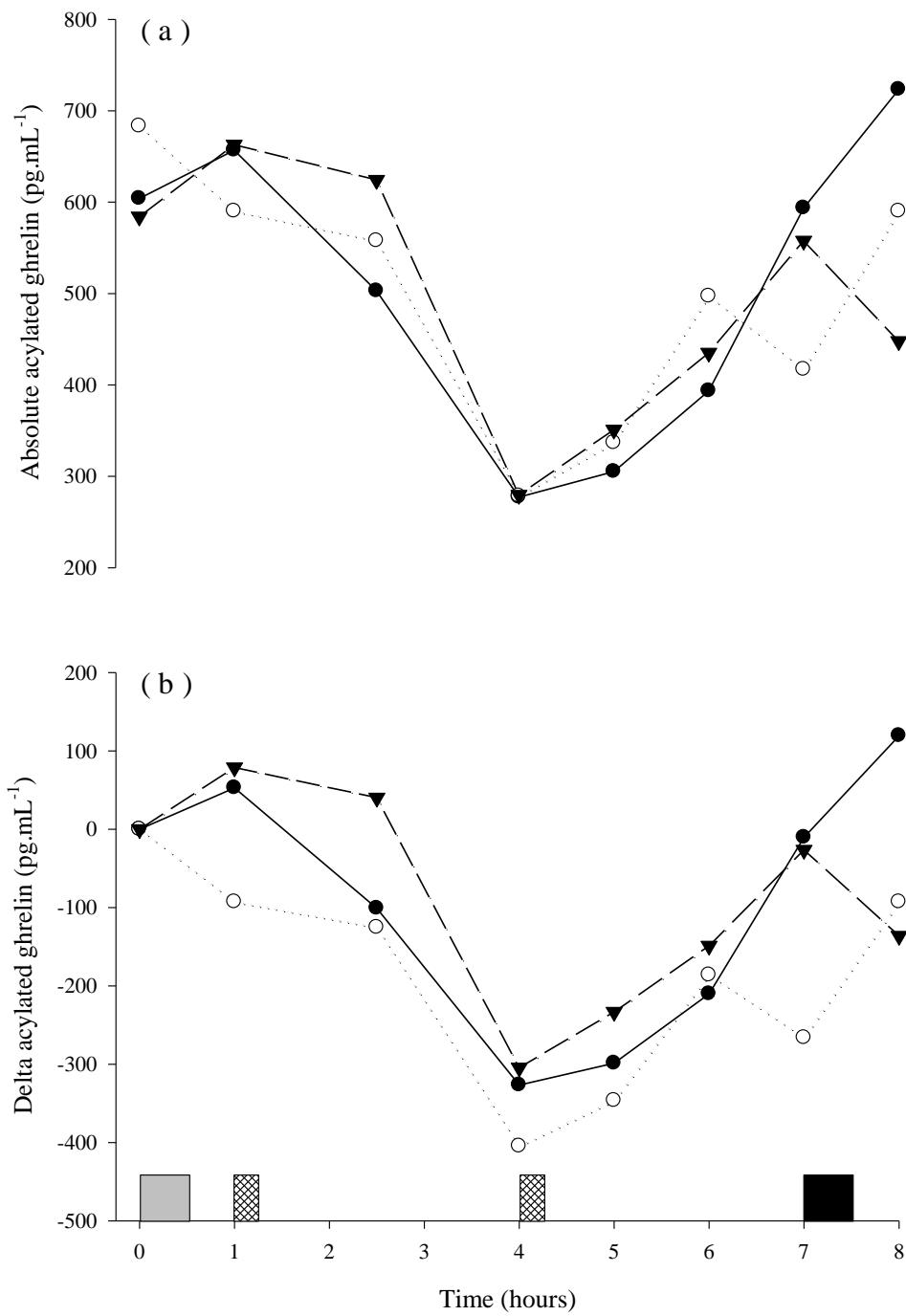


Figure 7.5. Absolute (a) and delta (b) acylated ghrelin concentrations for one of the outlying participants in Con (▼), Ex-Def (●) and Food-Def (○). Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.

### 7.3.5 Peptide YY<sub>3-36</sub> concentrations

Fasting PYY<sub>3-36</sub> concentrations did not differ significantly between trials at baseline (Con 93.5 (40.0); Ex-Def 87.1 (37.9); Food-Def 96.7 (46.0) pg.mL<sup>-1</sup>; P = 0.325). Two-factor ANOVA revealed a significant main effect of trial (P = 0.035) and time (P < 0.0005), and a trend towards a trial x time interaction (P = 0.054) for absolute PYY<sub>3-36</sub> concentrations (Figure 7.6a). Post-hoc analysis of between trial differences demonstrated higher PYY<sub>3-36</sub> concentrations in Ex-Def than Food-Def but this was not significant after Holm-Bonferroni adjustment.

Analysis of delta values confirmed these findings as a two factor ANOVA demonstrated a significant main effect of trial (P = 0.041) and time (P < 0.0005), and a trend towards a trial x time interaction (P = 0.054) (Figure 7.6b). Post-hoc analysis of between trial differences demonstrated higher PYY<sub>3-36</sub> concentrations in Ex-Def than Food-Def (P = 0.018).

Time-averaged area under the curve values for absolute and delta PYY<sub>3-36</sub> concentrations are presented in Table 7.2.

### 7.3.6 Correlations

Area under the curve values for delta PYY<sub>3-36</sub> concentrations were negatively correlated with changes in appetite for 0-1 h (r = -0.514; P = 0.001), 4-8 h (r = -0.340; P = 0.043) and for the entire 8 h trial (0-8 h; r = -0.349; P = 0.037). There was no significant correlation between acylated ghrelin and appetite AUCs for any time period.

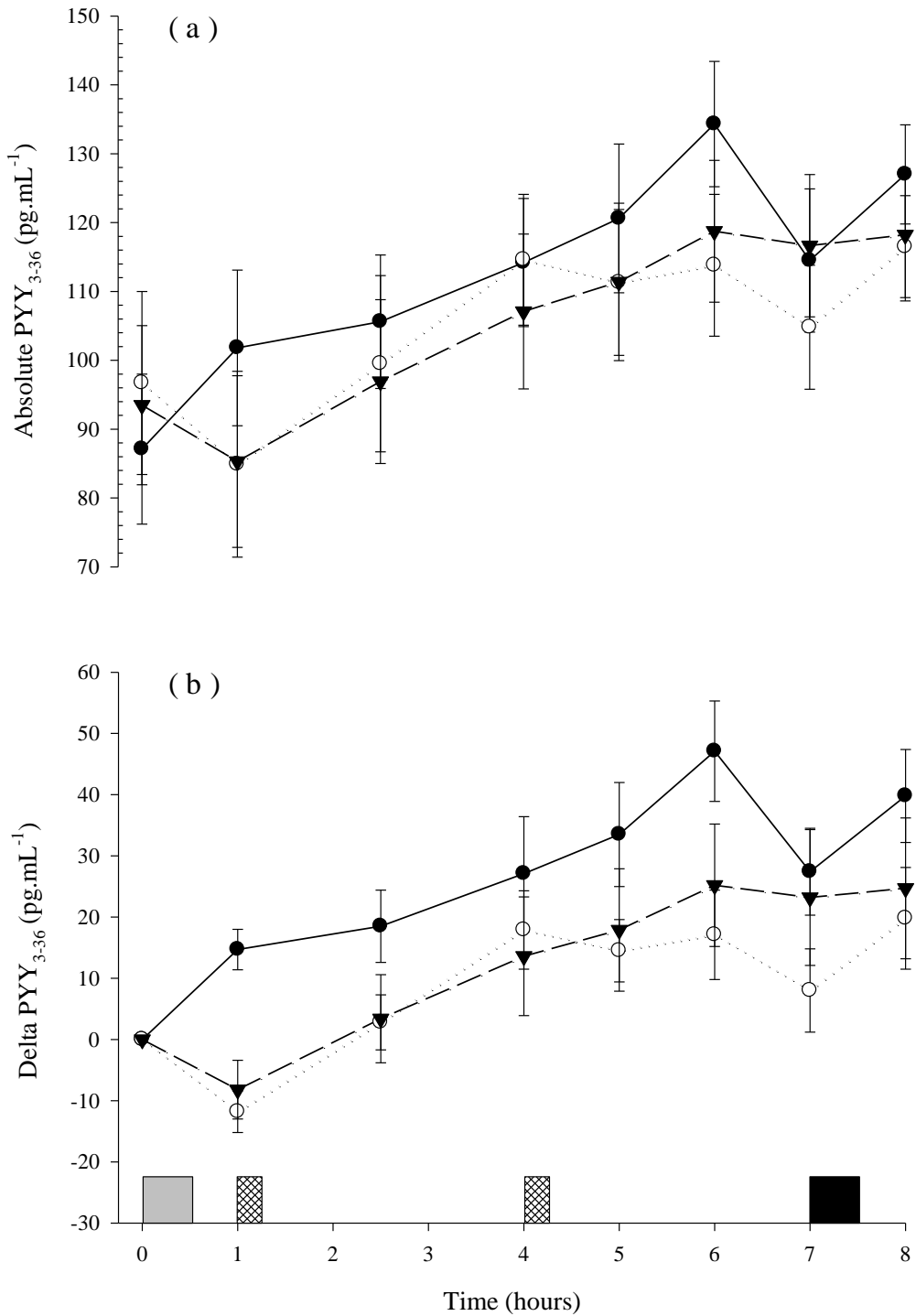


Figure 7.6. Absolute (a) and delta (b) PYY<sub>3-36</sub> concentrations in Con (▼), Ex-Def (●) and Food-Def (○). Values are mean (SEM), N = 12. Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.

Table 7.2. Time-averaged area under the curve values for absolute and delta PYY<sub>3-36</sub> concentrations in the Control, Ex-Def, and Food-Def trials.

	<b>Preprandial (0 – 1 h)</b>	<b>Intertest meal (1 – 4 h)</b>	<b>Posttest meals (4 – 8 h)</b>	<b>Total Trial (0 – 8 h)</b>
<b>Absolute</b>				
<b>PYY<sub>3-36</sub></b>				
<b>(pg.mL<sup>-1</sup>)</b>				
Control	89.4 (40.8)	96.6 (38.4)	114.9 (35.5)	104.8 (35.3)
Ex-Def	94.5 (38.1)	106.8 (31.5)	122.5 (30.5)	113.1 (30.4)
Food-Def	90.8 (45.9)	99.6 (40.5)	111.3 (32.0)	104.4 (35.8)
P	0.674	0.076	0.072	0.024
<b>Delta PYY<sub>3-36</sub></b>				
<b>(pg.mL<sup>-1</sup>)</b>				
Control	-4.1 (8.3)	3.1 (21.0)	21.4 (34.7)	11.3 (25.1)
Ex-Def	7.3 (5.7)	19.7 (16.9)	35.4 (24.2)	26.0 (17.7)
Food-Def	-5.9 (5.8)	2.9 (11.1)	14.6 (21.2)	7.7 (12.8)
P	< 0.0005 <sup>*†</sup>	0.039 <sup>*</sup>	0.086	0.036 <sup>*</sup>

Values are mean (SD), N = 12. <sup>\*</sup>Different between Ex-Def and Food-Def, <sup>†</sup>Different between Ex-Def and Control (One-way ANOVA: P < 0.05 after Holm-Bonferroni adjustment).

## 7.4 Discussion

The primary finding of this investigation is that an energy deficit of ~1475 kJ stimulated compensatory increases in appetite when induced via food restriction but not when achieved by an acute bout of exercise. Such divergent appetite responses appeared to be associated with changes in circulating concentrations of PYY<sub>3-36</sub> but were unrelated to changes in plasma acylated ghrelin and did not influence subsequent energy intake.

This study has extended the findings of previous research by demonstrating that appetite perceptions increase in response to subtle reductions in energy intake but do not change in response to an equivalent exercise-induced energy deficit (Hubert et al. 1998; King et al. 2011a). Increases in appetite occurred despite an average decrease in energy intake of only 682 kJ at breakfast and 796 kJ at lunch. This highlights the sensitivity of the appetite-regulating system to reductions in food intake and supports previous observations that dieting is often compromised by increases in appetite (Ikeda et al. 2004). Alternatively, appetite perceptions were unresponsive to an equivalent energy deficit induced through 30 min of moderate intensity exercise. This exercise bout represents the current UK government and ACSM guidelines for physical activity (British Heart Foundation 2010; Donnelly et al. 2009) and confirms previous findings from this thesis that continuous moderate intensity exercise does not stimulate compensatory increases in appetite during the subsequent 5 – 9 h monitoring period (Chapters 4 – 6).

In contrast with previous findings, the divergent appetite response to exercise and food restriction was not associated with concordant changes in plasma acylated ghrelin concentrations (King et al. 2011a). Furthermore, the acylated ghrelin profile of the participant displayed in Figure 7.5 exhibited an increase in response to the lunch meal in all trials despite reporting a simultaneous decrease in appetite. Such disassociation between appetite and ghrelin profiles in a single participant has previously been reported by Cummings et al. (2004), as one out of six participants did not demonstrate an increase in ghrelin prior to spontaneous meal request, despite exhibiting significant increases in appetite and a similar energy intake and meal request response as all other participants. The reasons for the occurrence of outlying participants in the present study are unclear as all outliers displayed an appetite, energy intake and PYY<sub>3-36</sub> response that

was consistent with the remainder of the sample. Furthermore, there was no difference between the outlying and non-outlying participants for any of the measured physiological characteristics.

The removal of outlying participants from the acylated ghrelin data revealed a marked suppression of this peptide during the hours after exercise, which supports the findings of previous authors (Broom et al. 2007; Kawano et al. 2013; Wasse et al. 2013). However, contrary to the hypothesis of the study and previous findings from our laboratory (King et al. 2011a), food restriction did not stimulate any compensatory increases in acylated ghrelin. This is likely to reflect the smaller food restriction employed in the present study as a similar reduction in energy intake of ~1218 kJ did not influence 24 h total ghrelin concentrations in a previous investigation (Weigle et al. 2003).

The findings of the present study contribute to the current debate about the importance of physiological changes in ghrelin as mediator of appetite. In this regard, a recent study by Lippl and colleagues (2012) reported that exogenous infusion of ghrelin at physiological and mildly supraphysiological doses does not influence appetite, spontaneous meal request or energy intake. Furthermore, recent studies of knockout mice that are deficient for either ghrelin, GHS-R or GOAT reported a similar feeding response between these knockout mice and wild type controls (Sun et al. 2008; Zhao et al. 2010). Alternatively, these authors suggested that the primary function of acylated ghrelin was to preserve blood glucose concentrations during food restriction as an absence of either acylated ghrelin or GHS-R elicited a significant reduction in blood glucose during 50 – 60 % calorie restriction. It seems plausible that the 69 % calorie restriction employed by King et al. (2011a) may have stimulated increases in acylated ghrelin to maintain blood glucose concentrations, whereas the 22 % energy deficit in the present study may have been insufficient to threaten blood glucose levels. Although this contributes to an interesting debate about the primary function of acylated ghrelin, these suggestions are speculative and require further investigation.

Alternatively, changes in PYY<sub>3-36</sub> concentrations were significantly negatively correlated with changes in appetite from 0-1 h, 4-8 h and for the entire 8 h trial. To the author's knowledge, only three experiments have previously measured the PYY<sub>3-36</sub> response to exercise beyond the provision of a single test meal (Chapter 5; Cheng et al.

2009; King et al. 2011a). The findings of the present study support previous findings by demonstrating a prolonged increase in PYY<sub>3-36</sub> after exercise. Furthermore, although not statistically significant, the increase in PYY<sub>3-36</sub> concentrations in response to the lunch meal appeared to be reduced during the food restriction trial. Although, these findings are far from conclusive, it seems plausible that the contrasting changes in PYY<sub>3-36</sub> in response to exercise and food restriction may be implicated in the divergent appetite response to these trials. It must be noted that appetite is regulated by the complex interaction of many physiological and psychological factors (King et al. 2007; Murphy & Bloom 2006). Therefore, the response of a single hormone to the subtle energy deficits employed in this study is unlikely to account for all of the variation in appetite between trials. Nevertheless, considering that obese participants have consistently been found to exhibit a blunted PYY and satiety response to feeding (Batterham et al. 2006; Korner et al. 2005; le Roux et al. 2006a; Stock et al. 2005), it would be useful for future experiments to investigate whether this response is improved with exercise.

Surprisingly, despite a significant increase in appetite in response to food restriction, energy intake at the ad libitum meal did not differ between trials. This contrasts with previous investigations that have demonstrated an increase in energy intake in response to food restriction compared with an equivalent energy deficit induced via exercise (Hubert et al. 1998; King et al. 2011a). However, this is likely to reflect the smaller changes in appetite observed in the present study due to the modest energy deficits employed. Such a disassociation between appetite and energy intake has been commonly reported within the scientific literature in response to modest experimental manipulations and is thought to represent an accruing degree of motivation prior to the initiation of a behavioural response (Stubbs et al. 2000). It seems reasonable to speculate that continued food restriction would elicit increases in energy intake over a longer monitoring period but this requires further investigation.

Although closely supervised interventions involving either exercise alone or dieting alone have been demonstrated to result in successful weight loss (King et al. 2008; Stewart & Fleming 1973), these interventions are largely unsuccessful when the participants are not closely supervised (Franz et al. 2007). This is likely to reflect a lack of adherence as changes in exercise participation and dietary practises represent challenging interventions for many individuals. In this regard, the findings of the



present study have demonstrated the sensitivity of the appetite-regulating system to reductions in food intake, which emphasises the need for significant willpower to resist increases in appetite during food restriction. Alternatively, fulfilment of the current physical activity guidelines requires a significant lifestyle change, time commitment and level of exertion for a sedentary individual. In this regard, 30 min of exercise that was perceived as ‘somewhat hard’ only induced an energy deficit of ~1469 kJ in the present study, which highlights the substantial time commitment that is required to induce larger energy deficits using exercise alone. Considering that the energy deficits utilised in the present study are below the recommended minimum of 2092 kJ.d<sup>-1</sup> for weight loss (NHS Choices 2011) and that larger energy deficits are required for greater weight loss, it seems logical to encourage a combined exercise and dietary approach to weight loss in order to compromise between the difficulties of each individual intervention. This supports findings from systematic reviews that combined diet and exercise interventions are the most effective non-surgical method of achieving sustained weight loss (Curioni & Lourenço 2005; Franz et al. 2007).

In conclusion, food restriction of ~1478 kJ across two meals stimulated compensatory increases in appetite that did not occur in response to a similar energy deficit induced by 30 min of moderate intensity exercise. Although the mechanisms underlying such a contrasting response are unclear, it does not appear to be influenced by changes in plasma acylated ghrelin concentrations. Alternatively, changes in PYY<sub>3-36</sub> were negatively correlated with changes in appetite, which supports the anorexigenic nature of this peptide. Future studies should be conducted to elucidate whether PYY<sub>3-36</sub> concentrations also increase in response to exercise in obese participants and if this improves the satiety response to a standardised meal.

## **CHAPTER VIII**

### **General Discussion**

#### **8.1 Introduction**

Recent years have witnessed significant interest from both the scientific community and the media regarding the influence of exercise on subsequent appetite and energy intake responses. The novel work presented throughout this thesis has sought to further investigate this topic by comparing the appetite and energy intake responses to energy deficits induced via different exercise protocols and food restriction. Circulating concentrations of acylated ghrelin and PYY have also been measured within these experiments in order to further elucidate the influence of these hormones in determining the appetite response to exercise. The purpose of this chapter is to reflect upon the main outcomes of this thesis by collectively discussing the findings that have been presented throughout the experiments. Table 8.1 provides a summary of the study protocols and variables measured during each experimental chapter.

Table 8.1. Summary of the study protocols presented within the experimental chapters of this thesis.

Chapter	Trials	Protocol	Net energy expenditure (kJ)	Measurements
4	Fasted exercise	Sixty min of treadmill running at 71.1 (2.1) % of VO <sub>2</sub> max.	3247 (423)	Appetite. Energy & macronutrient intake. Resting energy expenditure & substrate oxidation.
	Postprandial exercise	Sixty min of treadmill running at 71.9 (2.7) % of VO <sub>2</sub> max.	3234 (435)	
	Control	Rest.	-	
5	Endurance exercise	Sixty min of cycling at 68.1 (4.3) % of VO <sub>2</sub> max.	2640 (418)	Appetite. Energy & macronutrient intake. Resting energy expenditure & substrate oxidation. Acylated ghrelin. Total PYY.
	Sprint interval exercise	Six Wingate tests separated by 4 min recovery periods.	594 (50)	
	Control	Rest.	-	
6	Endurance exercise	Sixty min of cycling at 59.5 (1.6) % of VO <sub>2</sub> max.	2451 (208)	Appetite. Energy intake. PYY <sub>3-36</sub> .
	HIIE	Ten 4 min cycling intervals at 85.8 (4.0) % of VO <sub>2</sub> max separated by 2 min recovery periods.	2429 (266)	
	Control	Rest.	-	
7	Exercise	Thirty min of cycling at 64.5 (3.2) % of VO <sub>2</sub> max.	1469 (256)	Appetite. Energy intake. Acylated ghrelin. PYY <sub>3-36</sub> .
	Food restriction	Energy intake reduced by 682 (127) kJ at breakfast and 796 (148) kJ at lunch.	-	
	Control	Rest.	-	

Values are mean (SD).

## 8.2 Appetite

A longstanding body of literature has demonstrated that strenuous exercise ( $\geq 60\%$  of  $\text{VO}_2$  max) induces a transient suppression of appetite termed ‘exercise-induced anorexia’ (Blundell & King 1999; Blundell et al. 2003; Martins et al. 2008). The work presented within Chapters 5 and 6 of this thesis has further characterised this phenomenon by demonstrating that appetite suppression is enhanced when exercise is performed above an intensity of  $85\%$  of  $\text{VO}_2$  max compared with moderate intensity exercise at  $60 - 68\%$  of  $\text{VO}_2$  max. Furthermore, the findings from Chapter 4 confirmed preliminary evidence from Cheng and colleagues (2009) that exercise induces a greater and more prolonged appetite suppression when performed postprandially, rather than in the fasted state. However, despite enhancing exercise-induced anorexia with these manipulations, appetite perceptions returned to control values within 45 min of the cessation of exercise and prior to feeding opportunities in all studies. This confirms the transient nature of appetite suppression during exercise and further emphasises the importance of monitoring appetite perceptions for a prolonged period after exercise.

Several recent authors have suggested that HIIE may be a particularly effective protocol for minimising appetite perceptions during the post-exercise period (Boutcher 2011; Heydari et al. 2012; Trapp et al. 2008; Tremblay et al. 1994). However, to the author’s knowledge, Chapters 5 and 6 of this thesis represent the first experiments to investigate the appetite response to HIIE. Furthermore, in contrast with such postulations from previous authors, Chapter 5 demonstrated that a recently popularised sprint interval exercise protocol, consisting of six 30 s maximal sprints on a cycle ergometer, stimulated compensatory increases in appetite during the five hour monitoring period after exercise that did not occur in response to 60 min of continuous cycling at  $68\%$  of  $\text{VO}_2$  max. In order to further investigate this issue, a high volume, submaximal HIIE protocol was investigated in Chapter 6 and it was determined that compensatory increases in appetite did not occur during the five hour monitoring period after ten 4 min cycling intervals at  $85 - 90\%$  of  $\text{VO}_2$  max or 60 min of continuous cycling at  $60\%$  of  $\text{VO}_2$  max. The absence of any compensatory increases in appetite in this study suggests that the observed increases after supramaximal interval exercise are likely to be a result of the extreme intensity rather than the intermittent nature of exercise. It remains plausible that a threshold exercise intensity may exist for the stimulation of appetite during the post-exercise period but this requires further

investigation. It is also important to note that these studies employed exercise protocols that represented both extremes of the HIIE spectrum (i.e. very low volume, supramaximal interval exercise in Chapter 5 and high volume, submaximal interval exercise in Chapter 6) and therefore they provide convincing evidence that HIIE does not elicit lower appetite perceptions during the hours after exercise compared with traditional endurance exercise.

The experiments conducted throughout this thesis have consistently demonstrated that continuous moderate intensity exercise does not stimulate compensatory increases in appetite during the 5 – 9 h monitoring period after exercise (Chapters 4 - 7). However, despite previous speculation from Cheng and colleagues (2009), performing continuous exercise in the postprandial state did not elicit lower appetite perceptions than fasted exercise after the recovery from exercise-induced anorexia (Chapter 4). Nonetheless, the absence of any immediate increases in appetite after moderate intensity exercise supports the majority of studies within the scientific literature (Caudwell et al. 2013). The findings presented within this thesis also suggest that short-term increases in appetite are not driven by the energy expenditure of exercise as increases were only apparent in response to sprint exercise in Chapter 5, which induced the smallest energy deficit of all exercise protocols investigated (Table 8.1).

Although energy deficits of ~3247 kJ did not stimulate any increases in appetite when induced by exercise, Chapter 7 demonstrates that a reduction in food intake of ~1478 kJ across two meals stimulates rapid compensatory increases in appetite. These findings support previous evidence that human appetite is primarily mediated by the passage of food through the mouth and gastrointestinal tract (Borer et al. 2009; Hubert et al. 1998; King et al. 2011a) and support observations that dieting is often difficult to maintain due to increases in appetite (Ikeda et al. 2004).

### **8.3 Energy Intake**

The majority of experiments within the scientific literature have demonstrated that an acute bout of exercise does not stimulate increases in ad libitum energy intake during the subsequent 24 hours (King et al. 2010a; Martins et al. 2008). In support of these findings, daily energy intake did not differ from a resting control trial after any of the exercise protocols utilised throughout the experimental chapters of this thesis.

The apparent insensitivity of energy intake to exercise supports the appetite responses to moderate intensity exercise in Chapters 4 – 7 but represents a disassociation from the elevations in appetite observed during the hours after sprint interval exercise in Chapter 5 and food restriction in Chapter 7. Such divergence has been previously reported within the scientific literature (Borer et al. 2009; King et al. 2011b) and is thought to represent a greater sensitivity of appetite perceptions than energy intake responses to modest experimental manipulations (Stubbs et al. 2000). Furthermore, although the experiments performed throughout this thesis may be regarded as underpowered, the effect sizes for differences in total ad libitum energy intake between trials were small for all comparisons ( $d < 0.3$ ). It is also important to note that despite differences in appetite during the hours preceding the ad libitum meal in Chapters 5 and 7, these values reached a similar peak in all trials immediately prior to the ad libitum meal. It therefore seems plausible that this anticipatory increase in appetite may have negated any subtle differences in appetite arising from the experimental manipulations.

Nevertheless, previous experiments have reported increases in acute food intake in response to energy deficits of 1820 - 4820 kJ induced by food restriction (Hubert et al. 1998; King et al. 2011a). Furthermore, the findings of King et al. (2011a) were observed when using the same ad libitum meal and experimental protocol as that employed in Chapters 4 and 5 of this thesis. Subsequently, considering that the standard deviation of energy intake was lower using an ad libitum pasta meal in Chapters 6 and 7 of this thesis, the lack of difference in energy intake observed throughout this thesis is likely to represent a lack of effect rather than a lack of statistical power. Therefore, the absence of an increase in daily food intake in response to exercise-induced energy deficits of 594 - 3247 kJ (Chapters 4 - 7) supports a role for exercise in weight management. This consistent finding also counters claims from recent media articles that exercise stimulates rapid compensatory increases in appetite and food intake that prevent weight loss (Time Magazine 2009) and increase body fat (The Daily Telegraph 2009). It has been suggested that it may take 10 years to repair the damage caused by these media articles (Blundell 2009) and it is hoped that the research presented within this thesis can contribute to achieving this.

## **8.4 Acylated ghrelin**

During recent years, many studies have reported a temporal association between exercise-induced anorexia and a transient decrease in circulating concentrations of the orexigenic peptide acylated ghrelin (Becker et al. 2012; Broom et al. 2007; 2009; Kawano et al. 2013; King et al. 2010a; 2011a; 2011b; Wasse et al. 2012). This consistent finding has been replicated with the measurement of plasma acylated ghrelin concentrations in Chapters 5 and 7 of this thesis. Furthermore, Chapter 5 represents the first experiment to investigate the effect of supramaximal exercise on acylated ghrelin concentrations. This study revealed that enhanced appetite suppression during sprint interval exercise was associated with greater reductions in plasma acylated ghrelin concentrations. This extends the findings of previous investigations and further strengthens the relationship between exercise-induced decreases in appetite and acylated ghrelin.

However, during the hours after exercise, a marked divergence was observed between changes in appetite and plasma acylated ghrelin concentrations. In this regard, acylated ghrelin remained significantly suppressed during the 45 min after sprint interval exercise in Chapter 5, yet appetite perceptions returned to similar values in all trials. This resulted in a significant negative correlation between these variables and further divergence was observed during the subsequent hours as appetite was highest and acylated ghrelin concentrations were lowest during the afternoon of the sprint trial. This opposes the findings of the majority of studies within the current literature, which have demonstrated that appetite and acylated ghrelin concentrations do not differ from a resting control trial during the hours after exercise (Broom et al. 2009; Hagobian et al. 2013; Kelly et al. 2012; King et al. 2010a; 2010b; 2011a; Ueda et al. 2009b; Unick et al. 2010; Wasse et al. 2012). However, divergence between these variables has also been reported within the literature when acylated ghrelin levels have been suppressed beyond the exercise period (Broom et al. 2007; Kawano et al. 2013; Wasse et al. 2013). It therefore seems plausible that previous associations between acylated ghrelin concentrations and appetite ratings during the hours after exercise may have been correlational rather than causal as further decreases in acylated ghrelin do not appear to reduce appetite.

Further disassociations were observed in Chapter 7 as food restriction stimulated compensatory increases in appetite in the absence of any increases in circulating acylated ghrelin levels. Additionally, the acylated ghrelin profile of one outlying participant (see Figure 7.5) exhibited an increase in response to the lunch meal in all trials despite the participant reporting a simultaneous decrease in appetite. The reasons for such an altered acylated ghrelin response to feeding in this participant are unclear but similar findings have also been reported by Cummings and colleagues (2004) as one out of six participants did not demonstrate an increase in ghrelin prior to spontaneous meal request, despite exhibiting significant increases in appetite and a similar energy intake and meal request response as all other participants.

The findings of this thesis therefore question the importance of physiological changes in plasma acylated ghrelin as a mediator of appetite. This viewpoint is also supported by a recent infusion study, which demonstrated that physiological and mildly supraphysiological exogenous concentrations of acylated ghrelin do not influence appetite, energy intake or spontaneous meal request (Lippl et al. 2012). Furthermore, recent studies of knockout mice that are deficient for either ghrelin, GHS-R or GOAT reported a similar feeding response between these knockout mice and wild type controls (Sun et al. 2008; Zhao et al. 2010). It seems plausible that the mediating influence of ghrelin on appetite has been overstated due to the conspicuous nature of this peptide as the only circulating orexigen. It must be remembered that ghrelin is only one component of the appetite-regulating neuroendocrine system and that appetite is likely to reflect many hormonal and psychological factors (King et al. 2007; Murphy & Bloom 2006).

## **8.5 Peptide YY**

In accordance with previous research, the experiments conducted within Chapters 5 – 7 of this thesis reported a temporal association between elevations in circulating concentrations of the anorexigenic hormone PYY and reductions in appetite during exercise (Broom et al. 2009; Kawano et al. 2013; King et al. 2011a; Martins et al. 2007; Ueda et al. 2009a; Wasse et al. 2012). However, the comparison of different intensity exercise bouts in Chapters 5 and 6 demonstrated that changes in appetite during exercise are not solely dependent upon changes in this peptide. In this regard, sprint interval exercise induced greater appetite suppression than endurance exercise in



Chapter 5, yet plasma concentrations of total PYY increased more consistently upon completion of endurance exercise. This finding was also replicated in Chapter 6 with the measurement of PYY<sub>3-36</sub> as endurance exercise stimulated the most consistent increases in this peptide despite appetite suppression being greatest during HIIE.

To the author's knowledge, only two studies prior to this thesis have investigated the PYY<sub>3-36</sub> response to exercise for a prolonged period with the inclusion of test meals (Cheng et al. 2009; King et al. 2011a). In support of the findings from these studies, the experiments conducted in Chapters 6 and 7 demonstrated a prolonged elevation in PYY<sub>3-36</sub> during the hours after exercise. Conversely, increases in total PYY concentrations in response to exercise in Chapter 5 were transient, which supports the findings from the majority of previous investigations (Kawano et al. 2013; Martins et al. 2007; Ueda et al. 2009b; Wasse et al. 2012). Considering that the analysis of total PYY concentrations includes both PYY<sub>1-36</sub> and PYY<sub>3-36</sub>, it seems plausible that this measurement may have masked changes in PYY<sub>3-36</sub>. However, this requires future investigation via the measurement of both total PYY and PYY<sub>3-36</sub> in response to exercise.

The findings from Chapter 6 suggest that changes in PYY<sub>3-36</sub> immediately after exercise may not predict changes during the subsequent hours as concentrations increased most consistently upon cessation of endurance exercise but were elevated to a greater extent during the hours after HIIE. Although the reasons for this effect are unclear, it seems reasonable to postulate that greater elevations in response to HIIE may be a result of differences in sympathetic nervous system activity. In this regard, sympathetic activity has been demonstrated to increase with exercise intensity (Perini et al. 1989) and appears to stimulate PYY secretion (Brechet et al. 2001; Zhang et al. 1993). However, this theory does not explain the finding that endurance exercise stimulated more consistent increases in PYY<sub>3-36</sub> immediately upon completion of exercise compared with HIIE. This confliction may be a result of intestinal blood flow, which decreases with exercise intensity (Clausen 1977; Gil et al. 1998) and may have therefore reduced the transport of PYY<sub>3-36</sub> into the peripheral circulation immediately after exercise in HIIE (Mailman 1982). These are the first postulations to be made about the mechanisms of exercise-induced increases in PYY and although this would explain the findings presented in this thesis, it must be noted that this is speculation and requires future investigation.

Although continued elevations in PYY<sub>3-36</sub> did not elicit lower appetite responses during the hours after exercise in Chapter 6, elevated concentrations in response to exercise compared with food restriction were associated with lower appetite perceptions in Chapter 7. In accordance with this association, changes in PYY<sub>3-36</sub> concentrations were significantly negatively correlated with changes in appetite from 0 - 1 h, 4 - 8 h and for the entire 8 h trial. Although these findings are far from conclusive, it seems plausible that changes in circulating PYY concentrations may have acted in combination with a multitude of other physiological and psychological factors to influence appetite perceptions in the hours after exercise.

## **8.6 Limitations and future directions**

The experiments conducted throughout this thesis contain several common limitations. Firstly, all participants were young, physically active, healthy males. Although previous research suggests that exercise elicits similar appetite and energy intake responses in lean and obese participants (Ueda et al. 2009b), it remains important to perform further investigations in overweight and obese populations as this is where weight management strategies hold the most clinical relevance. It may also be beneficial for future research to investigate the appetite response to exercise in the elderly as little research has been performed in this area. A second major limitation of this work is that all experiments monitored the daily response to an acute bout of exercise. Subsequently, it remains important for future studies to measure the appetite and energy intake responses to repeated bouts of exercise in order to investigate whether differences and similarities observed in response to a single exercise bout continue in response to repeated bouts. Finally, from a mechanistic perspective, the measurement of acylated ghrelin and PYY is insufficient to explain changes in appetite in response to exercise. Considering the complex nature of appetite regulation, it may be beneficial for future investigations to utilise a multidisciplinary approach that includes the measurement of additional hormones, as well as brain region activity and psychological measures.

## REFERENCES

- Abbott, C. R., Small, C. J., Kennedy, A. R., Neary, N. M., Sajedi, A., Ghatei, M. A., & Bloom, S. R. (2005a). Blockade of the neuropeptide Y Y2 receptor with the specific antagonist BIIE0246 attenuates the effect of endogenous and exogenous peptide YY(3-36) on food intake. *Brain research*, *1043*(1-2), 139–44.
- Abbott, C. R., Monteiro, M., Small, C. J., Sajedi, A., Smith, K. L., Parkinson, J. R. C., Ghatei, M. A., et al. (2005b). The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain research*, *1044*(1), 127–31.
- Acuna-Goycolea, C., & Van den Pol, A. N. (2005). Peptide YY(3-36) inhibits both anorexigenic proopiomelanocortin and orexigenic neuropeptide Y neurons: implications for hypothalamic regulation of energy homeostasis. *Journal of neuroscience*, *25*(45), 10510–9.
- Adams, S. H., Lei, C., Jodka, C. M., Nikoulina, S. E., Hoyt, J. A., Gedulin, B., Mack, C. M., et al. (2006). PYY[3-36] administration decreases the respiratory quotient and reduces adiposity in diet-induced obese mice. *Journal of nutrition*, *136*(1), 195–201.
- Adrian, T. E., Ferri, G. L., Bacarese-Hamilton, A. J., Fuessl, H. S., Polak, J. M., & Bloom, S. R. (1985a). Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology*, *89*(5), 1070–7.
- Adrian, T. E., Savage, A. P., Sagor, G. R., Allen, J. M., Bacarese-Hamilton, A. J., Tatemoto, K., Polak, J. M., et al. (1985b). Effect of peptide YY on gastric, pancreatic, and biliary function in humans. *Gastroenterology*, *89*(3), 494–9.
- Adrian, T. E., Sagor, G. R., Savage, A. P., Bacarese-Hamilton, A. J., Hall, G. M., & Bloom, S. R. (1986). Peptide YY kinetics and effects on blood pressure and circulating pancreatic and gastrointestinal hormones and metabolites in man. *Journal of clinical endocrinology and metabolism*, *63*(4), 803–7.
- Air, E. L., Benoit, S. C., Blake Smith, K. A., Clegg, D. J., & Woods, S. C. (2002). Acute third ventricular administration of insulin decreases food intake in two paradigms. *Pharmacology, biochemistry, and behavior*, *72*(1-2), 423–9.

- Allen, J. M., Fitzpatrick, M. L., Yeats, J. C., Darcy, K., Adrian, T. E., & Bloom, S. R. (1984). Effects of peptide YY and neuropeptide Y on gastric emptying in man. *Digestion*, *30*(4), 255–62.
- Almada, C., Cataldo, L. R., Smalley, S. V., Diaz, E., Serrano, A., Hodgson, M. I., & Santos, J. L. (2013). Plasma levels of interleukin-6 and interleukin-18 after an acute physical exercise: relation with post-exercise energy intake in twins. *Journal of physiology and biochemistry*, *69*(1), 85–95.
- Alméras, N., Lavallée, N., Després, J. P., Bouchard, C., & Tremblay, A. (1995). Exercise and energy intake: effect of substrate oxidation. *Physiology & behavior*, *57*(5), 995–1000.
- Alvarez Bartolomé, M., Borque, M., Martínez-Sarmiento, J., Aparicio, E., Hernández, C., Cabrerizo, L., & Fernández-Represa, J. A. (2002). Peptide YY secretion in morbidly obese patients before and after vertical banded gastroplasty. *Obesity surgery*, *12*(3), 324–7.
- American College of Sports Medicine (2006). *ACSM's guidelines for exercise testing and prescription* (7th ed.). Baltimore, USA: Lippincott Williams & Wilkins.
- Arvat, E., Maccario, M., Di Vito, L., Broglio, F., Benso, A., Gottero, C., Papotti, M., et al. (2001). Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *Journal of clinical endocrinology and metabolism*, *86*(3), 1169–74.
- Asakawa, A., Inui, A., Yuzuriha, H., Ueno, N., Katsuura, G., Fujimiya, M., Fujino, M. A., et al. (2003). Characterization of the effects of pancreatic polypeptide in the regulation of energy balance. *Gastroenterology*, *124*(5), 1325–36.
- Baatar, D., Patel, K., & Taub, D. D. (2011). The effects of ghrelin on inflammation and the immune system. *Molecular and cellular endocrinology*, *340*(1), 44–58.
- Babraj, J. A., Volllaard, N. B. J., Keast, C., Guppy, F. M., Cottrell, G., & Timmons, J. A. (2009). Extremely short duration high intensity interval training substantially improves insulin action in young healthy males. *BMC endocrine disorders*, *9*, 3.

- Bahr, R. (1992). Excess postexercise oxygen consumption--magnitude, mechanisms and practical implications. *Acta physiologica Scandinavica. Supplementum*, 605, 1–70.
- Balaguera-Cortes, L., Wallman, K. E., Fairchild, T. J., & Guelfi, K. J. (2011). Energy intake and appetite-related hormones following acute aerobic and resistance exercise. *Applied physiology, nutrition, and metabolism*, 36(6), 958–66.
- Baldanzi, G., Filigheddu, N., Cutrupi, S., Catapano, F., Bonisconi, S., Fubini, A., Malan, D., et al. (2002). Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. *Journal of cell biology*, 159(6), 1029–37.
- Bartlett, J. D., Close, G. L., MacLaren, D. P. M., Gregson, W., Drust, B., & 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(6), 547–53.
- Barwell, N. D., Malkova, D., Leggate, M., & Gill, J. M. R. (2009). Individual responsiveness to exercise-induced fat loss is associated with change in resting substrate utilization. *Metabolism*, 58(9), 1320–8.
- Batterham, R. L., Cohen, M. A., Ellis, S. M., le Roux, C. W., Withers, D. J., Frost, G. S., Ghatei, M. A., et al. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. *New England journal of medicine*, 349(10), 941–8.
- Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., Wren, A. M., et al. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature*, 418(6898), 650–4.
- Batterham, R. L., Ffytche, D. H., Rosenthal, J. M., Zelaya, F. O., Barker, G. J., Withers, D. J., & Williams, S. C. R. (2007). PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. *Nature*, 450(7166), 106–9.
- Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., le Roux, C. W., et al. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell metabolism*, 4(3), 223–33.

- Becker, G. F., Macedo, R. C. O., Cunha, G. D. S., Martins, J. B., Laitano, O., & Reischak-Oliveira, A. (2012). Combined effects of aerobic exercise and high-carbohydrate meal on plasma acylated ghrelin and levels of hunger. *Applied physiology, nutrition, and metabolism*, *37*(1), 184–92.
- Belza, A., Ritz, C., Sørensen, M. Q., Holst, J. J., Rehfeld, J. F., & Astrup, A. (2013). Contribution of gastroenteropancreatic appetite hormones to protein-induced satiety. *American journal of clinical nutrition*.
- Benelam, B. (2009). Satiating, satiety and their effects on eating behaviour. *Nutrition bulletin*, *34*(2), 126–173.
- Blundell, J. (2009). Exercise makes you fat - what's going on? *Nutrition bulletin*, *34*(4), 380–382.
- Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Luch, A., Mela, D., et al. (2010). Appetite control: methodological aspects of the evaluation of foods. *Obesity reviews*, *11*(3), 251–70.
- Blundell, J. E., & King, N. A. (1999). Physical activity and regulation of food intake: current evidence. *Medicine and science in sports and exercise*, *31*(11 Suppl), S573–83.
- Blundell, J. E., Stubbs, R. J., Hughes, D. A., Whybrow, S., & King, N. A. (2003). Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proceedings of the nutrition society*, *62*(3), 651–61.
- Boey, D, Lin, S., Enriquez, R. F., Lee, N. J., Slack, K., Couzens, M., Baldock, P. A., et al. (2008). PYY transgenic mice are protected against diet-induced and genetic obesity. *Neuropeptides*, *42*(1), 19–30.
- Boey, D, Lin, S., Karl, T., Baldock, P., Lee, N., Enriquez, R., Couzens, M., et al. (2006). Peptide YY ablation in mice leads to the development of hyperinsulinaemia and obesity. *Diabetologia*, *49*(6), 1360–70.
- Borer, K. T., Wuorinen, E., Chao, C., & Burant, C. (2005). Exercise energy expenditure is not consciously detected due to oro-gastric, not metabolic, basis of hunger sensation. *Appetite*, *45*(2), 177–81.

- Borer, K. T., Wuorinen, E., Ku, K., & Burant, C. (2009). Appetite responds to changes in meal content, whereas ghrelin, leptin, and insulin track changes in energy availability. *Journal of clinical endocrinology and metabolism*, *94*(7), 2290–8.
- Borg, G. A. (1973). Perceived exertion: a note on “history” and methods. *Medicine and science in sports*, *5*(2), 90–3.
- Boutcher, S. H. (2011). High-intensity intermittent exercise and fat loss. *Journal of obesity*, *2011*, 868305.
- Bray, G. A. (2004). Medical consequences of obesity. *Journal of clinical endocrinology and metabolism*, *89*(6), 2583–9.
- Brechet, S., Plaisancié, P., Dumoulin, V., Chayvialle, J. A., Cuber, J. C., & Claustre, J. (2001). Involvement of beta1- and beta2- but not beta3-adrenoceptor activation in adrenergic PYY secretion from the isolated colon. *Journal of endocrinology*, *168*(1), 177–83.
- British Heart Foundation (2010). Physical Activity Guidelines in the UK: Review & Recommendations. Available at:  
[https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/213743/dh\\_128255.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/213743/dh_128255.pdf).
- Broom, D. R., Batterham, R. L., King, J. A., & Stensel, D. J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American journal of physiology. Regulatory, integrative and comparative physiology*, *296*(1), R29–35.
- Broom, D. R., Stensel, D. J., Bishop, N. C., Burns, S. F., & Miyashita, M. (2007). Exercise-induced suppression of acylated ghrelin in humans. *Journal of applied physiology*, *102*(6), 2165–71.
- Burgomaster, K. A, Cermak, N. M., Phillips, S. M., Benton, C. R., Bonen, A., & Gibala, M. J. (2007). Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *American journal of physiology. Regulatory, integrative and comparative physiology*, *292*(5), R1970–6.

- Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., Macdonald, M. J., McGee, S. L., & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *Journal of physiology*, *586*(1), 151–60.
- Burns, S. F., Broom, D. R., Miyashita, M., Mundy, C., & Stensel, D. J. (2007). A single session of treadmill running has no effect on plasma total ghrelin concentrations. *Journal of sports sciences*, *25*(6), 635–42.
- Børsheim, E., & Bahr, R. (2003). Effect of exercise intensity, duration and mode on post-exercise oxygen consumption. *Sports medicine*, *33*(14), 1037–60.
- Cabrele, C., & Beck-Sickinger, A. G. (2000). Molecular characterization of the ligand-receptor interaction of the neuropeptide Y family. *Journal of peptide science*, *6*(3), 97–122.
- Cahill, F., Shea, J. L., Randell, E., Vasdev, S., & Sun, G. (2011). Serum peptide YY in response to short-term overfeeding in young men. *American journal of clinical nutrition*, *93*(4), 741–7.
- Callahan, H. S., Cummings, D. E., Pepe, M. S., Breen, P. A., Matthys, C. C., & Weigle, D. S. (2004). Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *Journal of clinical endocrinology and metabolism*, *89*(3), 1319–24.
- Carlini, V. P., Martini, A. C., Schiöth, H. B., Ruiz, R. D., Fiol de Cuneo, M., & De Barioglio, S. R. (2008). Decreased memory for novel object recognition in chronically food-restricted mice is reversed by acute ghrelin administration. *Neuroscience*, *153*(4), 929–34.
- Caudwell, P., Gibbons, C., Finlayson, G., Näslund, E., & Blundell, J. (2013). Physical activity, energy intake, and obesity: the links between exercise and appetite. *Current obesity reports*, *2*(2), 185–190.
- Challis, B., Pinnock, S., Coll, A., Carter, R., Dickson, S., & O’Rahilly, S. (2003). Acute effects of PYY3–36 on food intake and hypothalamic neuropeptide expression in the mouse. *Biochemical and biophysical research communications*, *311*(4), 915–919.



- Chan, J. L., Stoyneva, V., Kelesidis, T., Raciti, P., & Mantzoros, C. S. (2006a). Peptide YY levels are decreased by fasting and elevated following caloric intake but are not regulated by leptin. *Diabetologia*, *49*(1), 169–73.
- Chan, J. L., Mun, E. C., Stoyneva, V., Mantzoros, C. S., & Goldfine, A. B. (2006b). Peptide YY levels are elevated after gastric bypass surgery. *Obesity*, *14*(2), 194–8.
- Chandarana, K., Gelegen, C., Karra, E., Choudhury, A. I., Drew, M. E., Fauveau, V., Viollet, B., et al. (2011). Diet and gastrointestinal bypass-induced weight loss: the roles of ghrelin and peptide YY. *Diabetes*, *60*(3), 810–8.
- Chelikani, P. K., Haver, A. C., Reeve, J. R., Keire, D. A., & Reidelberger, R. D. (2006). Daily, intermittent intravenous infusion of peptide YY(3-36) reduces daily food intake and adiposity in rats. *American journal of physiology. Regulatory, integrative and comparative physiology*, *290*(2), R298–305.
- Chelikani, P. K., Haver, A. C., & Reidelberger, R. D. (2005). Intravenous infusion of peptide YY(3-36) potentially inhibits food intake in rats. *Endocrinology*, *146*(2), 879–88.
- Chen, H. Y., Trumbauer, M. E., Chen, A. S., Weingarth, D. T., Adams, J. R., Frazier, E. G., Shen, Z., et al. (2004). Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology*, *145*(6), 2607–12.
- Cheng, M. H-Y., Bushnell, D., Cannon, D. T., & Kern, M. (2009). Appetite regulation via exercise prior or subsequent to high-fat meal consumption. *Appetite*, *52*(1), 193–8.
- Christ, E. R., Zehnder, M., Boesch, C., Trepp, R., Mullis, P. E., Diem, P., & Décombaz, J. (2006). The effect of increased lipid intake on hormonal responses during aerobic exercise in endurance-trained men. *European journal of endocrinology*, *154*(3), 397–403.
- Clausen, J. P. (1977). Effect of physical training on cardiovascular adjustments to exercise in man. *Physiological reviews*, *57*(4), 779–815.
- Cohen, M. A., Ellis, S. M., le Roux, C. W., Batterham, R. L., Park, A., Patterson, M., Frost, G. S., et al. (2003). Oxyntomodulin suppresses appetite and reduces food intake in humans. *Journal of clinical endocrinology and metabolism*, *88*(10), 4696–701.

- Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., et al. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England journal of medicine*, *334*(5), 292–5.
- Cox, J. E., & Randich, A. (2004). Enhancement of feeding suppression by PYY(3-36) in rats with area postrema ablations. *Peptides*, *25*(6), 985–9.
- Cummings, D. E., Frayo, R. S., Marmonier, C., Aubert, R., & Chapelot, D. (2004). Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *American journal of physiology. Endocrinology and metabolism*, *287*(2), E297–304.
- Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., & Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, *50*(8), 1714–9.
- Cummings, D. E., Weigle, D. S., Frayo, R. S., Breen, P. A., Ma, M. K., Dellinger, E. P., & Purnell, J. Q. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New England journal of medicine*, *346*(21), 1623–30.
- Curioni, C. C., & Lourenço, P. M. (2005). Long-term weight loss after diet and exercise: a systematic review. *International journal of obesity*, *29*(10), 1168–74.
- Dakin, C. L., Small, C. J., Batterham, R. L., Neary, N. M., Cohen, M. A., Patterson, M., Ghatei, M. A., et al. (2004). Peripheral oxyntomodulin reduces food intake and body weight gain in rats. *Endocrinology*, *145*(6), 2687–95.
- Dall, R., Kanaley, J., Hansen, T. K., Møller, N., Christiansen, J. S., Hosoda, H., Kangawa, K., et al. (2002). Plasma ghrelin levels during exercise in healthy subjects and in growth hormone-deficient patients. *European journal of endocrinology*, *147*(1), 65–70.
- Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Nijjima, A., Matsuo, H., Kangawa, K., et al. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology*, *123*(4), 1120–8.

De Bock, K., Richter, E. A., Russell, A. P., Eijnde, B. O., Derave, W., Ramaekers, M., Koninckx, E., et al. (2005). Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *Journal of physiology*, *564*(Pt 2), 649–60.

De Silva, A., Salem, V., Long, C. J., Makwana, A., Newbould, R. D., Rabiner, E. A., Ghatei, M. A., et al. (2011). The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. *Cell metabolism*, *14*(5), 700–6.

De Souza, C. T., Araujo, E. P., Bordin, S., Ashimine, R., Zollner, R. L., Boschero, A. C., Saad, M. J. A., et al. (2005). Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology*, *146*(10), 4192–9.

De Vriese, C., Gregoire, F., Lema-Kisoka, R., Waelbroeck, M., Robberecht, P., & Delporte, C. (2004). Ghrelin degradation by serum and tissue homogenates: identification of the cleavage sites. *Endocrinology*, *145*(11), 4997–5005.

Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., Drewe, J., & Beglinger, C. (2005). Effect of peptide YY3-36 on food intake in humans. *Gastroenterology*, *129*(5), 1430–6.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of applied physiology*, *37*(2), 247–8.

Donnelly, J. E., Blair, S. N., Jakicic, J. M., Manore, M. M., Rankin, J. W., & Smith, B. K. (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*(2), 459–71.

Druce, M. R., Neary, N. M., Small, C. J., Milton, J., Monteiro, M., Patterson, M., Ghatei, M. A., et al. (2006). Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *International journal of obesity*, *30*(2), 293–6.

- Druce, M. R., Wren, A. M., Park, A. J., Milton, J. E., Patterson, M., Frost, G., Ghatei, M. A., et al. (2005). Ghrelin increases food intake in obese as well as lean subjects. *International journal of obesity*, 29(9), 1130–6.
- Durnin, J. V., & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British journal of nutrition*, 32(1), 77–97.
- Eberlein, G. A., Eysselein, V. E., Schaeffer, M., Layer, P., Grandt, D., Goebell, H., Niebel, W., et al. (1989). A new molecular form of PYY: structural characterization of human PYY(3-36) and PYY(1-36). *Peptides*, 10(4), 797–803.
- Elder, S. J., & Roberts, S. B. (2007). The effects of exercise on food intake and body fatness: a summary of published studies. *Nutrition reviews*, 65(1), 1–19.
- Erdmann, J., Tahbaz, R., Lippl, F., Wagenpfeil, S., & Schusdziarra, V. (2007). Plasma ghrelin levels during exercise - effects of intensity and duration. *Regulatory peptides*, 143(1-3), 127–35.
- Evero, N., Hackett, L. C., Clark, R. D., Phelan, S., & Hagobian, T. A. (2012). Aerobic exercise reduces neuronal responses in food reward brain regions. *Journal of applied physiology*, 112(9), 1612–9.
- Farah, N. M. F., Brunstrom, J. M., & Gill, J. M. R. (2012). Using a novel computer-based approach to assess the acute effects of exercise on appetite-related measures. *Appetite*, 58(1), 196–204.
- Farooqi, I. S., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., Sanna, V., et al. (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *Journal of clinical investigation*, 110(8), 1093–103.
- Faul, F., Erdfelder, E., Lang, A.G., & Buchner, A. (2007). G\*Power 3: a flexible statistical power analysis program for the social, behavioural, and biomedical sciences. *Behaviour research methods* 39: 175-91.

- Febbraio, M. A., Chiu, A., Angus, D. J., Arkininstall, M. J., & Hawley, J. A. (2000). Effects of carbohydrate ingestion before and during exercise on glucose kinetics and performance. *Journal of applied physiology*, *89*(6), 2220–6.
- Febbraio, M. A., & Stewart, K. L. (1996). CHO feeding before prolonged exercise: effect of glycemic index on muscle glycogenolysis and exercise performance. *Journal of applied physiology*, *81*(3), 1115–20.
- Finlayson, G., Bryant, E., Blundell, J. E., & King, N. A. (2009). Acute compensatory eating following exercise is associated with implicit hedonic wanting for food. *Physiology & behavior*, *97*(1), 62–7.
- Finucane, M. M., Stevens, G. A., Cowan, M. J., Danaei, G., Lin, J. K., Paciorek, C. J., Singh, G. M., 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. *Lancet*, *377*(9765), 557–67.
- Flatt, J. P. (1987). Dietary fat, carbohydrate balance, and weight maintenance: effects of exercise. *American journal of clinical nutrition*, *45*(1 Suppl), 296–306.
- Flint, A., Raben, A., Blundell, J. E., & 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*, *24*(1), 38–48.
- Flint, A., Raben, A., Ersbøll, A. K., Holst, J. J., & Astrup, A. (2001). The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. *International journal of obesity and related metabolic disorders*, *25*(6), 781–92.
- Franz, M. J., VanWormer, J. J., Crain, A. L., Boucher, J. L., Histon, T., Caplan, W., Bowman, J. D., et al. (2007). Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. *Journal of the american dietetic association*, *107*(10), 1755–67.
- Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of applied physiology*, *55*(2), 628–34.

- Freese, E. C., Levine, A. S., Chapman, D. P., Hausman, D. B., & Cureton, K. J. (2011). Effects of acute sprint interval cycling and energy replacement on postprandial lipemia. *Journal of applied physiology*, *111*(6), 1584–9.
- Gatta, B., Zuberbuehler, C., Arnold, M., Aubert, R., Langhans, W., & Chapelot, D. (2009). Acute effects of pharmacological modifications of fatty acid metabolism on human satiety. *British journal of nutrition*, *101*(12), 1867–77.
- George, V. A., & Morganstein, A. (2003). Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite*, *40*(1), 43–6.
- Ghigo, E., Broglio, F., Arvat, E., Maccario, M., Papotti, M., & Muccioli, G. (2005). Ghrelin: more than a natural GH secretagogue and/or an orexigenic factor. *Clinical endocrinology*, *62*(1), 1–17.
- Gibala, M. J., Little, J. P., Macdonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *Journal of physiology*, *590*(Pt 5), 1077–84.
- Gibbons, C., Caudwell, P., Finlayson, G., Webb, D-L., Hellström, P. M., Näslund, E., & Blundell, J. E. (2013). Comparison of postprandial profiles of ghrelin, active GLP-1, and total PYY to meals varying in fat and carbohydrate and their association with hunger and the phases of satiety. *Journal of clinical endocrinology and metabolism*.
- Gibney, M.J., Lanham-New, S.A., Cassidy, A., & Vorster H.H. (2009). *Introduction to human nutrition (second edition)*. Wiley-Blackwell, Oxford UK.
- Gil, S. M., Yazaki, E., & Evans, D. F. (1998). Aetiology of running-related gastrointestinal dysfunction. How far is the finishing line? *Sports medicine*, *26*(6), 365–78.
- Gonzalez, J. T., Veasey, R. C., Rumbold, P. L. S., & Stevenson, E. J. (2013). Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *British journal of nutrition*, 1–12.
- Gregersen, N. T., Flint, A., Bitz, C., Blundell, J. E., Raben, A., & Astrup, A. (2008). Reproducibility and power of ad libitum energy intake assessed by repeated single meals. *American journal of clinical nutrition*, *87*(5), 1277–81.

- Guo, Y., Ma, L., Enriori, P. J., Koska, J., Franks, P. W., Brookshire, T., Cowley, M. A., et al. (2006). Physiological evidence for the involvement of peptide YY in the regulation of energy homeostasis in humans. *Obesity*, *14*(9), 1562–70.
- Hagberg, J. M., Hickson, R. C., McLane, J. A., Ehsani, A. A., & Winder, W. W. (1979). Disappearance of norepinephrine from the circulation following strenuous exercise. *Journal of applied physiology*, *47*(6), 1311–4.
- Hagberg, J. M., Mullin, J. P., & Nagle, F. J. (1980). Effect of work intensity and duration on recovery O<sub>2</sub>. *Journal of applied physiology*, *48*(3), 540–4.
- Hagobian, T. A., Yamashiro, M., Hinkel-Lipsker, J., Streder, K., Evero, N., & Hackney, T. (2013). Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. *Applied physiology, nutrition, and metabolism*, *38*(1), 66–72.
- Halatchev, I. G., & Cone, R. D. (2005). Peripheral administration of PYY(3-36) produces conditioned taste aversion in mice. *Cell metabolism*, *1*(3), 159–68.
- Halatchev, I. G., Ellacott, K. L. J., Fan, W., & Cone, R. D. (2004). Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. *Endocrinology*, *145*(6), 2585–90.
- Hanlon, B., Larson, M. J., Bailey, B. W., & LeCheminant, J. D. (2012). Neural response to pictures of food after exercise in normal-weight and obese women. *Medicine and science in sports and exercise*, *44*(10), 1864–70.
- Hansen, T. K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J. S., & Jørgensen, J. O. L. (2002). Weight loss increases circulating levels of ghrelin in human obesity. *Clinical endocrinology*, *56*(2), 203–6.
- Hargreaves, M., Costill, D. L., Katz, A., & Fink, W. J. (1985). Effect of fructose ingestion on muscle glycogen usage during exercise. *Medicine and science in sports and exercise*, *17*(3), 360–3.
- Harrold, J. A., Dovey, T. M., Blundell, J. E., & Halford, J. C. G. (2012). CNS regulation of appetite. *Neuropharmacology*, *63*(1), 3–17.

- Helgerud, J., Høydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., Simonsen, T., et al. (2007). Aerobic high-intensity intervals improve VO<sub>2</sub>max more than moderate training. *Medicine and science in sports and exercise*, 39(4), 665–71.
- Henderson, G. C., Fattor, J. A, Horning, M. A, Faghihnia, N., Johnson, M. L., Mau, T. L., Luke-Zeitoun, M., et al. (2007). Lipolysis and fatty acid metabolism in men and women during the postexercise recovery period. *Journal of physiology*, 584(Pt 3), 963–81.
- Heydari, M., Freund, J., & Boutcher, S. H. (2012). The effect of high-intensity intermittent exercise on body composition of overweight young males. *Journal of obesity*, 2012, 480467.
- Hill, B. R., De Souza, M. J., & Williams, N. I. (2011). Characterization of the diurnal rhythm of peptide YY and its association with energy balance parameters in normal-weight premenopausal women. *American journal of physiology. Endocrinology and metabolism*, 301(2), E409–15.
- Hoentjen, F., Hopman, W. P., & Jansen, J. B. (2001). Effect of circulating peptide YY on gallbladder emptying in humans. *Scandinavian journal of gastroenterology*, 36(10), 1086–91.
- Hopkins, M., Jeukendrup, A., King, N. A., & Blundell, J. E. (2011). The relationship between substrate metabolism, exercise and appetite control: does glycogen availability influence the motivation to eat, energy intake or food choice? *Sports medicine*, 41(6), 507–21.
- Hosoda, H., Doi, K., Nagaya, N., Okumura, H., Nakagawa, E., Enomoto, M., Ono, F., et al. (2004). Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clinical chemistry*, 50(6), 1077–80.
- Hosoda, H., Kojima, M., Matsuo, H., & Kangawa, K. (2000). Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochemical and biophysical research communications*, 279(3), 909–13.



- Howard, A. D., Feighner, S. D., Cully, D. F., Arena, J. P., Liberator, P. A., Rosenblum, C. I., Hamelin, M., et al. (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science*, *273*(5277), 974–7.
- Hubert, P., King, N. A., & 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*(1), 9–19.
- Hunter, G. R., Weinsier, R. L., Bamman, M. M., & Larson, D. E. (1998). A role for high intensity exercise on energy balance and weight control. *International journal of obesity and related metabolic disorders*, *22*(6), 489–93.
- Ikeda, J. P., Lyons, P., Schwartzman, F., & Mitchell, R. A. (2004). Self-reported dieting experiences of women with body mass indexes of 30 or more. *Journal of the american dietetic association*, *104*(6), 972–4.
- Imamura, M. (2002). Effects of surgical manipulation of the intestine on peptide YY and its physiology. *Peptides*, *23*(2), 403–7.
- Imbeault, P., Saint-Pierre, S., Alméras, N., & Tremblay, A. (1997). Acute effects of exercise on energy intake and feeding behaviour. *British journal of nutrition*, *77*(4), 511–21.
- Irving, B. A., Davis, C. K., Brock, D. W., Weltman, J. Y., Swift, D., Barrett, E. J., Gaesser, G. A., et al. (2008). Effect of exercise training intensity on abdominal visceral fat and body composition. *Medicine and science in sports and exercise*, *40*(11), 1863–72.
- Jakicic, J. M., Marcus, B. H., Lang, W., & Janney, C. (2008). Effect of exercise on 24-month weight loss maintenance in overweight women. *Archives of internal medicine*, *168*(14), 1550–9.
- Janssen, P., Vanden Berghe, P., Verschueren, S., Lehmann, A., Depoortere, I., & Tack, J. (2011). Review article: the role of gastric motility in the control of food intake. *Alimentary pharmacology & therapeutics*, *33*(8), 880–94.

- Jeacocke, N.A., & Burke, L. (2010). Methods to standardize dietary intake before performance testing. *International journal of sport nutrition and exercise metabolism*, 20, 87-103.
- Jesudason, D. R., Monteiro, M. P., McGowan, B. M. C., Neary, N. M., Park, A. J., Philippou, E., Small, C. J., et al. (2007). Low-dose pancreatic polypeptide inhibits food intake in man. *British journal of nutrition*, 97(3), 426–9.
- Jokisch, E., Coletta, A., & Raynor, H. A. (2012). Acute energy compensation and macronutrient intake following exercise in active and inactive males who are normal weight. *Appetite*, 58(2), 722–9.
- Jones, A. M., & Doust, J. (1996). Communications to the annual conference of the British Association of Sport and Exercise Sciences (BASES). *Journal of sports sciences*, 14(1), 89.
- Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., & Wakabayashi, I. (2001). Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and agouti-related protein mRNA levels and body weight in rats. *Diabetes*, 50(11), 2438–43.
- Karra, E., & Batterham, R. L. (2010). The role of gut hormones in the regulation of body weight and energy homeostasis. *Molecular and cellular endocrinology*, 316(2), 120–8.
- Karra, E., Chandarana, K., & Batterham, R. L. (2009). The role of peptide YY in appetite regulation and obesity. *Journal of physiology*, 587(Pt 1), 19–25.
- Kawano, H., Mineta, M., Asaka, M., Miyashita, M., Numao, S., Gando, Y., Ando, T., et al. (2013). Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite*, 66, 26–33.
- Keire, D. A., Mannon, P., Kobayashi, M., Walsh, J. H., Solomon, T. E., & Reeve, J. R. (2000). Primary structures of PYY, [Pro(34)]PYY, and PYY-(3-36) confer different conformations and receptor selectivity. *American journal of physiology. Gastrointestinal and liver physiology*, 279(1), G126–31.

- Kelly, P. J., Guelfi, K. J., Wallman, K. E., & Fairchild, T. J. (2012). Mild dehydration does not reduce postexercise appetite or energy intake. *Medicine and science in sports and exercise*, *44*(3), 516–24.
- Kennedy, G. C. (1953). The role of depot fat in the hypothalamic control of food intake in the rat. *Proceedings of the royal society of london. Series B, containing papers of a biological character. Royal Society*, *140*(901), 578–96.
- King, J. A., Miyashita, M., Wasse, L. K., & Stensel, D. J. (2010a). Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite*, *54*(3), 492–8.
- King, J. A., Wasse, L. K., Broom, D. R., & Stensel, D. J. (2010b). Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Medicine and science in sports and exercise*, *42*(3), 485–92.
- King, J. A., Wasse, L. K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R. L., & 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 and metabolism*, *96*(4), 1114–21.
- King, J. A., Wasse, L. K., & Stensel, D. J. (2011b). The acute effects of swimming on appetite, food intake, and plasma acylated ghrelin. *Journal of obesity*, 351628.
- King, J. A., Wasse, L. K., & Stensel, D. J. (2013). Acute exercise increases feeding latency in healthy normal weight young males but does not alter energy intake. *Appetite*, *61*(1), 45–51.
- King, N. A., & Blundell, J. E. (1995). High-fat foods overcome the energy expenditure induced by high-intensity cycling or running. *European journal of clinical nutrition*, *49*(2), 114–23.
- King, N. A., Burley, V. J., & Blundell, J. E. (1994). Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *European journal of clinical nutrition*, *48*(10), 715–24.

King, N. A., Caudwell, P., Hopkins, M., Byrne, N. M., Colley, R., Hills, A. P., Stubbs, J. R., et al. (2007). Metabolic and behavioral compensatory responses to exercise interventions: barriers to weight loss. *Obesity, 15*(6), 1373–83.

King, N. A., Caudwell, P., Hopkins, M., Stubbs, J. R., Naslund, E., & Blundell, J. E. (2009). Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. *American journal of clinical nutrition, 90*(4), 921–7.

King, N. A., Hopkins, M., Caudwell, P., Stubbs, R. J., & Blundell, J. E. (2008). Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. *International journal of obesity, 32*(1), 177–84.

King, N. A., Horner, K., Hills, A. P., Byrne, N. M., Wood, R. E., Bryant, E., Caudwell, P., et al. (2012). Exercise, appetite and weight management: understanding the compensatory responses in eating behaviour and how they contribute to variability in exercise-induced weight loss. *British journal of sports medicine, 46*(5), 315–22.

King, N. A., Lluch, A., Stubbs, R. J., & Blundell, J. E. (1997). High dose exercise does not increase hunger or energy intake in free living males. *European journal of clinical nutrition, 51*(7), 478–83.

Kissileff, H. R., Pi-Sunyer, F. X., Segal, K., Meltzer, S., & Foelsch, P. A. (1990). Acute effects of exercise on food intake in obese and nonobese women. *American journal of clinical nutrition, 52*(2), 240–5.

Kissileff, H. R., Pi-Sunyer, F. X., Thornton, J., & Smith, G. P. (1981). C-terminal octapeptide of cholecystokinin decreases food intake in man. *American journal of clinical nutrition, 34*(2), 154–60.

Klausen, B., Toubro, S., Ranneries, C., Rehfeld, J. F., Holst, J. J., Christensen, N. J., & Astrup, A. (1999). Increased intensity of a single exercise bout stimulates subsequent fat intake. *International journal of obesity and related metabolic disorders, 23*(12), 1282–7.

- Klem, M. L., Wing, R. R., McGuire, M. T., Seagle, H. M., & Hill, J. O. (1997). A descriptive study of individuals successful at long-term maintenance of substantial weight loss. *American journal of clinical nutrition*, *66*(2), 239–46.
- Kluge, M., Riedl, S., Uhr, M., Schmidt, D., Zhang, X., Yassouridis, A., & Steiger, A. (2010). Ghrelin affects the hypothalamus-pituitary-thyroid axis in humans by increasing free thyroxine and decreasing TSH in plasma. *European journal of endocrinology*, *162*(6), 1059–65.
- Koda, S., Date, Y., Murakami, N., Shimbara, T., Hanada, T., Toshinai, K., Nijjima, A., et al. (2005). The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. *Endocrinology*, *146*(5), 2369–75.
- Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*(6762), 656–60.
- Kojima, M., & Kangawa, K. (2005). Ghrelin: structure and function. *Physiological reviews*, *85*(2), 495–522.
- Kojima, S., Ueno, N., Asakawa, A., Sagiya, K., Naruo, T., Mizuno, S., & Inui, A. (2007). A role for pancreatic polypeptide in feeding and body weight regulation. *Peptides*, *28*(2), 459–63.
- Korner, J., Bessler, M., Cirilo, L. J., Conwell, I. M., Daud, A., Restuccia, N. L., & Wardlaw, S. L. (2005). Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. *Journal of clinical endocrinology and metabolism*, *90*(1), 359–65.
- Laan, D. J., Leidy, H. J., Lim, E., & 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*(6), 842–7.
- Laforgia, J., Withers, R. T., & Gore, C. J. (2006). Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *Journal of sports sciences*, *24*(12), 1247–64.

- Laforgia, J., Withers, R. T., Shipp, N. J., & Gore, C. J. (1997). Comparison of energy expenditure elevations after submaximal and supramaximal running. *Journal of applied physiology*, *82*(2), 661–6.
- Larhammar, D. (1996). Structural diversity of receptors for neuropeptide Y, peptide YY and pancreatic polypeptide. *Regulatory peptides*, *65*(3), 165–74.
- Larson-Meyer, D. E., Palm, S., Bansal, A., Austin, K. J., Hart, A. M., & Alexander, B. M. (2012). Influence of running and walking on hormonal regulators of appetite in women. *Journal of obesity*, 730409.
- le Roux, C. W., Batterham, R. L., Aylwin, S. J. B., Patterson, M., Borg, C. M., Wynne, K. J., Kent, A., et al. (2006a). Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology*, *147*(1), 3–8.
- le Roux, C. W., Aylwin, S. J. B., Batterham, R. L., Borg, C. M., Coyle, F., Prasad, V., Shurey, S., et al. (2006b). Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Annals of surgery*, *243*(1), 108–114.
- Leidy, H. J., Dougherty, K. A., Frye, B. R., Duke, K. M., & Williams, N. I. (2007). Twenty-four-hour ghrelin is elevated after calorie restriction and exercise training in non-obese women. *Obesity*, *15*(2), 446–55.
- Liddle, R. A., Goldfine, I. D., Rosen, M. S., Taplitz, R. A., & Williams, J. A. (1985). Cholecystikinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. *Journal of clinical investigation*, *75*(4), 1144–52.
- Lim, C. T., Kola, B., & Korbonits, M. (2011). The ghrelin/GOAT/GHS-R system and energy metabolism. *Reviews in endocrine & metabolic disorders*, *12*(3), 173–86.
- Lippl, F., Erdmann, J., Steiger, A., Lichter, N., Czogalla-Peter, C., Bidlingmaier, M., Tholl, S., et al. (2012). Low-dose ghrelin infusion--evidence against a hormonal role in food intake. *Regulatory peptides*, *174*(1-3), 26–31.
- Livingstone, M. B. E., & Black, A. E. (2003). Markers of the validity of reported energy intake. *Journal of nutrition*, *133* (Suppl 3), 895S–920S.

- Lluch, A., King, N. A., & Blundell, J. E. (1998). Exercise in dietary restrained women: no effect on energy intake but change in hedonic ratings. *European journal of clinical nutrition*, 52(4), 300–7.
- Luquet, S., Perez, F. A., Hnasko, T. S., & Palmiter, R. D. (2005). NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science*, 310(5748), 683–5.
- Mailman, D. (1982). Blood flow and intestinal absorption. *Federation proceedings*, 41(6), 2096–100.
- Malkova, D., McLaughlin, R., Manthou, E., Wallace, A. M., & Nimmo, M. A. (2008). Effect of moderate-intensity exercise session on preprandial and postprandial responses of circulating ghrelin and appetite. *Hormone and metabolic research*, 40(6), 410–5.
- Maraki, M., Tsofliou, F., Pitsiladis, Y. P., Malkova, D., Mutrie, N., & Higgins, S. (2005). Acute effects of a single exercise class on appetite, energy intake and mood. Is there a time of day effect? *Appetite*, 45(3), 272–8.
- Martins, C., Morgan, L., Bloom, S. R., & Robertson, M. D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *Journal of endocrinology*, 193(2), 251–8.
- Martins, C., Morgan, L., & Truby, H. (2008). A review of the effects of exercise on appetite regulation: an obesity perspective. *International journal of obesity (2005)*, 32(9), 1337–47.
- Marzullo, P., Salvadori, A., Brunani, A., Verti, B., Walker, G. E., Fanari, P., Tovaglieri, I., et al. (2008). Acylated ghrelin decreases during acute exercise in the lean and obese state. *Clinical endocrinology*, 69(6), 970–1.
- Masuda, Y., Tanaka, T., Inomata, N., Ohnuma, N., Tanaka, S., Itoh, Z., Hosoda, H., et al. (2000). Ghrelin stimulates gastric acid secretion and motility in rats. *Biochemical and biophysical research communications*, 276(3), 905–8.
- Maughan, R. J., Fallah, J., & Coyle, E. F. (2010). The effects of fasting on metabolism and performance. *British journal of sports medicine*, 44(7), 490–4.

- McGarvey, W., Jones, R., & Petersen, S. (2005). Excess post-exercise oxygen consumption following continuous and interval cycling exercise. *International journal of sport nutrition and exercise metabolism*, 15(1), 28–37.
- McGowan, M. K., Andrews, K. M., & Grossman, S. P. (1992). Chronic intrahypothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat. *Physiology & behavior*, 51(4), 753–66.
- Medbø, J. I., Mohn, A. C., Tabata, I., Bahr, R., Vaage, O., & Sejersted, O. M. (1988). Anaerobic capacity determined by maximal accumulated O<sub>2</sub> deficit. *Journal of applied physiology*, 64(1), 50–60.
- Mentlein, R. (1999). Dipeptidyl-peptidase IV (CD26)--role in the inactivation of regulatory peptides. *Regulatory peptides*, 85(1), 9–24.
- Mentlein, R., Dahms, P., Grandt, D., & Krüger, R. (1993). Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regulatory peptides*, 49(2), 133–44.
- Mifflin, M. D., St Jeor, S. T., Hill, L. A., Scott, B. J., Daugherty, S. A., & Koh, Y. O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. *American journal of clinical nutrition*, 51(2), 241–7.
- Moholdt, T. T., Amundsen, B. H., Rustad, L. A., Wahba, A., Løvø, K. T., Gullikstad, L. R., Bye, A., et al. (2009). Aerobic interval training versus continuous moderate exercise after coronary artery bypass surgery: a randomized study of cardiovascular effects and quality of life. *American heart journal*, 158(6), 1031–7.
- Morínigo, R., Moizé, V., Musri, M., Lacy, A. M., Navarro, S., Marín, J. L., Delgado, S., et al. (2006). Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. *Journal of clinical endocrinology and metabolism*, 91(5), 1735–40.
- Morris, C. J., Fullick, S., Gregson, W., Clarke, N., Doran, D., MacLaren, D., & Atkinson, G. (2010). Paradoxical post-exercise responses of acylated ghrelin and leptin during a simulated night shift. *Chronobiology international*, 27(3), 590–605.



- Morton, G. J., Cummings, D. E., Baskin, D. G., Barsh, G. S., & Schwartz, M. W. (2006). Central nervous system control of food intake and body weight. *Nature*, *443*(7109), 289–95.
- Murphy, K. G., & Bloom, S. R. (2004). Gut hormones in the control of appetite. *Experimental physiology*, *89*(5), 507–16.
- Murphy, K. G., & Bloom, S. R. (2006). Gut hormones and the regulation of energy homeostasis. *Nature*, *444*(7121), 854–9.
- Nagaya, N., Kojima, M., Uematsu, M., Yamagishi, M., Hosoda, H., Oya, H., Hayashi, Y., et al. (2001a). Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *American journal of physiology. Regulatory, integrative and comparative physiology*, *280*(5), R1483–7.
- Nagaya, N., Uematsu, M., Kojima, M., Date, Y., Nakazato, M., Okumura, H., Hosoda, H., et al. (2001b). Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation*, *104*(17), 2034–2038.
- Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., & Matsukura, S. (2001). A role for ghrelin in the central regulation of feeding. *Nature*, *409*(6817), 194–8.
- Näslund, E., Grybäck, P., Hellström, P. M., Jacobsson, H., Holst, J. J., Theodorsson, E., & Backman, L. (1997). Gastrointestinal hormones and gastric emptying 20 years after jejunioileal bypass for massive obesity. *International journal of obesity and related metabolic disorders*, *21*(5), 387–92.
- National Institute of Health (1998). Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. *Obesity research*, *6* (Suppl 2), 51S–209S.
- Neary, N. M., Druce, M. R., Small, C. J., & Bloom, S. R. (2006). Acylated ghrelin stimulates food intake in the fed and fasted states but desacylated ghrelin has no effect. *Gut*, *55*(1), 135.

- Neary, N. M., Goldstone, A. P., & Bloom, S. R. (2004). Appetite regulation: from the gut to the hypothalamus. *Clinical endocrinology*, *60*(2), 153–160.
- Neary, N. M., Small, C. J., Druce, M. R., Park, A. J., Ellis, S. M., Semjonous, N. M., Dakin, C. L., et al. (2005). Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively. *Endocrinology*, *146*(12), 5120–7.
- NHS Choices (2011). Why most of us should eat fewer calories. Available at: <http://www.nhs.uk/Livewell/Goodfood/Pages/eat-less.aspx>.
- NHS Information Centre (2012). Statistics on obesity, physical activity and diet: England, 2012. Available at: <https://catalogue.ic.nhs.uk/publications/public-health/obesity/obes-phys-acti-diet-eng-2012/obes-phys-acti-diet-eng-2012-rep.pdf>.
- Nicolaïdis, S., & Rowland, N. (1976). Metering of intravenous versus oral nutrients and regulation of energy balance. *American journal of physiology*, *231*(3), 661–8.
- Nybo, L., Sundstrup, E., Jakobsen, M. D., Mohr, M., Hornstrup, T., Simonsen, L., Bülow, J., et al. (2010). High-intensity training versus traditional exercise interventions for promoting health. *Medicine and science in sports and exercise*, *42*(10), 1951–8.
- Nygaard, R., Nielbo, S., Schwartz, T. W., & Poulsen, F. M. (2006). The PP-fold solution structure of human polypeptide YY and human PYY3-36 as determined by NMR. *Biochemistry*, *45*(27), 8350–7.
- Otto, B., Cuntz, U., Fruehauf, E., Wawarta, R., Folwaczny, C., Riepl, R. L., Heiman, M. L., et al. (2001). Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *European journal of endocrinology*, *145*(5), 669–73.
- Paik, K. H., Jin, D.K., Lee, K. H., Armstrong, L., Lee, J. E., Oh, Y. J., Kim, S., et al. (2007). Peptide YY, cholecystokinin, insulin and ghrelin response to meal did not change, but mean serum levels of insulin is reduced in children with Prader-Willi syndrome. *Journal of korean medical science*, *22*(3), 436–41.
- Paoli, A., Marcolin, G., Zonin, F., Neri, M., Sivieri, A., & Pacelli, Q. F. (2011). Exercising fasting or fed to enhance fat loss? Influence of food intake on respiratory ratio and excess postexercise oxygen consumption after a bout of endurance training. *International journal of sport nutrition and exercise metabolism*, *21*(1), 48–54.

- Pedersen, C., Lefevre, S., Peters, V., Patterson, M., Ghatei, M. A., Morgan, L. M., & Frost, G. S. (2013). Gut hormone release and appetite regulation in healthy non-obese participants following oligofructose intake. A dose-escalation study. *Appetite*, *66*, 44–53.
- Perini, R., Orizio, C., Comandè, A., Castellano, M., Beschi, M., & Veicsteinas, A. (1989). Plasma norepinephrine and heart rate dynamics during recovery from submaximal exercise in man. *European journal of applied physiology and occupational physiology*, *58*(8), 879–83.
- Perry, C. G. R., Heigenhauser, G. J. F., Bonen, A., & Spriet, L. L. (2008). High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. *Applied physiology, nutrition, and metabolism*, *33*(6), 1112–23.
- Pfluger, P. T., Kampe, J., Castaneda, T. R., Vahl, T., D'Alessio, D. A., Kruthaupt, T., Benoit, S. C., et al. (2007). Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. *Journal of clinical endocrinology and metabolism*, *92*(2), 583–8.
- Pittner, R. A., Moore, C. X., Bhavsar, S. P., Gedulin, B. R., Smith, P. A., Jodka, C. M., Parkes, D. G., et al. (2004). Effects of PYY[3-36] in rodent models of diabetes and obesity. *International journal of obesity and related metabolic disorders*, *28*(8), 963–71.
- Playford, R. J., Mehta, S., Upton, P., Rentch, R., Moss, S., Calam, J., Bloom, S., et al. (1995). Effect of peptide YY on human renal function. *American journal of physiology*, *268*(4 Pt 2), F754–9.
- Poehlman, E. T. (1989). A review: exercise and its influence on resting energy metabolism in man. *Medicine and science in sports and exercise*, *21*(5), 515–25.
- Pomerleau, M., Imbeault, P., Parker, T., & Doucet, E. (2004). Effects of exercise intensity on food intake and appetite in women. *American journal of clinical nutrition*, *80*(5), 1230–6.
- Purnell, J. Q., Cummings, D., & Weigle, D. S. (2007). Changes in 24-h area-under-the-curve ghrelin values following diet-induced weight loss are associated with loss of fat-

free mass, but not with changes in fat mass, insulin levels or insulin sensitivity. *International journal of obesity*, 31(2), 385–9.

Rakobowchuk, M., Tanguay, S., Burgomaster, K. A., Howarth, K. R., Gibala, M. J., & MacDonald, M. J. (2008). Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *American journal of physiology. Regulatory, integrative and comparative physiology*, 295(1), R236–42.

Ravussin, E., Tschöp, M., Morales, S., Bouchard, C., & Heiman, M. L. (2001). Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *Journal of clinical endocrinology and metabolism*, 86(9), 4547–51.

Richards, J. C., Johnson, T. K., Kuzma, J. N., Lonac, M. C., Schweder, M. M., Voyles, W. F., & Bell, C. (2010). Short-term sprint interval training increases insulin sensitivity in healthy adults but does not affect the thermogenic response to beta-adrenergic stimulation. *Journal of physiology*, 588(Pt 15), 2961–72.

Riediger, T., Bothe, C., Becskei, C., & Lutz, T. A. (2004). Peptide YY directly inhibits ghrelin-activated neurons of the arcuate nucleus and reverses fasting-induced c-Fos expression. *Neuroendocrinology*, 79(6), 317–26.

Rognmo, Ø., Hetland, E., Helgerud, J., Hoff, J., & Slørdahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *European journal of cardiovascular prevention and rehabilitation*, 11(3), 216–22.

Ross, R., Berentzen, T., Bradshaw, A. J., Janssen, I., Kahn, H. S., Katzmarzyk, P. T., Kuk, J. L., et al. (2008). Does the relationship between waist circumference, morbidity and mortality depend on measurement protocol for waist circumference? *Obesity reviews*, 9(4), 312–25.

Royal College of Physicians (2013). Action on obesity: Comprehensive care for all. Available at: <http://www.rcplondon.ac.uk/sites/default/files/action-on-obesity.pdf>.

Russel, R. R., Willis, K. S., Ravussin, E., & Larson-Meyer, E. D. (2009). Effects of endurance running and dietary fat on circulating ghrelin and peptide YY. *Journal of sports science & medicine*, 8(4), 574–583.

- Sahu, A. (2003). Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. *Frontiers in neuroendocrinology*, 24(4), 225–53.
- Sato, T., Nakamura, Y., Shiimura, Y., Ohgusu, H., Kangawa, K., & Kojima, M. (2012). Structure, regulation and function of ghrelin. *Journal of biochemistry*, 151(2), 119–28.
- Satou, M., Nishi, Y., Yoh, J., Hattori, Y., & Sugimoto, H. (2010). Identification and characterization of acyl-protein thioesterase 1/lysophospholipase I as a ghrelin deacylation/lysophospholipid hydrolyzing enzyme in fetal bovine serum and conditioned medium. *Endocrinology*, 151(10), 4765–75.
- Savage, A. P., Adrian, T. E., Carolan, G., Chatterjee, V. K., & Bloom, S. R. (1987). Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut*, 28(2), 166–70.
- Scharrer, E., & Langhans, W. (1986). Control of food intake by fatty acid oxidation. *American journal of physiology*, 250(6 Pt 2), R1003–6.
- Scheid, J. L., De Souza, M. J., Leidy, H. J., & Williams, N. I. (2011). Ghrelin but not peptide YY is related to change in body weight and energy availability. *Medicine and science in sports and exercise*, 43(11), 2063–71.
- Schwartz, M. W., & Porte, D. (2005). Diabetes, obesity, and the brain. *Science*, 307(5708), 375–9.
- Schwartz, M. W., Woods, S. C., Porte, D., Seeley, R. J., & Baskin, D. G. (2000). Central nervous system control of food intake. *Nature*, 404(6778), 661–71.
- Scott, C. B., Roby, F. B., Lohman, T. G., & Bunt, J. C. (1991). The maximally accumulated oxygen deficit as an indicator of anaerobic capacity. *Medicine and science in sports and exercise*, 23(5), 618–24.
- Scott, V., Kimura, N., Stark, J. A., & Luckman, S. M. (2005). Intravenous peptide YY3-36 and Y2 receptor antagonism in the rat: effects on feeding behaviour. *Journal of neuroendocrinology*, 17(7), 452–7.
- Shi, Y.C., Hämmerle, C. M., Lee, I.C. J., Turner, N., Nguyen, A. D., Riepler, S. J., Lin, S., et al. (2012). Adult-onset PYY overexpression in mice reduces food intake and increases lipogenic capacity. *Neuropeptides*, 46(4), 173–82.

Shiia, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M. S., Tanaka, M., Nozoe, S.I., et al. (2002). Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *Journal of clinical endocrinology and metabolism*, 87(1), 240–4.

Shorten, A. L., Wallman, K. E., & Guelfi, K. J. (2009). Acute effect of environmental temperature during exercise on subsequent energy intake in active men. *American journal of clinical nutrition*, 90(5), 1215–21.

Siri, W. E. (1956). The gross composition of the body. *Advances in biological and medical physics*, 4, 239–80.

Sloth, B., Holst, J. J., Flint, A., Gregersen, N. T., & Astrup, A. (2007). Effects of PYY1-36 and PYY3-36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects. *American journal of physiology. Endocrinology and metabolism*, 292(4), E1062–8.

St Jeor, S. T., Brunner, R. L., Harrington, M. E., Scott, B. J., Daugherty, S. A., Cutter, G. R., Brownell, K. D., et al. (1997). A classification system to evaluate weight maintainers, gainers, and losers. *Journal of the american dietetic association*, 97(5), 481–8.

Stewart, W. K., & Fleming, L. W. (1973). Features of a successful therapeutic fast of 382 days' duration. *Postgraduate medical journal*, 49(569), 203–9.

Stock, S., Lechner, P., Wong, A. C. K., Ghatei, M. A., Kieffer, T. J., Bloom, S. R., & Chanoine, J.P. (2005). Ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents. *Journal of clinical endocrinology and metabolism*, 90(4), 2161–8.

Stoeckel, L. E., Weller, R. E., Giddings, M., & Cox, J. E. (2008). Peptide YY levels are associated with appetite suppression in response to long-chain fatty acids. *Physiology & behavior*, 93(1-2), 289–95.

Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Rowley, E., Reid, C., Elia, M., Stratton, R., et al. (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. *British journal of nutrition*, 84(4), 405–15.

Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Whybrow, S., Horgan, G. W., King, N., & Blundell, J. (2004). Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. *American journal of physiology. Regulatory, integrative and comparative physiology*, 286(2), R350–8.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of psychosomatic research*, 29(1), 71–83.

Sun, Y., Butte, N. F., Garcia, J. M., & Smith, R. G. (2008). Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology*, 149(2), 843–50.

Talanian, J. L., Galloway, S. D. R., Heigenhauser, G. J. F., Bonen, A., & Spriet, L. L. (2007). Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *Journal of applied physiology*, 102(4), 1439–47.

Talanian, J. L., Holloway, G. P., Snook, L. A, Heigenhauser, G. J. F., Bonen, A., & Spriet, L. L. (2010). Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle. *American journal of physiology. Endocrinology and metabolism*, 299(2), E180–8.

Talsania, T., Anini, Y., Siu, S., Drucker, D. J., & Brubaker, P. L. (2005). Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. *Endocrinology*, 146(9), 3748–56.

Tatemoto, K., & Mutt, V. (1980). Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature*, 285(5764), 417–8.

The Daily Telegraph. (2009). Health warning: exercise makes you fat. By R. Gray, August 25, 2009. Available at: <http://www.telegraph.co.uk/science/6083234/Health-warning-exercise-makes-you-fat.html>.

- Thompson, D. A., Wolfe, L. A., & Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medicine and science in sports and exercise*, 20(3), 222–7.
- Time Magazine. (2009). Why exercise won't make you thin. By J. Cloud, August 9, 2009. Available at: <http://www.time.com/time/health/article/0,8599,1914857,00.html>.
- Tjønnå, A. E., Lee, S. J., Rognmo, Ø., Stølen, T. O., Bye, A., Haram, P. M., Loennechen, J. P., et al. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation*, 118(4), 346–54.
- Toshinai, K., Kawagoe, T., Shimbara, T., Tobina, T., Nishida, Y., Mondal, M. S., Yamaguchi, H., et al. (2007). Acute incremental exercise decreases plasma ghrelin level in healthy men. *Hormone and metabolic research*, 39(11), 849–51.
- Track, N. S., McLeod, R. S., & Mee, A. V. (1980). Human pancreatic polypeptide: studies of fasting and postprandial plasma concentrations. *Canadian journal of physiology and pharmacology*, 58(12), 1484–9.
- Trapp, E. G., Chisholm, D. J., Freund, J., & Boutcher, S. H. (2008). The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *International journal of obesity*, 32(4), 684–91.
- Tremblay, A., Simoneau, J. A., & Bouchard, C. (1994). Impact of exercise intensity on body fatness and skeletal muscle metabolism. *Metabolism*, 43(7), 814–8.
- Tremblay, A., & Therrien, F. (2006). Physical activity and body functionality: implications for obesity prevention and treatment. *Canadian journal of physiology and pharmacology*, 84(2), 149–56.
- Trost, S. G., Owen, N., Bauman, A. E., Sallis, J. F., & Brown, W. (2002). Correlates of adults' participation in physical activity: review and update. *Medicine and science in sports and exercise*, 34(12), 1996–2001.
- Tschöp, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature*, 407(6806), 908–13.



- Tschöp, M., Weyer, C., Tataranni, P. A., Devanarayan, V., Ravussin, E., & Heiman, M. L. (2001). Circulating ghrelin levels are decreased in human obesity. *Diabetes*, *50*(4), 707–9.
- Tsilchorozidou, T., Batterham, R. L., & Conway, G. S. (2008). Metformin increases fasting plasma peptide tyrosine tyrosine (PYY) in women with polycystic ovarian syndrome (PCOS). *Clinical endocrinology*, *69*(6), 936–42.
- Tsofliou, F., Pitsiladis, Y. P., Malkova, D., Wallace, A. M., & Lean, M. E. J. (2003). Moderate physical activity permits acute coupling between serum leptin and appetite-satiety measures in obese women. *International journal of obesity and related metabolic disorders*, *27*(11), 1332–9.
- Turton, M. D., O’Shea, D., Gunn, I., Beak, S. A., Edwards, C. M., Meeran, K., Choi, S. J., et al. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature*, *379*(6560), 69–72.
- Tymitz, K., Engel, A., McDonough, S., Hendy, M. P., & Kerlakian, G. (2011). Changes in ghrelin levels following bariatric surgery: review of the literature. *Obesity surgery*, *21*(1), 125–30.
- Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T., & 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*(3), 357–64.
- Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., & Fujimoto, S. (2009b). Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *Journal of endocrinology*, *201*(1), 151–9.
- Unick, J. L., Otto, A. D., Goodpaster, B. H., Helsel, D. L., Pellegrini, C. A., & Jakicic, J. M. (2010). Acute effect of walking on energy intake in overweight/obese women. *Appetite*, *55*(3), 413–9.
- Van den Hoek, A. M., Heijboer, A. C., Voshol, P. J., Havekes, L. M., Romijn, J. A., Corssmit, E. P. M., & Pijl, H. (2007). Chronic PYY3-36 treatment promotes fat oxidation and ameliorates insulin resistance in C57BL6 mice. *American journal of physiology. Endocrinology and metabolism*, *292*(1), E238–45.

- Verdich, C., Flint, A., Gutzwiller, J. P., Näslund, E., Beglinger, C., Hellström, P. M., Long, S. J., et al. (2001). A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *Journal of clinical endocrinology and metabolism*, 86(9), 4382–9.
- Verger, P., Lanteaume, M. T., & Louis-Sylvestre, J. (1994). Free food choice after acute exercise in men. *Appetite*, 22(2), 159–64.
- Vestergaard, E. T., Dall, R., Lange, K. H. W., Kjaer, M., Christiansen, J. S., & Jorgensen, J. O. L. (2007). The ghrelin response to exercise before and after growth hormone administration. *Journal of clinical endocrinology and metabolism*, 92(1), 297–303.
- Votruba, S. B., Atkinson, R. L., Hirvonen, M. D., & Schoeller, D. A. (2002). Prior exercise increases subsequent utilization of dietary fat. *Medicine and science in sports and exercise*, 34(11), 1757–65.
- Vrang, N., Madsen, A. N., Tang-Christensen, M., Hansen, G., & Larsen, P. J. (2006). PYY(3-36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity. *American journal of physiology. Regulatory, integrative and comparative physiology*, 291(2), R367–75.
- Wade, G. N., & Jones, J. E. (2004). Neuroendocrinology of nutritional infertility. *American journal of physiology. Regulatory, integrative and comparative physiology*, 287(6), R1277–96.
- Warburton, D. E. R., McKenzie, D. C., Haykowsky, M. J., Taylor, A., Shoemaker, P., Ignaszewski, A. P., & Chan, S. Y. (2005). Effectiveness of high-intensity interval training for the rehabilitation of patients with coronary artery disease. *American journal of cardiology*, 95(9), 1080–4.
- Warren, A., Howden, E. J., Williams, A. D., Fell, J. W., & Johnson, N. A. (2009). Postexercise fat oxidation: effect of exercise duration, intensity, and modality. *International journal of sport nutrition and exercise metabolism*, 19(6), 607–23.
- Wasse, L. K., Sunderland, C., King, J. A., Batterham, R. L., & Stensel, D. J. (2012). Influence of rest and exercise at a simulated altitude of 4,000 m on appetite, energy

intake, and plasma concentrations of acylated ghrelin and peptide YY. *Journal of applied physiology*, 112(4), 552–9.

Wasse, L. K., Sunderland, C., King, J. A., Miyashita, M., & Stensel, D. J. (2013). 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), 1–6.

Weigle, D. S., Cummings, D. E., Newby, P. D., Breen, P. A., Frayo, R. S., Matthys, C. C., Callahan, H. S., et al. (2003). Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet. *Journal of clinical endocrinology and metabolism*, 88(4), 1577–86.

West, D. B., Fey, D., & Woods, S. C. (1984). Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *American journal of physiology*, 246(5 Pt 2), R776–87.

Westerberp-Plantenga, M. S., Verwegen, C. R., Ijedema, M. J., Wijckmans, N. E., & Saris, W. H. (1997). Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiology & behavior*, 62(6), 1345–54.

Whyte, L. J., Gill, J. M. R., & Cathcart, A. J. (2010). Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. *Metabolism*, 59(10), 1421–8.

Wisløff, U., Støylen, A., Loennechen, J. P., Bruvold, M., Rognum, Ø., Haram, P. M., Tjønnå, A. E., et al. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation*, 115(24), 3086–94.

Woods, S. C., & Seeley, R. J. (1998). Dietary interventions in noninsulin-dependent diabetes mellitus: new approaches. *Nutrition*, 14(6), 527–8.

World Health Organisation (2009). Global health risks. Available at: [http://www.who.int/healthinfo/global\\_burden\\_disease/GlobalHealthRisks\\_report\\_full.pdf](http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf).

- Wortley, K. E., Del Rincon, J.P., Murray, J. D., Garcia, K., Iida, K., Thorner, M. O., & Sleeman, M. W. (2005). Absence of ghrelin protects against early-onset obesity. *Journal of clinical investigation*, *115*(12), 3573–8.
- Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., Dhillo, W. S., et al. (2001). Ghrelin enhances appetite and increases food intake in humans. *Journal of clinical endocrinology and metabolism*, *86*(12), 5992.
- Wren, A. M., Small, C. J., Ward, H. L., Murphy, K. G., Dakin, C. L., Taheri, S., Kennedy, A. R., et al. (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology*, *141*(11), 4325–8.
- Wynne, K., Stanley, S., McGowan, B., & Bloom, S. (2005a). Appetite control. *Journal of endocrinology*, *184*(2), 291–318.
- Wynne, K., Park, A. J., Small, C. J., Patterson, M., Ellis, S. M., Murphy, K. G., Wren, A. M., et al. (2005b). Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes*, *54*(8), 2390–5.
- Wynne, K., Park, A. J., Small, C. J., Meeran, K., Ghatei, M. A., Frost, G. S., & Bloom, S. R. (2006). Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *International journal of obesity*, *30*(12), 1729–36.
- Yamamoto, H., Kishi, T., Lee, C. E., Choi, B. J., Fang, H., Hollenberg, A. N., Drucker, D. J., et al. (2003). Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. *Journal of neuroscience*, *23*(7), 2939–46.
- Yang, J., Brown, M. S., Liang, G., Grishin, N. V., & Goldstein, J. L. (2008). Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell*, *132*(3), 387–96.
- Zhang, T., Uchida, T., Gomez, G., Lluís, F., Thompson, J. C., & Greeley, G. H. (1993). Neural regulation of peptide YY secretion. *Regulatory peptides*, *48*(3), 321–8.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505), 425–32.

Zhao, T.J., Liang, G., Li, R. L., Xie, X., Sleeman, M. W., Murphy, A. J., Valenzuela, D. M., et al. (2010). Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proceedings of the national academy of sciences of the united states of america*, 107(16), 7467–72.

Zigman, J. M., Nakano, Y., Coppari, R., Balthasar, N., Marcus, J. N., Lee, C. E., Jones, J. E., et al. (2005). Mice lacking ghrelin receptors resist the development of diet-induced obesity. *Journal of clinical investigation*, 115(12), 3564–72.

## APPENDIX A

### INFORMED CONSENT FORM (to be completed after Participant Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Advisory Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my participation.

I understand that I am under no obligation to take part in the study.

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers.

I agree to participate in this study.

Your name \_\_\_\_\_

Your signature \_\_\_\_\_

Signature of investigator \_\_\_\_\_

Date \_\_\_\_\_

## APPENDIX B

### Health Screen Questionnaire for Study Volunteers

As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

**Please complete this brief questionnaire to confirm your fitness to participate:**

**1. At present**, do you have any health problem for which you are:

- |  |     |                          |    |                          |
|--|-----|--------------------------|----|--------------------------|
| (a) on medication, prescribed or otherwise ..... | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (b) attending your general practitioner .....    | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (c) on a hospital waiting list .....             | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |

**2. In the past two years**, have you had any illness which required you to:

- |   |     |                          |    |                          |
|---|-----|--------------------------|----|--------------------------|
| (a) consult your GP .....                         | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (b) attend a hospital outpatient department ..... | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (c) be admitted to hospital .....                 | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |

**3. Have you ever** had any of the following:

- |   |     |                          |    |                          |
|---|-----|--------------------------|----|--------------------------|
| (a) Convulsions/epilepsy .....                | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (b) Asthma .....                              | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (c) Eczema .....                              | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (d) Diabetes .....                            | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (e) A blood disorder .....                    | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (f) Head injury .....                         | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (g) Digestive problems .....                  | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (h) Heart problems .....                      | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (i) Problems with bones or joints .....       | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (j) Disturbance of balance/coordination ..... | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (k) Numbness in hands or feet .....           | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (l) Disturbance of vision .....               | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (m) Ear / hearing problems .....              | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (n) Thyroid problems .....                    | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| (o) Kidney or liver problems .....            | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |

(p) Allergy to nuts ..... Yes  No

4. **Has any**, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? ..... Yes  No

**If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)**

.....  
.....  
.....

5. Allergy Information

(a) are you allergic to any food products? Yes  No   
(b) are you allergic to any medicines? Yes  No   
(c) are you allergic to plasters? Yes  No

**If YES to any of the above, please provide additional information on the allergy**

.....  
.....

6. Please provide contact details of a suitable person for us to contact in the event of any incident or emergency.

Name:

.....  
.....

Telephone Number:

.....  
.....

Work  Home  Mobile

Relationship to

Participant:.....  
.....

7. Are you currently involved in any other research studies at the University or elsewhere?

Yes  No

If yes, please provide details of the study

.....  
.....  
.....



## APPENDIX C

### PHYSICAL ACTIVITY QUESTIONNAIRE

During one week, how many times on average do you do the following kinds of exercise for more than 15 minutes?

(a) **Strenuous exercise** (heart beats rapidly)

For example; running, jogging, squash, hockey, football, volleyball, vigorous swimming, vigorous long distance cycling.

\_\_\_\_\_times per week.

(b) **Moderate exercise** (not exhausting)

For example; fast walking, tennis, easy cycling, badminton, easy swimming, dancing.

\_\_\_\_\_ times per week.

(c) **Mild exercise** (minimal effort)

For example; yoga, archery, fishing, bowling, golf, easy walking.

\_\_\_\_\_ times per week.

## APPENDIX D

### Part 1: please answer true/false

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

- 14. I have a pretty good idea of the number of calories in common food**  
True  False
- 15. Sometimes when I start eating, I just can't seem to stop**  
True  False
- 16. It is not difficult for me to leave something on my plate**  
True  False
- 17. At certain times of the day, I get hungry because I have gotten used to eating then**  
True  False
- 18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it**  
True  False
- 19. Being with someone who is eating often makes me hungry enough to eat also**  
True  False
- 20. When I feel blue, I often overeat**  
True  False
- 21. I enjoy eating too much to spoil it by counting calories or watching my weight**  
True  False
- 22. When I see a real delicacy I often get so hungry that I have to eat it right away**  
True  False
- 23. I often stop eating when I am not really full as a conscious means of limiting what I eat**  
True  False
- 24. I get so hungry that my stomach often feels like a bottomless pit**  
True  False
- 25. My weight has hardly changed at all in the last ten years**  
True  False
- 26. I am always hungry so it is hard for me to stop eating before I finish all the food on my plate**  
True  False
- 27. When I feel lonely, I console myself by eating**  
True  False
- 28. I consciously hold back at meals in order not to gain weight**  
True  False

**29. I sometimes get very hungry late in the evening or at night**

True  False

**30. I eat anything I want, anytime I want**

True  False

**31. Without even thinking about it, I take a long time to eat**

True  False

**32. I count calories as a conscious means of controlling my weight**

True  False

**33. I do not eat some foods because they make me fat**

True  False

**34. I am always hungry enough to eat at any time**

True  False

**35. I pay a great deal of attention to changes in my figure**

True  False

**36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods**

True  False

**Part 2:**

**37. How often are you dieting in a conscious effort to control your weight?**

1 (rarely) 2(sometimes) 3(usually) 4(always)

**38. Would a weight fluctuation of 5 lbs affect the way you live your life?**

1(not at all) 2(lightly) 3(moderately) 4(very much)

**39. How often do you feel hungry?**

1(only at meal times) 2(sometimes between meals) 3(often between meals) 4(almost always)

**40. Do your feelings of guilt about overeating help you to control your food intake?**

1(never) 2(rarely) 3(often) 4(always)

**41. How difficult would it be for you to stop eating half way through dinner and not eat again for four hours?**

1(easy) 2(lightly difficult) 3(moderately difficult) 4(very difficult)

**42. How conscious are you of what you are eating?**

1(not at all) 2(lightly) 3(moderately) 4(extremely)

**43. How frequently do you avoid 'stocking up' on tempting foods?**

1 (almost never) 2(seldom) 3(usually) 4(almost always)

**44. How likely are you to shop for low calorie foods?**

1(unlikely) 2(slightly unlikely) 3(moderately likely) 4(very likely)

**45. Do you eat sensibly in front of others and splurge alone?**

1(never) 2(rarely) 3(often) 4(always)

**46. How likely are you to consciously eat slowly in order to cut down on how much you eat?**

1(unlikely) 2(slightly likely) 3(moderately likely) 4(very likely)

**47. How frequently do you skip desert because you are no longer hungry?**

1(almost never) 2(seldom) 3(at least once a week) 4(almost every day)

**48. How likely are you to consciously eat less than you want?**

1(unlikely) 2(slightly likely) 3(moderately likely) 4(very likely)

**49. Do you go on eating binges though you are not hungry?**

1(never) 2(rarely) 3(sometimes) 4(at least once a week)

**50. On a scale of 0-5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?**

0

Eat whatever you want, whenever you want it

1

Usually eat whatever you want, whenever you want it

2

Often eat whatever you want, whenever you want it

3

Often limit food intake, but often 'give in'

4

Usually limit food intake, rarely 'give in'

5

Constantly limiting food intake, never 'give in'

**51. To what extent does this statement describe your eating behaviour? 'I start dieting in the morning, but because of a number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'**

1(not like me) 2(little like me) 3(pretty good description of me) 4(describes me perfectly)

## APPENDIX E

### Food Preference Questionnaire (Chapter 4)

#### Breakfast

Orange Juice  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

White Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Margarine  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Strawberry Jam  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Banana  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

#### Buffet meals

Frosties  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Corn Flakes  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Co-co Pops  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Milk  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange Juice  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

White Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Brown Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cheese  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ham  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tuna  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Banana  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Apple  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ready Salted Crisps  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Butter  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Margarine  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mayonnaise  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Nutri-grain  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mars bar  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cookies  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Muffins  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Choc-Chip Muffins  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mini-rolls  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

## APPENDIX F

### Food Preference Questionnaire (Chapter 5)

#### Breakfast

Orange Juice  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

White Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Margarine  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Strawberry Jam  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Banana  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

#### Cold buffet meal

Frosties  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Corn Flakes  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Co-co Pops  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Milk  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange Juice  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

White Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Brown Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cheese  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ham  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tuna  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Banana  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)



Apple  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ready Salted Crisps  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Butter  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Margarine  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mayonnaise  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Nutri-grain  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mars bar  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cookies  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Muffins  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Choc-Chip Muffins  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mini-rolls  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

**Hot buffet meal**

Pasta  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tomato Pasta Sauce  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chicken  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

White Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Brown Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Cheese

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Raspberry Jelly

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Rice Pudding

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Strawberry Yoghurt

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Raspberry Yoghurt

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Black Cherry Yoghurt

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Milk

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange Juice

(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

## APPENDIX G

### Food Preference Questionnaire (Chapters 6 & 7)

#### Breakfast

White Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Margarine  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Strawberry Jam  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Banana  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Orange Juice  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

#### Lunch

White Bread  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tuna  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Mayonnaise  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Ready Salted Crisps  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Apple  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Chocolate Muffin  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

#### Ad libitum meal

Pasta  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

Tomato Pasta Sauce  
(Dislike extremely) 1 2 3 4 5 6 7 8 9 10 (Like extremely)

## APPENDIX H

Subject Number: \_\_\_\_\_ Trial \_\_\_\_\_ Date: \_\_\_\_\_

<p><b>Visual Analogue Scale</b></p> <p>Time: _____</p>
--

Place a mark on the horizontal lines below after considering the following questions:

I am not hungry at all | **How hungry do you feel?** | I have never been more hungry

I am completely empty | **How satisfied do you feel?** | I cannot eat another bite

Not at all full | **How full do you feel?** | Totally full

Nothing at all | **How much do you think you can eat?** | A lot