Apparatus-free, high-intensity intermittent training (HIIT) for the regulation of appetite, energy balance and metabolic health in inactive, overweight and obese females

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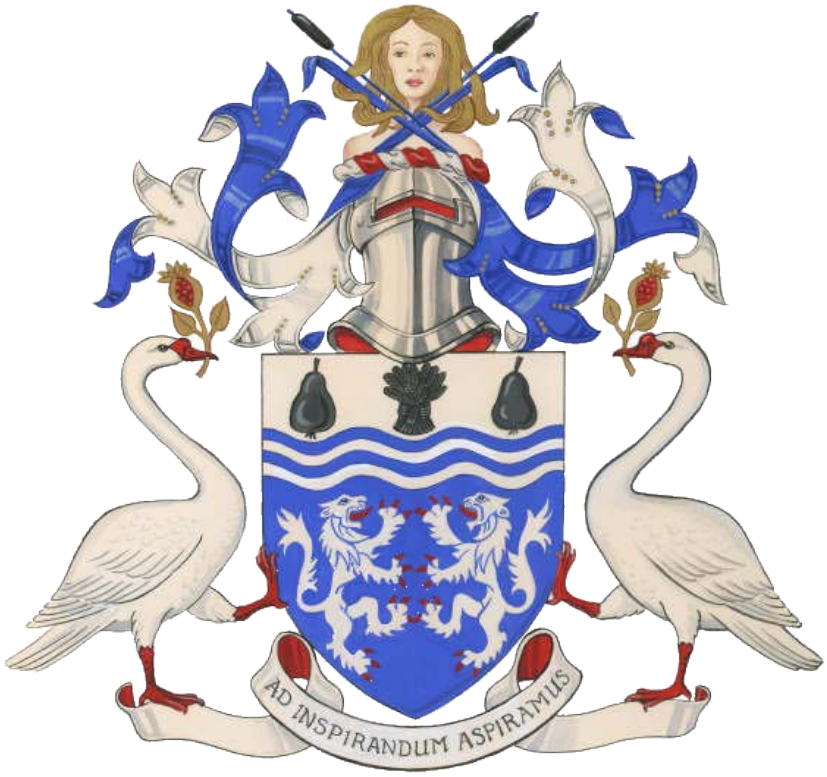

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A. E. Burgin

A thesis submitted in partial fulfilment of the University's requirements for the Degree of Doctor of Philosophy

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University of Worcester

DECLARATION

I declare that this thesis is a presentation of my own original research work and all the written work and investigations are entirely my own. Wherever contributions of others are involved, this is clearly acknowledged and referenced.

I declare that no portion of the work referred to in this thesis has been submitted for another degree or qualification of any comparable award at this or any other university or other institution of learning.

Signed 

Date 27/09/2019

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PUBLICATIONS AND COMMUNICATIONS

Conference presentations

- Burgin, A., Blannin, A.K., Peters, D.M. & Holliday, A. (2017) Acute physiological, affective and enjoyment responses to apparatus-free protocols of high-intensity intermittent exercise in inactive females. *Annual meeting of Health Enhancing Physical Activity Europe*, *Zagreb,* 15–17th November. (Study 1, oral presentation)

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Unrelated publications

- Holliday, A., Burgin, A., Fenton, S.A.M., Vargas-Fernandez, E., & Blannin, A.K. (2018) 'Points-based physical activity: a novel approach to facilitate changes in body composition in inactive women with overweight and obesity', *BMC Public Health*, 18(261), pp. 1-13.

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LIST OF ABBREVIATIONS

µlU	micro international units
%kcal	percentage of kilocalories
%LI	percentage of time spent in light-intensity physical activity
%MOD	percentage of time spent in moderate-intensity physical activity
%SED	percentage of time spent sedentary
%VIG	percentage of time spent in vigorous-intensity physical activity
%V-VIG	percentage of time spent in very vigorous-intensity physical activity
ΔAG	change in acylated ghrelin concentration from rest
ΔGLP-1	change in glucagon-like peptide 1 concentration from rest
ΔPV	change in plasma volume from rest
ΔPYY	change in peptide YY concentration from rest
95% CI	95 percent confidence interval
ACSM	American College of Sports Medicine
AEE	activity energy expenditure
AG	acylated ghrelin
AMPK	5'-AMP-activated protein kinase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AUC	area under the curve
BLa	blood lactate concentration
BMI	body mass index
BMR	basal metabolic rate
CCK	Cholecystokinin
CHO	carbohydrate
CO_2	carbon dioxide
COEQ	Control of Eating Questionnaire (Hill, Weaver and Blundell, 1991)
CV	coefficient of variation
DEBQ	Dutch Eating Behaviour Questionnaire (van Strien *et al.*, 1986a)
EDTA	ethylenediaminetetraacetic acid

EE	energy expenditure
EI	energy intake
ELISA	enzyme-linked immunosorbent assay
FAT	fat
FFM	fat-free mass
FS	Feeling scale (Hardy and Rejeski, 1989)
GLP-1	glucagon-like peptide 1
HbA1c	glycated haemoglobin
HDL-C	high-density lipoprotein cholesterol
HIFT	high-intensity functional training
HIIE	high-intensity intermittent exercise
HIIT	high-intensity intermittent training
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HR	heart rate
HR_{max}/HR_{peak}	maximum/peak heart rate
IPAQ	International Physical Activity Questionnaire (Craig *et al.*, 2003)
ISAK	International Society for the Advancement of Kinanthropometry
IV	intravenous
kcal	kilocalories
kg	kilograms
L	litre(s)
LDL-C	low-density lipoprotein cholesterol
M	molar
min	minute(s)
mL	millilitres
mmol	millimoles
MET	metabolic equivalent(s) of task
MVPA	moderate-vigorous-intensity physical activity
NEFA	non-esterified fatty acids
O_2	oxygen
PA	physical activity

PACES	Physical Activity Enjoyment Scale (Kendzierski and DeCarlo, 1991)
PANAS	Positive and Negative Affect Scale (Watson, Clark and Tellegen, 1988)
PAR-Q	Physical Activity Readiness Questionnaire (Thomas, Reading and Shephard, 1992)
PAR-Q+	Physical Activity Readiness Questionnaire for Everyone (Warburton *et al.*, 2011)
pg	picograms
pM	picomolar
PP	Pancreatic polypeptide
PPO	peak power output
PRO	protein
PYY	peptide YY
RER	respiratory exchange ratio
RPE	rating of perceived exertion
RPM	revolutions per minute
SIT	sprint interval training
SPSS	Statistical Package for Social Sciences
TAG	triglycerides
TC	total cholesterol
TEE	total energy expenditure
TTE	time to exhaustion
VAS	Visual Analogue Scale (Hill and Blundell, 1982)
VCO_2	volume of carbon dioxide
VO_2	volume of oxygen
VO_{2peak}	maximum rate of oxygen consumption measured
VO_{2max}	peak rate of oxygen consumption measured
W	Watts

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Appendix 25 Acceptability of the Intervention Scale (using methods previously described by Boereboom *et al.*, 2016)

ABSTRACT

Globally, one in four males and one in three females self-report not meeting physical activity guidelines (150 minutes of moderate-intensity physical activity per week or 75 minutes of vigorous-intensity physical activity per week, or a combination of moderate and vigorous physical activity). In turn, levels of overweight and obesity continue to rise which are resultant of prolonged and repeated periods of positive energy balance, where energy intake exceeds energy expenditure.

High-intensity intermittent exercise (HIIE) consists of brief, repeated bursts of relatively intense exercise interspersed with periods of rest and, specifically, low volume HIIE protocols are those totalling ≤30 minutes. As appetite and energy intake can be transiently reduced following low volume high-intensity intermittent exercise, strategically timing such exercise bouts prior to meal times may promote energy deficit. Over the longer term, this could be of benefit to weight management in an overweight and/or obese population. However, while often perceived as time efficient, the affective and enjoyment responses to high-intensity exercise in an inactive and overweight/obese population are contentious. Other barriers to physical activity, including lack of access to apparatus and facilities need to be considered if low volume high-intensity intermittent exercise is to be adopted in a free-living setting by an inactive population. Therefore, the aim of this thesis was to assess appetite, energy balance, physiological, affective and enjoyment responses to acute and chronic apparatus-free high-intensity intermittent exercise in inactive, overweight/obese females.

Study 1 demonstrated that not only were the physiological responses to 4 x 30 seconds of “all out” star jumping (jumping jacks) comparable to 4 x 30 seconds of “all out” (maximal effort) cycling, this mode of exercise also induced preferable affective and enjoyment responses. Findings of study 2 then showed a tendency for a condition effect with on relative energy intake ($p=0.064$; $n^2_p=0.221$) such that there was a reduction in relative energy intake with a medium effect size of 121kcal following 4 x 30 seconds of “all out” star jumping compared with the resting condition ($p=0.086$; $d=0.52$). Importantly, participants were free to request to eat at any point following the condition, as opposed to energy intake being assessed at a pre-determined time point.

When 4 x 30 seconds of “all out” star jumping (jumping jacks) was undertaken twice daily on three days per week for eight weeks, either within thirty minutes (‘pre-meal’ group) or outside of one hour prior to a meal time (‘anytime group’), no differences in body mass or mean daily energy intake were found (study 3; all $p>0.05$). However, during week eight of the intervention mean daily energy intake was reduced on exercise days across both groups by a mean of 426kcal, compared with non-exercise days ($p=0.033$), although this occurred in both groups and was therefore independent of exercise timing. Compared with baseline, change in subjective appetite increased following the intervention in those undertaking exercise within thirty minutes prior to a meal ($p=0.033$), while postprandial subjective appetite decreased at 120 minutes following the standardised meal ($p=0.026$) in those undertaking exercise outside of one hour prior to a meal. In the ‘anytime’ group only, GLP AUC concentration was significantly increased by 21% following the intervention ($p=0.037$).

Regardless of exercise group, mean cardiorespiratory fitness improved by a mean of 8% ($p=0.007$) and mean waist circumference reduced by a mean of 1.3cm ($p=0.011$), highlighting possible reductions in abdominal fat. Mean hip circumference improved by a mean of 1.4cm in the ‘anytime’ group only ($p=0.04$). No changes in glycaemic control or fasted lipid profiles occurred (all $p>0.05$). Preferable enjoyment responses were seen in the ‘anytime’ group in weeks 6 ($p=0.033$), 7 ($p=0.002$) and 8 ($p=0.018$) in the group undertaking exercise outside of one hour prior to a meal.

This thesis concludes that 4 x 30 seconds of “all out” star jumping, twice a day on three days per week over eight weeks improves cardiorespiratory fitness, waist circumference and daily energy intake on exercise days regardless of exercise timing, while inducing positive affective responses. Timing exercise outside of one hour prior to meal times induces preferable modulations in postprandial subjective appetite as well as greater improvements in hip circumference and more preferable enjoyment responses. Such findings inform strategies to effectively improve markers of health and regulation of energy balance on exercising days in a manner that addresses commonly-reported barriers in inactive, overweight and obese females.

Future research is warranted, specifically for further insight into the effects of exercise timing around meal times, as well as the optimisation of low volume HIIT for health in a free-living setting that offers greater flexibility with regards to exercise mode and timing, while it could

also interrupt prolonged sedentary behaviour. Interventions that incorporate HIIE modes that also promote improvements in lean mass and muscle strength are also merited.

Chapter 1

Background and Rationale

Instances of overweight (a body mass index (BMI) of 25-29.9kg·m^{-2}) and obesity (BMI ≥30.0 kg·m^{-2}) have continued to increase globally since 1980 (Finucane *et al.*, 2011). The prevalence of overweight (including obesity) increased from 57.6% to 67.1% in males and from 48.6% to 57.2% in females between the years of 1993 and 2013 in England (Health and Social Care Information Centre, 2015). Being overweight or obese increases the risk of many potentially fatal chronic diseases including type II diabetes, coronary heart disease, stroke and several cancers (Wang *et al.*, 2011). Current trends in the UK predict 11 million further obese adults in the UK by 2030, which will instigate 545,000 further cases of type II diabetes, 331,000 further cases of coronary heart disease and stroke and 87,000 further cases of cancers (Wang *et al.*, 2011). Furthermore, for every further 5kg·m^{-2} of BMI gained, risk of mortality increases by as much as 39% for a BMI greater than 25kg·m^{-2} (Prospective Studies Collaboration, 2009).

Becoming overweight or obese results from prolonged and repeated periods of positive energy balance, with energy intake (EI) exceeding energy expenditure (EE). Weight management strategies therefore focus on reducing EI through caloric restriction and/or increasing EE through physical activity (PA) and exercise in order to prevent an energy surplus. PA is defined as any bodily movement produced by skeletal muscle that results in EE above that of resting (Caspersen, Powell and Christenson, 1985), while 'exercise' is recognised as a subset of PA with planned, structured and repetitive characteristics as well as an objective to improve or maintain fitness (Caspersen, Powell and Christenson, 1985). PA guidelines for adults recommend 150 minutes of moderate-intensity PA or 75 minutes of vigorous-intensity PA per week, or a combination of moderate and vigorous PA (Department of Health & Social Care, 2019).

Globally, 23.4% of males and 31.7% of females self-report to be physical inactivity, defined as not meeting PA guidelines (Guthold *et al.*, 2018). In the UK, 67% of men and 55% of women self-reportedly meet PA guidelines (Health and Social Care Information Centre, 2015), hence there is a clear disparity between sexes with females less active than males. Such a disparity has also been demonstrated by a recent global investigation of physical activity levels; physical inactivity is greater in females compared with males across most countries with

58.0% and 68.69% of males and females, respectively, self-reportedly not meeting recommended PA guidelines in the UK (Hallal *et al.*, 2012). Being female also predicts attrition while being male predicts adherence to regular PA (Burgess, Hassmén and Pumpa, 2017), suggesting that greater insight into both efficacious and effective physical activity strategies is required for females.

Furthermore, females are found to be significantly underrepresented in sport and exercise medicine research (Costello, Bieuzen and Bleakley, 2014). The average percentage of female participants per article (a total of 1382 articles) was 39% with the average percentage of male participants being 61% (Costello, Bieuzen and Bleakley, 2014). Additionally, observed reductions in body mass in response to exercise interventions are greater in males than female, which is suggested to be due to greater compensation in EI and reduced EE during exercise for females (Donnelly and Smith, 2005). This gives rationale to explore this population further with regard to effective energy balance manipulation with PA and exercise.

Moreover, when measured objectively through the use of accelerometers, it has been demonstrated that just ~5% of UK adults are meeting PA guidelines (Craig and Hirani, 2009). Globally, the extent of physical inactivity accounts for 9% of premature mortality (Lee *et al.*, 2012), making it the fourth largest independent cause of death worldwide (Kohl *et al.*, 2012). In the UK alone, physical inactivity is responsible for 10.5% of cases of coronary heart disease, 13% of cases of type II diabetes and 17.9% of cases of breast cancer (Lee *et al.*, 2012).

Given the risk of non-communicable disease and premature mortality associated with both physical inactivity and overweight/obesity, increasing PA is warranted in the interest of directly preventing these risks as well as attenuating an accumulation of positive energy balance that will likely lead to overweight and obesity. It is imperative that such PA strategies are not only efficacious in doing so but are also effective. In order to be effective, strategies must be informed by commonly-reported barriers to regular PA. Such barriers include a perceived lack of time (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017), a lack of access to apparatus or facilities as well as limited access to apparatus at home (Trost *et al.*, 2002; Cerin *et al.*, 2010). Furthermore, a perceived lack of time is a more prominent barrier in overweight/obese females and a lack of access to specialist facilities was a more prominent barrier in a female population (Cerin *et al.*, 2010), further demonstrating the requirement for both efficacious and effective physical activity

programmes for an overweight/obese female population that approach such barriers in order to increase PA levels.

High-intensity intermittent exercise (HIIE) consists of "brief, repeated bursts of relatively intense exercise interspersed with periods of rest" (Gillen and Gibala, 2014; p. 409) and, specifically, low volume HIIE protocols are those totalling ≤30 minutes, including recovery time. Low volume HIIE has been demonstrated to be efficacious in improving markers of metabolic health, such as cardiorespiratory fitness and insulin sensitivity (Babraj *et al.*, 2009; Cocks *et al.*, 2016; Gillen *et al.*, 2016), which have long been demonstrated strong predictors of all-cause mortality (Myers *et al.*, 2002; Kodama *et al.*, 2009; Taylor *et al.*, 2013; Mandsanger *et al.*, 2018).

Low volume HIIE is therefore potentially an effective PA strategy for improving metabolic health, given its posed time-efficiency. However, protocols typically consist of 4-7 x 30 seconds of "all-out" (maximal effort) cycling on an ergometer against set resistance, interspersed with ~3-4 minutes light-intensity recovery (Gibala, 2007). Therefore, the total time required, as well as the requirement of specialised apparatus (typically treadmills or cycle ergometers) or facilities (and the time required to travel to such facilities), arguably limits their practicality and effectiveness in a free-living environment (Gray *et al.*, 2016). This, therefore, limits the potential for HIIE to be effectively translated into a public health strategy. Low volume HIIE that does not require specialised apparatus nor facilities and that can be completed in a minimal amount of time (if benefits to health are observed) is warranted. It has also been posed that an inactive, overweight/obese population may experience negative affective and enjoyment responses to exercise at a high-intensity, which is likely to deter such a population from sustained participation in HIIE (Hardcastle *et al.*, 2014; Biddle and Batterham, 2015). Affective and enjoyment responses to HIIE protocols should therefore be assessed and considered, ideally seeking low volume HIIE protocols that promote positive responses.

In addition, it is acknowledged that the caloric expenditure of low volume HIIE protocols is, by nature, small and likely less than a typical moderate-intensity, continuous bout of exercise. Nonetheless, a growing body of evidence demonstrates a potential suppression in appetite immediately following an acute bout of exercise at a high-intensity; this is an observation originally coined as 'exercise-induced anorexia' by King, Burley and Blundell (1994). For

example, Holliday and Blannin (2017a) found a suppression of subjective and objective markers of appetite following 4 x 30 seconds of high-intensity cycling which accumulated an EE of ~152 kilocalories (kcal; total duration 17 minutes). A ~20% reduction in composite appetite score (area under the curve; AUC) was seen with exercise compared with rest, while a 42% reduction in acylated ghrelin (AG) concentration AUC was also demonstrated with exercise compared with rest (Holliday and Blannin, 2017a). Beaulieu *et al.* (2015) also estimated an EE of ~145kcal with 4 x 30 seconds of high-intensity running (total duration 25 minutes) which was followed by a suppression in both subjective and objective markers of appetite. Actual reductions in EI are less often shown, but this may be due to the design of particular studies, with EI assessed via *ad libitum* feeding at pre-determined time points after exercise which limits broader assessment of eating behaviour such as eating initiation and free-living food choice.

Should there be less of a time, apparatus and facility requirement, low volume HIIE may be an efficacious and an effective strategy to improve metabolic health. Utilisation of low volume HIIE protocols to induce an energy deficit through simultaneously increasing EE and reducing EI in an acute period of time are of interest, with regard to manipulating energy balance for effective weight management in an overweight/obese and inactive population. How any accumulated effects can then truly influence weight management, appetite and eating behaviour in the longer term, as well as effects on metabolic health, is of paramount interest. The purpose of this thesis is to explore the efficacy and effectiveness of apparatus-free HIIE on energy balance, through manipulation of both EE and EI, appetite and eating behaviour as well as on other markers of metabolic health in an inactive, overweight/obese and female population.

Chapter 2

Literature Review

2.1 Efficacy of high-intensity intermittent training to improve physical activity levels and metabolic health

Maximal oxygen uptake (VO_{2max}) is a common measure used to assess cardiorespiratory fitness. It is documented as a very strong predictor of mortality (Myers *et al.*, 2002; Kodama *et al.*, 2009; Mandsanger *et al.*, 2018), while increasing (or at least preventing decline in) VO_{2max} is associated with reductions in all-cause mortality risk (Lee *et al.*, 2011). For every 1 metabolic equivalent (MET) improvement in VO_{2max} (equal to a 3.5mL·min^{-1}·kg^{-1} increase in oxygen consumption; American College of Sports Medicine (ACSM), 2006), there is an associated 15% reduction in risk of all-cause mortality and a 19% reduction in cardiovascular disease mortality (Lee *et al.*, 2011). High-intensity intermittent training (HIIT) interventions made up of acute HIIE sessions are efficacious, and often more so than continuous, moderate-intensity exercise interventions, in inducing large improvements in cardiorespiratory fitness (Kessler, Sisson and Short, 2012; Milanovic, Sporis and Weston, 2015).

It has also been demonstrated that HIIT interventions are as, if not more, efficacious in improving markers of insulin sensitivity (fasted glucose, fasted insulin, glycated haemoglobin (HbA1c) and measures of insulin resistance) in adults compared with moderate-intensity, continuous exercise interventions (Kessler, Sisson and Short, 2012; Jellyman *et al.*, 2015). Indeed, a 23% improvement in insulin sensitivity has been seen following 4-6 x 30 seconds "all-out" laboratory-based cycling against a set resistance (three times a week for two weeks) in young, healthy men (Babraj *et al.*, 2009). This was a low volume HIIT intervention; however, the time required per session for this intervention totalled 26 minutes per session and almost 80 minutes per week, which is notably not dissimilar from current PA guidelines. Additionally, these findings were seen in young, healthy, active males. Thus, this therefore questions the effectiveness of such implications for public health as similar responses cannot simply be extrapolated to a female, inactive and/or overweight/obese population.

While such findings have been observed in lean individuals, an 11% improvement in insulin sensitivity and a 13% improvement in peak VO_2 uptake (VO_{2peak}; 33.9±1.2mL·min^{-1}·kg^{-1} to

36.3±1.6mL·min^{-1}·kg^{-1}) were also observed with 4-7 x 30 seconds "all-out" laboratory-based cycling in inactive, overweight/obese men, undertaken five times a week over just 4 weeks (Cocks *et al.*, 2016). Time requirement for sessions in the study of Cocks *et al.* (2016) was 17.5 minutes and thus offers greater time efficiency than the sessions undertaken in Babraj *et al.* (2009), although this still totalled >80 minutes per week. However, a 19% improvement in VO_{2peak} (2.6±0.8L·min^{-1} to 3.0±0.7 L·min^{-1}) over 12 weeks of 3 x 20 seconds "all-out" laboratory-based cycling (three times per week) in inactive men, alongside a 53% improvement in insulin sensitivity has also been demonstrated in this population with a time commitment of only 12 minutes per session and ~36 minutes per week (Gillen *et al.*, 2016). Similarly, a ~15% improvement in VO_{2max} (4.4±2.2mL·kg^{-1}·min^{-1} increase) was achieved in inactive and obese individuals with repeated laboratory-based cycling for 8 seconds at 85-90% maximum heart rate (HR_{max}; interspersed with 12 seconds recovery) for ~10 minutes (three times a week for twelve weeks; Martins *et al.*, 2016). This magnitude of improvement was similar to that seen with both ~20 minutes of intermittent sprint cycling and ~30 minutes of cycling at a moderate-intensity (70% HR_{max}). Hence, the efficacy of low volume HIIT interventions for improving metabolic health is still clear despite the reduced exercise volume and time commitment. However, low volume HIIT interventions implemented in a free-living setting outside of the laboratory have been seldom explored. Understanding the effectiveness of these interventions in the real-world setting is limited, but of great importance.

2.2 Effectiveness of high-intensity intermittent training to improve physical activity levels and metabolic health

For seemingly efficacious HIIE approaches to translate into effective free-living exercise programmes and increase PA levels, the likely adherence to such exercise must be considered. Even with minimal time commitment required, the majority of low volume HIIE protocols explored rely upon the requirement of specialised apparatus. Given that a lack of access to apparatus and facilities, as well as limited access to apparatus at home, are both barriers to regular PA participation (Trost *et al.*, 2002; Cerin *et al.*, 2010), the effectiveness of such HIIE protocols can be contended. HIIE protocols that do not require specific apparatus or facilities

are warranted for greater effectiveness in a free-living setting (Gray *et al.*, 2016). In turn, the additional time that would likely be required to access such apparatus and/or facilities (such as travelling to a gym or other exercise facility) would further attenuate their potential time efficiency, which is notable given that a perceived lack of time is also a prominent barrier to regular PA and exercise (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017).

Affective and enjoyment responses to high-intensity exercise are also important to consider in the context of adherence. However, they have only recently begun to be deliberated specifically to HIIE and an inactive population. The 'dual-mode' theory states that exercise above the ventilatory threshold induces greater responses from interoceptive factors such as increased respiration (experienced as breathlessness) and blood lactate (BLa) accumulation (associated with an accumulation of hydrogen ions in the muscle and consequent muscle ache and burning sensations; Stork *et al.*, 2017). Such physiological stress with high-intensity exercise stimulates negative affective responses in the brain (Ekkekakis, 2003) and hence, it is posed that HIIE would also induce aversive affective responses in this way, which are associated with reduced adherence (Hardcastle *et al.*, 2014; Biddle and Batterham, 2015). This further queries the effectiveness of HIIE.

Indeed, reduced affective responses have been noted in inactive women as exercise intensity increases above the ventilatory threshold (Ekkekakis, Lind and Vazou, 2009). Notably, in the obese cohort of the study population of Ekkekakis, Lind and Vazou (2009), affective valence continually declined from the start of the treadmill exercise protocol until volitional exhaustion. However, it is now recognised that such findings have often only been explored specifically in high-intensity continuous and not intermittent protocols (Stork *et al.*, 2017; Stork, Gibala and Martin Ginis, 2018) and as such, affective responses to HIIE may not necessarily reflect those seen with high-intensity continuous exercise. It also remains of interest, especially given the previously discussed barriers, whether affective and enjoyment responses differ in apparatus-free protocols compared with cycle ergometer or treadmill-based protocols.

It is also important to note that affective responses to HIIE are predicted by a range of factors including BLa concentration, but also by enjoyment and baseline affective valence (Astorino and Vella, 2018). As such, there is marked individual variability in affective and enjoyment

responses to HIIE (Astorino and Vella, 2018). In turn, length of intervals should be acknowledged when considering implications of affective and enjoyment responses to HIIE. Decker and Ekkekakis (2017) found a reduction in affective and enjoyment responses following 4 x 3 minutes at 90±13% VO_{2peak} (interspersed with 2 minutes recovery) in inactive, obese females compared with a continuous, moderate-intensity (25 minutes at 66±10% VO_{2peak}). However, it is possible that intervals of this duration at a high-intensity are responsible for this, as enjoyment responses have been shown to be higher during and following shorter intervals (such as 30 seconds), compared with longer intervals of HIIE or high-intensity continuous exercise (Martinez *et al.*, 2015). Moreover, affective responses also rebound more positively following shorter duration intervals, such as 30 seconds, compared with longer intervals of HIIE or high-intensity continuous exercise (Martinez *et al.*, 2015).

It can therefore be suggested that shorter intervals (such as 30 seconds) of HIIE could offer more positive affective responses and therefore increased PA adherence (Kwan and Bryan, 2010a), as well as greater potential time efficiency. Given the prevalence, implications and determinants of physical inactivity and the documented health benefits of HIIE, for effective PA strategies it can be argued that further research should explore low volume HIIE protocols that do not require specialised apparatus, require only a short time commitment and induce preferable affective and enjoyment responses for optimising approaches for adherence in free-living settings. In turn, this will likely improve the effectiveness of HIIE for increasing PA levels, bettering metabolic health outcomes and possibly facilitating weight-loss in those with overweight and obesity.

2.3 The role of physical activity and exercise in weight management

The 'obesity systems map' presents the complex aetiology of obesity whereby energy balance is directly and indirectly influenced by over 100 different variables (see figure 2.1; Butland *et al.*, 2007). Nonetheless, given the current public health climate it is important to understand to what extent PA plays a role in weight management.

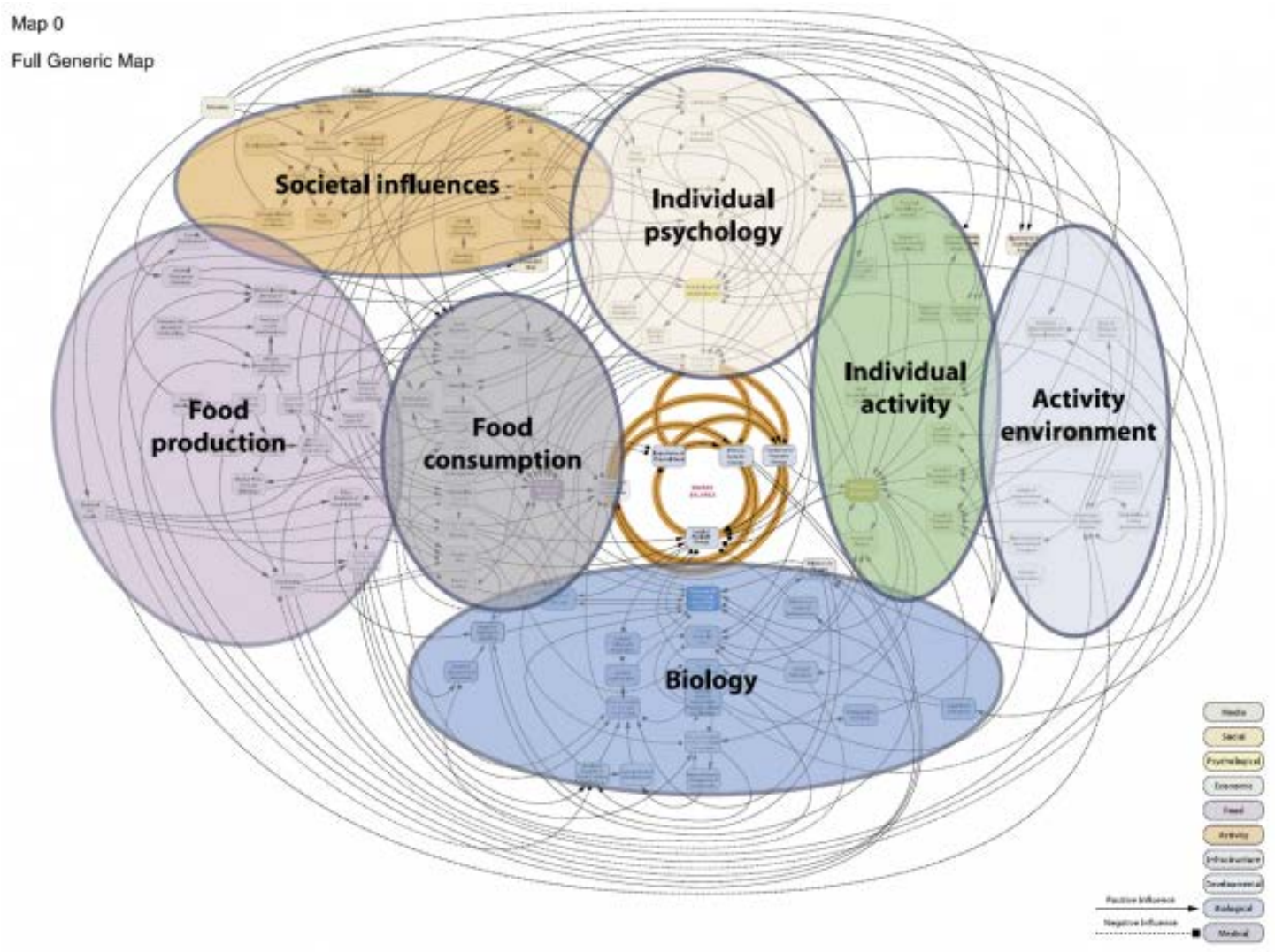

Figure 2.1 Obesity Systems Map (from Butland et al. (2007)

Compared with no treatment, prescription of exercise can induce loss in body mass (Shaw *et al.*, 2006), while high levels of PA and exercise also aid with the maintenance of reductions in body mass (Andersen *et al.*, 1999). Moreover, exercise combined with caloric restriction (diet) sees greater body mass loss than caloric restriction alone, such that the addition of exercise increases loss in body mass by ~1.1kg over ≥12 weeks (Shaw *et al.*, 2006; Franz *et al.*, 2007). Such an approach to weight management is not surprising given that it manipulates both EE and EI, aiming to induce an energy deficit through modulating both sides of the energy balance equation. Therefore, it is acknowledged that PA or exercise alone is not the most effective solution to achieve weight loss, as calorie restriction alone also results in greater fat loss than PA alone (Christiansen *et al.*, 2009; Parr, Coffey and Hawley, 2013). Nonetheless, PA

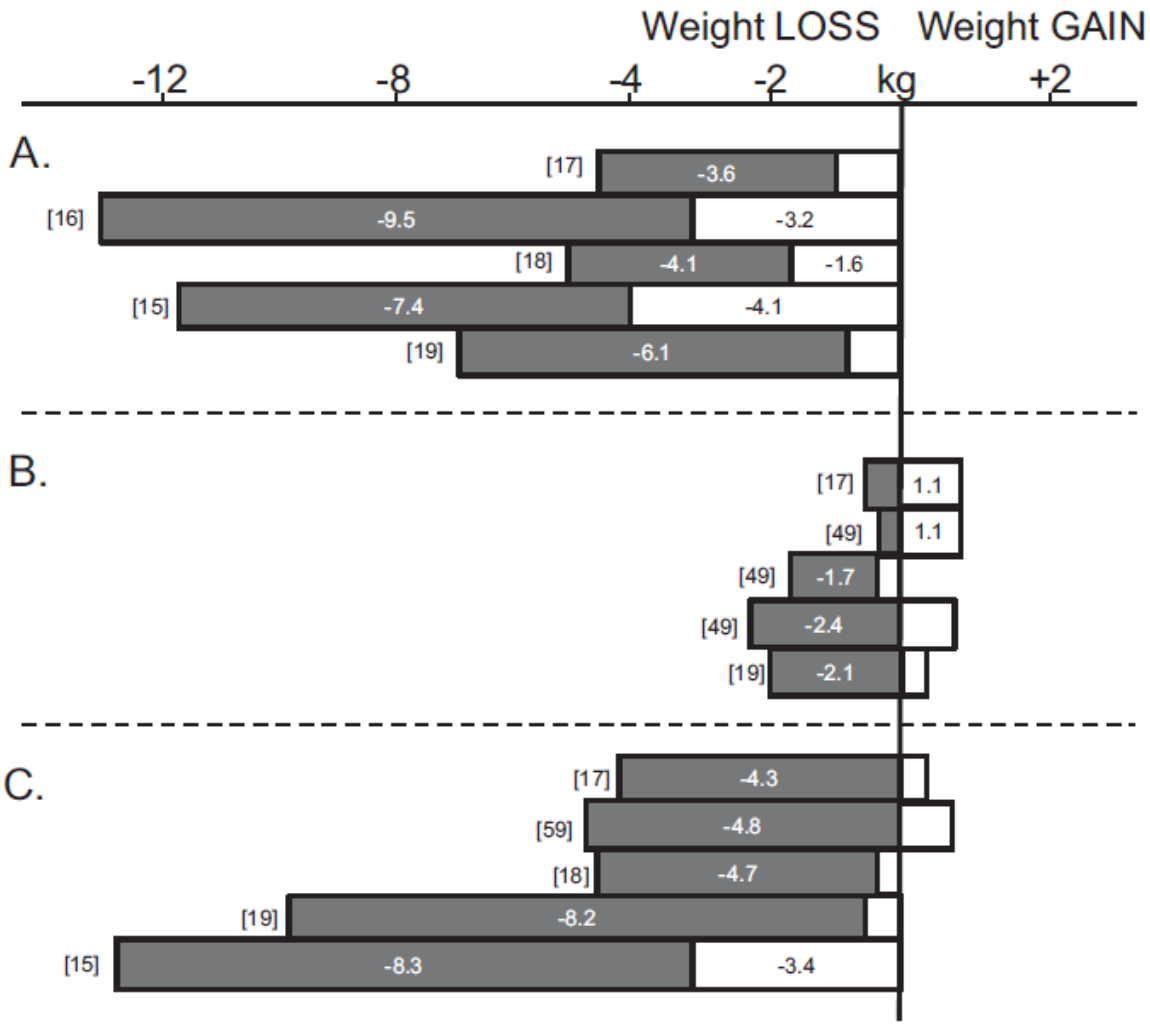

Figure 2.2 Data representing loss in body mass (total length of bar), lean mass (white bar) and fat mass (shaded bar) from studies employing calorie restriction alone (A), exercise alone (B) and a combination of calorie restriction and exercise (C; from Parr, Coffey and Hawley (2013)

and exercise can encourage the maintenance and promotion of lean mass, which can be lost with calorie restriction (Parr, Coffey and Hawley, 2013; see figure 2.2). It is noted that many studies in figure 2.2 did not necessarily control for energy deficit, however when this did occur greater lean mass was still lost with a diet only intervention compared with a diet and exercise intervention (Nicklas *et al.*, 2009). Exercise can also induce greater reductions in visceral adipose tissue (Verheggen *et al.*, 2016), which is often shown to be a preferable predictor of morbidity and mortality than total body mass itself (Ross and Bradshaw, 2009). In turn, preservation of lean mass can promote maintenance of basal metabolic rate (Ross *et al.*, 2000), preventing a potentially resultant reduction in daily EE. As the loss of fat mass and maintenance and/or gain of lean mass both represent favourable changes in body composition, and given the importance of PA for weight management and energy balance it is therefore reasonable to suggest that both EE and EI should be manipulated for effective and favourable energy balance regulation and management of body composition and bodyweight.

Notably, a greater loss in total body mass is seen as exercise intensity increases such that exercise ≥60% VO_{2max} increases loss in body mass by ~1.5kg compared with exercise at an intensity below this threshold (Shaw *et al.*, 2006). However, such observations have been seen largely with continuous exercise and less is known regarding such responses to HIIT interventions. However, those that have been explored have been shown to be similarly effective to moderate-intensity continuous exercise interventions in reducing body fat percentage and superior for absolute fat loss (Viana *et al.*, 2019). For example, ~1.5kg of body mass was lost in a group undertaking repeated twelve seconds of high-intensity exercise, separated by 8 seconds of recovery for a duration of twenty minutes, three times a week over fifteen weeks (Trapp *et al.*, 2008). Furthermore, similar losses in body mass were not seen in a group undertaking continuous cycling at 60% VO_{2peak} for up to forty minutes, while the HIIT group lost ~2.5kg fat mass compared with the continuous exercise group. These findings align with those of Viana *et al.* (2019), who demonstrated an overall modest reduction in body fat percentage of 1.44% and 1.50% for moderate-intensity and HIIT-based interventions, respectively.

Viana *et al.* (2019) demonstrated that when studies were sub-categorised into sprint interval training (SIT) on this occasion (the low- volume protocols typically consisting of repeated 30

seconds of “all-out” exercise) further improved total body mass loss (but not fat loss) compared with moderate-intensity, continuous interventions. This is of great interest given the typical low caloric expenditure of such exercise bouts. Given the demonstrated effectiveness on fat loss and weight management with low volume HIIE, it is pertinent to consider its efficaciousness and effectiveness for an inactive, overweight/obese population. With high rates of adherence in a real-world setting, HIIE may facilitate weight loss through the promotion of a negative energy balance.

2.4 Energy balance and its regulation

Energy balance can be depicted as a fairly simple phenomenon; EE can be subtracted from EI, with a surplus of EE resulting in a negative energy balance and a surplus of EI resulting in a positive energy balance. An accumulation of positive energy balance leads to weight gain and hence likely leading to overweight and obesity; it is estimated that an energy surplus of ~100kJ (~24kcal) per day would result in 1kg of body weight gain within three years (Hall *et al.*, 2011). Both EI and EE, however, are subject to a dynamic, complex network of control and a level of reciprocal interaction to determine resulting energy balance (Blundell *et al*., 2015; Beaulieu *et al.*, 2018; see figure 2.3). Indeed, how both acute and chronic PA and exercise (with a particular focus on HIIE) influences energy balance within the described network will be the focus of the remainder of this review, set within the context of physical inactivity, overweight and obesity set out earlier in this thesis.

2.5 Regulation and assessment of appetite, eating behaviour and energy intake

Appetite can be simply defined as the desire to eat (Janwitz and Grossman, 1949). This is a psychological drive that can be determined by, and manifests as, sensations of hunger. Often, in literature, the terms ‘appetite’ and ‘hunger’ are used interchangeably but for the remainder of this thesis, ‘appetite’ will refer to the general term encompassing hunger as well as desire to eat. Conversely, satiation can be defined as the process leading to meal termination and satiety as a feeling of fullness (Benelam, 2009). while the most common measure for subjective appetite is the use of a Visual Analogue Scale (VAS; Hill and Blundell, 1982) which

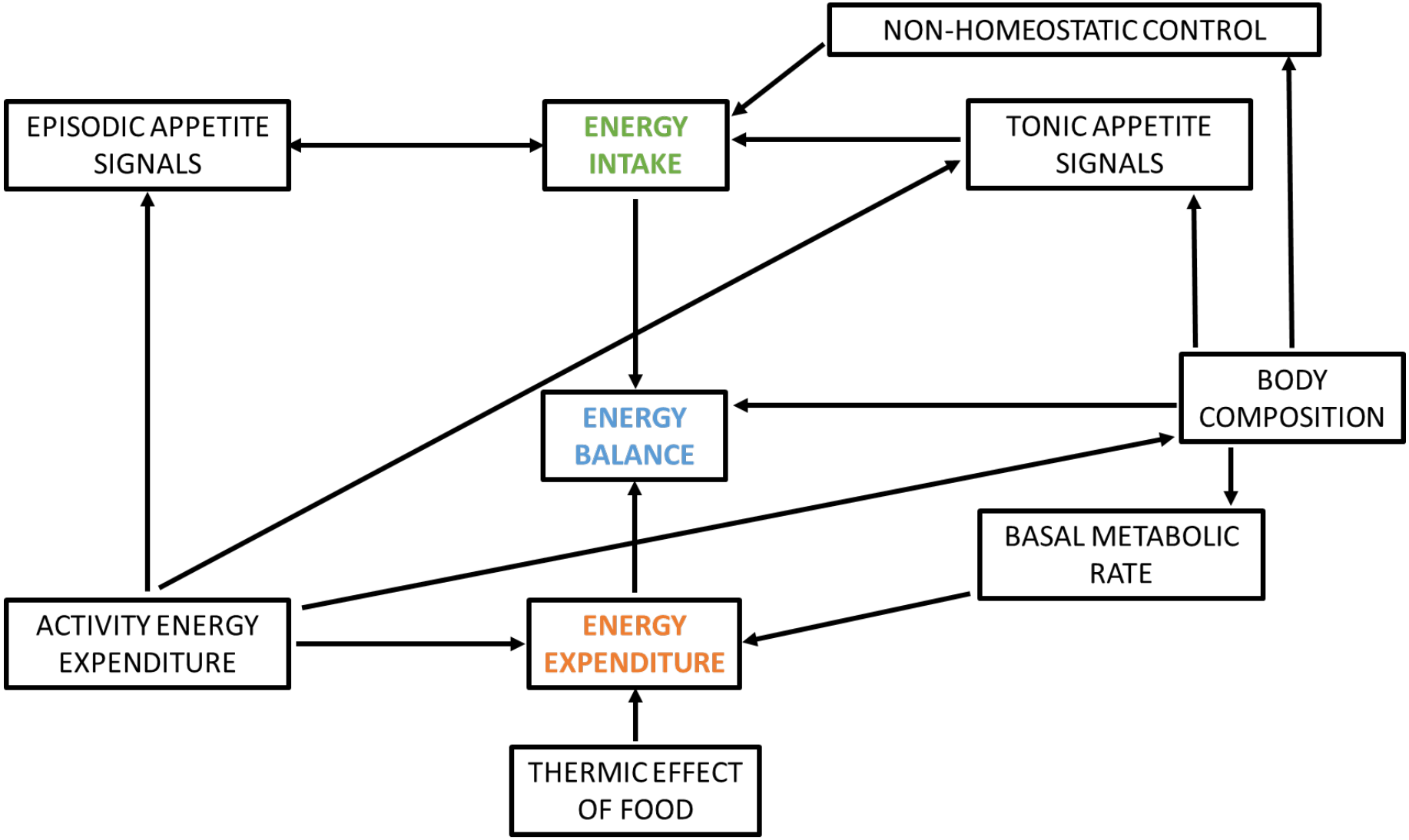

Figure 2.3 Network of influences on energy intake, energy expenditure and energy balance; adapted from Blundell et al. (2015)

individually assess hunger, satisfaction, fullness and prospective food consumption. Measuring caloric value of food intake (EI) is also a common objective assessment of appetite and eating behaviour. Food intake can also encompass the macronutrient contribution of a given EI at a meal. Appetite, hunger, satiety and EI are determined by the strength of the combination of objective (physiological) and subjective (psychological) responses (Stubbs *et al.*, 2000) otherwise known as homeostatic and non-homeostatic inputs, respectively.

Non-homeostatic inputs are those concerning behavioural traits and food hedonics (Blundell and Finlayson, 2004). Homeostatic inputs include tonic and episodic appetite hormones, which control the matrix of events and behaviours that are related to hunger, satiation and satiety. Tonic hormones are released from tissue and via cellular metabolic processes, signalling energy availability and requirements to the central nervous system (Morton *et al.*, 2006). Tonic hormones include leptin and other adipokines (Blundell *et al.*, 2015). Episodic signals are released from the gastrointestinal tract to the arcuate nucleus in the hypothalamus in the brain which influence meal initiation, termination and satiety (Beaulieu *et al.*, 2018). As such, gut peptide episodic hormones can be orexigenic (excitatory or appetite stimulating) and consist of acylated ghrelin (AG), or anorexigenic (inhibitory or appetite suppressing) including glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) (Blundell *et al.*, 2015). Together, this balance of gut peptide orexigenic and anorexigenic hormones influence appetite and control food intake (see figure 2.4), therefore the assessment of blood plasma concentrations of episodic and tonic hormones is used in the objective assessment of appetite, It is acknowledged that food intake is controlled by a vast network of processes and mechanisms additional to the gut peptide hormones discussed below and throughout this thesis and depicted in figure 2.4, but figure 2.4 represents a simplified version, adapted from Speakman (2014) for the purpose of reflecting measures taken within the thesis.

2.5.1 Acylated ghrelin

Ghrelin is a 28-amino acid episodic hormone released predominantly from the stomach (see figure 2.4) which is mainly composed of des-acylated ghrelin. The acylation of ghrelin results in the activated form of ghrelin, acylated ghrelin (AG), to be released into blood vessels (Kojima *et al.*, 2001) and it is only in this form that it can bind to the growth-hormone-

secretagogue receptor and cross the cross blood brain barrier to exert orexigenic effects on the arcuate nucleus (Kojima, Hosoda and Date, 1999; Murphy, Dhillo and Bloom, 2006).

Ghrelin is the only episodic hormone found to be orexigenic, such that circulating levels rise in the preprandial state and fall in the postprandial state in humans (Cummings *et al.*, 2001). In rats, ghrelin administration was shown to stimulate food intake (Wren *et al.*, 2000), while intravenous administration of ghrelin resulting in a two fold increase in plasma ghrelin in humans significantly increased subjective appetite as well as EI at an *ad libitum* buffet by 28% (Wren *et al.*, 2001). Furthermore, despite an increased EI, an accompanying reduction in feelings of subjective appetite 1 hour following feeding did not occur with infusion of ghrelin (Wren *et al.*, 2001), demonstrating its appetite-stimulating effects.

Acute exercise can suppress the release of AG, which was first demonstrated by Broom *et al.* (2007). The concentration of plasma circulating AG was significantly reduced immediately following 60 minutes of running at 72% VO_{2max} compared with resting control (Broom *et al.*, 2007). Moreover, area under the curve concentration (AUC) of AG was 38% lower during the 3 hours encompassing both the exercise condition and 2 hours following, as well as being 35% lower in the 9 hours encompassing both the exercise condition and 8 hours following compared with the resting control condition (Broom *et al.*, 2007). Of note, subjective appetite responses were parallel with the responses of AG (Broom *et al.*, 2007) further demonstrating its orexigenic role.

In response to incremental exercise to exhaustion, circulating levels of total ghrelin remained unchanged whilst levels of AG were significantly reduced in both healthy weight and obese individuals (Marzullo *et al.*, 2008). Therefore, this demonstrates the importance of assessing AG as opposed to total ghrelin. In turn, however, resting AG concentrations were demonstrated to be reduced in obese individuals compared with healthy weight individuals (Marzullo *et al.*, 2008). This would suggest reduced circulating AG with excess body mass, but it has been noted that it is energy stores that negatively correlate with concentrations of ghrelin (Cummings, Overduin and Foster-Schubert, 2005), demonstrating that it may not be body mass *per se*, but energy balance that regulates ghrelin concentrations. Furthermore, it has been demonstrated that increasing PA levels alongside reducing body mass may increase fasted concentrations of AG, but reduce postprandial concentrations (Martins *et al.*, 2010),

thereby suggesting improved appetite sensitivity to feeding with increased PA and reducing body mass.

2.5.2 Peptide YY

Peptide YY (PYY) is a 36-amino acid peptide hormone released from the L cells of the gastrointestinal tract, with the largest concentrations found in terminal ileum, colon and rectum (Adrian *et al.*, 1985). From here it acts directly on the arcuate nucleus in the hypothalamus to exhibit anorexigenic effects (Murphy, Dillo and Bloom, 2006). PYY exists in two endogenous forms as PYY_{1-36} and PYY_{3-36}; PYY_{3-36} is produced from PYY_{1-36} through the action of dipeptidyl peptidase IV and is the more common form of circulating PYY (le Roux *et al.*, 2006).

PYY is released in proportion to the caloric value of food intake (le Roux *et al.*, 2006), although its release is also dependent upon type of macronutrient, such that PYY release is greatest following meals high in protein compared with those high in fat or carbohydrate (van der Klaauw *et al.*, 2012) PYY is released postprandially and is demonstrated to peak in the second hour following food intake (Batterham *et al.*, 2002), further supporting its role in postprandial satiety. Indeed, the anorexigenic role of PYY was first demonstrated to reduce hunger by Okada and colleagues (1993). Furthermore, Batterham *et al.* (2002) showed that both peripheral and direct administration of PYY_{3-36} inhibited EI in rats, while in humans direct stimulation similarly suppressed appetite and reduced EI by 33% over 24 hours. Similarly, dose infusions of PYY_{3-36} stimulated a graded reduction in EI in humans (le Roux *et al.*, 2006). In the postprandial state, concentrations of PYY_{3-36} are greater than those of PYY_{1-36}, while the reverse is true during the fasted state (Grandt *et al.*, 1994), suggesting that it is preferable to assess PYY_{3-36} concentrations for measures of satiety in the postprandial state.

Acute exercise has only recently been demonstrated to influence circulating concentrations of PYY, such that 60 minutes of cycling at 50% VO_{2max} increased PYY concentration during and immediately following exercise compared with a resting control condition (Ueda *et al.*, 2009a). Moreover, Ueda and colleagues (2009b) then demonstrated that PYY_{3-36} release was greater immediately following 30 minutes of cycling at 75% VO_{2max} compared with at 50%

VO_{2max} and a resting control condition, suggesting an intensity-dependent role of PYY_{3-36} on satiety. In turn, this suggests that it may be of particular interest to explore PYY responses to HIIE with regards to suppressing appetite following HIIE. It is noted that PYY responses to food intake can be attenuated in obese individuals (le Roux *et al.*, 2006). This would suggest that reductions in body mass may lead to improved sensitivity of PYY responses to feeding, however, improved PYY responses were not seen with reductions in body mass following 12 weeks of moderate-intensity exercise in inactive, overweight/obese individuals (Martins et al., 2010).

2.5.3 GLP-1

GLP-1 is a neuropeptide hormone again stored and released alongside PYY in the postprandial state from the L cells of the gastrointestinal tract and the pancreas (Mojsov *et al.*, 1986). GLP-1 acts directly on the arcuate nucleus in the hypothalamus to exhibit anorexigenic effects (Murphy, Dillo and Bloom, 2006). Early work demonstrated that when administered peripherally, GLP-1 significantly increased satiety and fullness in response to a standardised breakfast, while EI at an *ad libitum* meal following breakfast was also significantly reduced (Flint *et al.*, 1998), thereby demonstrating the role of GLP-1 on satiety in healthy weight humans. The role of GLP-1 on satiety is further demonstrated by the findings of a meta-analysis where with GLP-1 administration, subsequent EI was reduced on average by 11% which was negatively correlated with GLP-1 dose (Verdich *et al.*, 2001a). Moreover, GLP-1 administration significantly reduced the rate of gastric emptying when compared with saline infusion (Verdich *et al.*, 2001a), suggesting that it is GLP-1's influence on rate of gastric emptying that at least partly explains its role in satiety given its action on the arcuate nucleus, too.

Ueda and colleagues (2009a) again demonstrated the role of acute exercise on circulating concentrations of GLP-1 as GLP-1 was significantly increased during and immediately following 60 minutes of cycling at 50% VO_{2max} and remained elevated up to 1 hour following exercise, compared with a resting control condition. However, unlike PYY, GLP-1 was not shown to be intensity dependent as concentrations of GLP-1 were similar between 30 minutes of cycling at both 50% and 75% VO_{2max} (Ueda *et al.*, 2009b). Of note, GLP-1 concentration was

significantly and negatively correlated with reduced EI following both exercise sessions (Ueda *et al.*, 2009b), suggesting a possible role of GLP-1 in satiation as well as satiety.

An attenuated GLP-1 response has been demonstrated in obese individuals (Ranganath *et al.*, 1996; Verdich *et al.*, 2001b). Moreover, such a response was improved following weight reduction (Verdich *et al.*, 2001b), suggesting a role for weight loss on appetite sensitivity in response to feeding. Furthermore, a tendency for an increased GLP-1 response following a standardised meal was demonstrated following increased PA levels and reduction in body mass over 12 weeks (Martins *et al.*, 2010), further supporting the role of PA and reduction in body mass for improved appetite sensitivity.

2.5.4 Pancreatic polypeptide

Other hormones have been shown to regulate appetite and food intake, including pancreatic polypeptide (PP) which is a 36-amino acid peptide released in the postprandial state from the pancreas (Murphy, Dillo and Bloom, 2006). Similar to PYY and GLP-1, peripheral administration of PP reduced subjective appetite and *ad libitum* EI by ~22% in healthy weight individuals (Batterham *et al.*, 2003). Moreover, reduction in EI was sustained into the morning of the following day such that cumulative 24-hour EI was reduced by ~25% with PP administration (Batterham *et al.*, 2003), demonstrating the role of PP in satiety.

However, the mechanisms of the anorexigenic effect of PP remain of contention. Although circulating PP concentrations were significantly increased following 3 x 17 minutes of cycling at 65% HR_{max} (2 minutes recovery) in healthy weight individuals (Martins *et al.*, 2007), fewer studies have explored the role of acute exercise and long-term increases in PA levels on PP concentrations and hence, PP will not be discussed or assessed further in this thesis.

2.5.5 Cholecystokinin

Cholecystokinin (CCK) was the first gut peptide hormone to be implicated in the role of controlling appetite. Only its sulphated form can bind to receptors to exert orexigenic effects and it is released from the small intestine in response to digestive products and nutrients

detected within the gut (Murphy and Bloom, 2004; Wren and Bloom, 2007). CCK release is up to five times greater in the postprandial state compared with the preprandial state and its main roles include delaying gastric emptying as well as stimulating pancreatic enzyme secretion and gall bladder contraction (Wren and Bloom, 2007). Indeed, peripheral CCK administration in humans significantly reduced food intake, while meals were also terminated sooner compared with saline administration (Kissilef *et al.*, 1981). However, CCK does have a short half-life (Wren and Bloom, 2007) which may explain as to why fewer exercise studies have explored the role of CCK in response to acute exercise and increased PA levels. As such, CCK will not be discussed or assessed further in this thesis in favour of gut peptide hormones with a greater exercise and PA-related evidence base.

2.5.6 Leptin

Leptin is a 167 amino-acid tonic hormone released from adipocytes in white adipose tissue in response to longer term regulation of appetite and EI. Therefore, circulating concentrations of leptin are proportional to fat mass. When released from adipocytes into the blood vessels, leptin acts as a signal to the arcuate nucleus in the hypothalamus during times of both adequate and insufficient energy stores to regulate EI in the longer term, demonstrating its role in fuel homestasis (Tuominen *et al.*, 1997). However, leptin is also rapidly released from the stomach in response to short-term feeding and delays release of satiety hormones as well as delaying gastric emptying (Cammisotto *et al.*, 2010).

2.6 Regulation and assessment of energy expenditure

EE itself is determined by a combination of the energy requirements for all biological processes of and within the body. Basal metabolic rate (BMR) makes up between 50-70% of total EE, demonstrated in a representative population of UK individuals (Mifflin *et al.*, 1990; Johnstone *et al.*, 2005) and is defined as the energy required to maintain metabolic activity of cells and tissues as well as blood circulation, respiration, gastrointestinal and renal function (Butte, Ekelund and Westerterp, 2012). BMR is assessed in a post-absorptive state, resting, awake and supine in a thermoneutral environment with indirect or direct calorimetry (Levine,

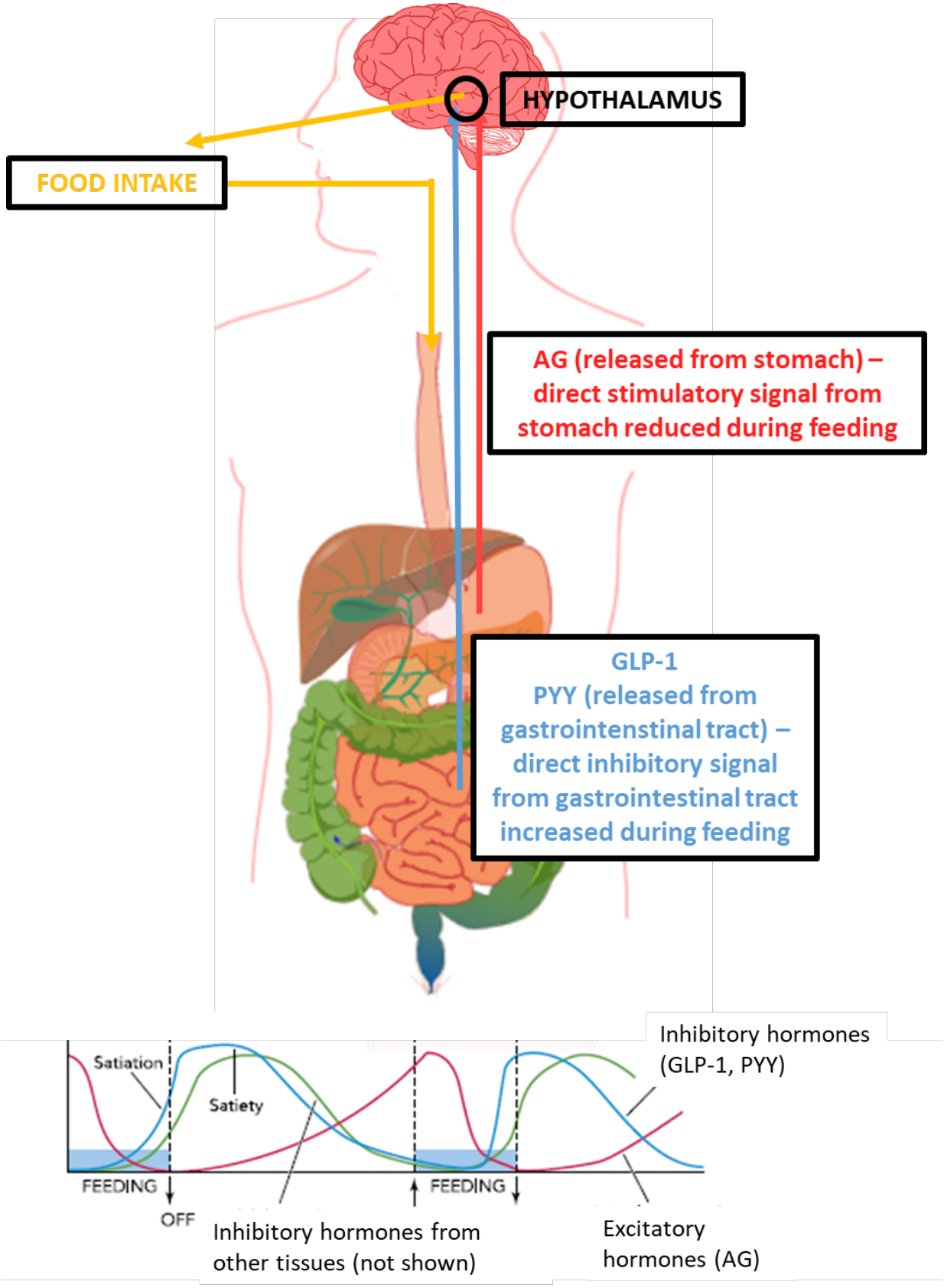

Figure 2.4 Origins and roles of episodic and tonic hormones where the balance of inhibitory and excitatory hormones switches food intake "on" and "off"; diagram adapted from Speakman (2014)

2005). The thermic effect of food is defined as the EE associated with digestion and processing of food and represents an assumed proportion of 10% of total EE (Hall *et al.*, 2012; Butte, Ekelund and Westerterp, 2012). Finally, activity energy expenditure (AEE), or the total EE during activity (including exercise/PA and non-exercise activity) makes up the remainder of contributions to total energy expenditure (TEE) but is the most variable of components of EE across individuals at 5-50% (Westerterp, 2003; Butte, Ekelund and Westerterp, 2012), as this ultimately depends on an individual's level of PA. The doubly labelled water method of assessing EE requires the enriching of an individual with a measured amount of doubly labelled water (consisting of heavy hydrogen and oxygen) where the difference in rates of turnover of hydrogen and oxygen are assessed by radio mass spectrometry through samples of blood, saliva and urine collected over one to three weeks to derive the rate of oxygen consumption (Westerterp, 2017). This method does not interfere with the behaviour (or EE) of the individual and so is considered the "gold standard" measure for assessing TEE and AEE (Westerterp, 2017), however it can be considered intrusive for individuals and requires a reasonable work load for the researcher. As a result, AEE (or PA levels) can also be assessed both subjectively (PA questionnaires, 24-hour recall, activity diaries) or objectively through the use of accelerometers and/or other technological devices such as pedometers.

2.7 The role and assessment of acute exercise on appetite, eating behaviour and energy balance

2.7.1 Exercise intensity

Early studies revealed an intensity-dependent relationship between acute exercise, appetite and eating behaviour responses. Seminal work from King, Burley and Blundell (1994) coined the term 'exercise-induced anorexia'; this term was used to describe the transient suppression in hunger seen following acute exercise that is of a high-intensity (>60% VO_{2max}). Indeed, this development was preceded by similar work in the field including that of Reger, Allison and Kuruca (1984) who showed that a bout of acute high-intensity intermittent running consisting of ~30 minutes of 1 minute at 70% VO_{2max}, interspersed with 3 minutes at 40% VO_{2max}, briefly suppressed hunger immediately following exercise when compared with

low-intensity, continuous running for either 30 or 60 minutes at 50% VO_{2max}. Similarly, feelings of hunger were briefly suppressed following high-intensity (mean 28.7±3 minutes at 68% VO_{2max}) compared with low-intensity (mean 57.5±6 minutes at 35% VO_{2max}) continuous cycling (Thompson, Wolfe and Eikelboom, 1988). Furthermore, a growing body of evidence confirms that 'exercise-induced anorexia' is typically only seen at an intensity ≥60% $VO_{2max,}$ (King, Burley and Blundell, 1994; King and Blundell, 1995; Blundell *et al.*, 2003; Broom *et al.*, 2009; Dorling *et al.*, 2018). Many studies that have seen a lack of change in appetite and eating behaviour post-exercise include protocols that did not meet this threshold, such as walking at 70-75% HR_{max} (Unick *et al.*, 2010) and cycling at 50% VO_{2max} (Ueda *et al.*, 2009a). Kissilef *et al.* (1990) also only demonstrated a reduction in EI following 40 minutes 'strenuous' (90W) cycling compared with moderate-intensity (30W) cycling (in healthy weight individuals), further supporting the intensity-dependent relationship between acute PA, appetite and eating behaviour. For the purpose of classification for this review, studies have been classified for review on the basis of this ≥60% $VO_{2max,}$ threshold (or ≥77% HR_{max} according to intensity guidelines from the ACSM (Garber *et al*., 2011).

Given the supporting evidence for an intensity-dependent 'exercise-induced anorexia', it is possible that HIIE can offer a time-efficient method for suppressing appetite post-exercise. At present, there are no studies that assess the effect of intensity of intermittent exercise and appetite responses *per se* (for example, moderate-intensity intermittent exercise versus HIIE), which would further elucidate the role of an intermittent natured protocol. Nonetheless, duration and mode of exercise are also important to consider, especially if postulating benefits of apparatus-free, low volume HIIE for appetite regulation and reduced EI.

2.7.2 Exercise duration

Duration of exercise has not been a great focus of investigation in current literature with regard to its influence on appetite, eating behaviour and energy balance responses. However, a greater suppression of subjective hunger following longer duration (51.8±2.4 minutes) was seen when compared with shorter duration (25.9±1.9 minutes) high-intensity (74±7-77±9% VO_{2max}) continuous cycling (King, Burley and Blundell, 1994). In contrast, subjective appetite

and AG responses were similar when participants undertook either 45 or 90 minutes of running at 70% VO_{2peak} although authors concluded that the suppression of AG may last longer with a greater duration of acute exercise (Broom *et al.*, 2017). In turn, continuous high-intensity (~76% VO_{2max}) cycling for 15, 30 or 45 minutes duration did not influence subjective appetite, nor absolute EI (Holliday and Blannin, 2017b). The authors demonstrated that all durations transiently suppressed AG, with no differences between durations (Holliday and Blannin, 2017b). However, it should be noted that all of the discussed findings are limited to healthy, active males. As appetite suppression has been observed with just 4 x 30 seconds of high-intensity running or cycling (Beaulieu *et al.*, 2015; Holliday and Blannin, 2017a), but not always with longer duration exercise when intensity did not far exceed 74% VO_{2max}, this demonstrates that intensity, but likely not duration, is important for appetite suppression in the transient period post-exercise. Low volume (and duration) HIIE again may offer a time-efficient method for suppressing appetite post-exercise. Nonetheless, given the barriers discussed in section 2.2, mode of exercise is also imperative to consider.

2.7.3 Exercise mode

The majority of studies exploring appetite and eating behaviour responses to acute exercise have used cycling (ergometer) or running (treadmill) protocols (see table 2.1). Indeed, King, Burley and Blundell (1994) proposed that weight-bearing activities may lead to greater postural disturbance. In turn, this may result in greater physiological distress and/or gastrointestinal disturbance and a greater direct induction of suppression of hunger and/or willingness to eat with this mode of exercise compared with non-weight-bearing exercise. Again, this is important to consider with regards to appetite and eating behaviour responses. Nonetheless, 6 x 7 minutes of moderate-intensity swimming (3 minutes recovery) has been explored in habitually active males, whereby, subjective appetite was suppressed during and immediately following exercise (King, Wasse and Stensel, 2010). Similarly, AG was significantly suppressed during swimming and prior to the buffet meal that followed 1 hour following each condition. However, AG concentrations were not correlated with EI at the buffet meal, and absolute EI did not differ between conditions. Moreover, subjective appetite was greater than resting control levels in the hours following swimming, although relative EI was significantly

greater in the resting control condition compared with swimming (King, Wasse and Stensel, 2010). Nonetheless, it is difficult to ascertain whether the lack of difference in absolute EI between conditions is a result of the mode of exercise *per se*, or a result of the exercise being only of a moderate-intensity.

Of note, when an acute protocol of 6.5km sculling (~30 minutes) was undertaken by elite male rowers both below and above individual anaerobic thresholds, total ghrelin concentrations did not change during exercise, although they did tend to be greater 30 minutes following exercise above the anaerobic threshold (Jürimäe *et al.*, 2007). Similarly, total ghrelin concentration increased immediately following a maximal 6000m rowing test (mean duration 1192±16 seconds) in elite male rowers (Jürimäe, Jürimäe and Purge, 2007). Although in contrast to many previously discussed acute high-intensity exercise studies, it may be that investigating such responses an elite population is responsible for the differing ghrelin responses and findings are certainly not transferable to an inactive, overweight/obese population. Moreover, it may be due to this mode of exercise being non-weight-bearing, given that Broom *et al.* (2009) demonstrated a significant suppression in hunger and AG during both 1 hour of running at 70% VO_{2max} and 90 minutes of resistance exercise, although resistance exercise did not increase PYY concentrations to the same extent as were observed with running. Indeed, this may align with the proposal by King, Burley and Blundell (1994) that weight-bearing activities may lead to greater postural disturbance.

Similarly, Kawano and colleagues (2013) demonstrated that 3 x 10 minutes of rope skipping at ~65% VO_{2max} suppressed hunger and AG as well as increase PYY compared with 3 x 10 minutes of ergometer cycling at ~65% VO_{2max} (Kawano *et al.*, 2013). Although neither condition were performed at a high-intensity, hunger was suppressed to a greater extent during skipping compared with cycling, again suggesting that weight-bearing exercise may be preferable for inducing greater appetite suppression. However, possible sex differences were noted between males and females with rope skipping exercise such that appetite increased following 2 x 10 minutes of rope skipping in females but not in males (Kawano *et al.*, 2012), although again it is difficult to ascertain whether the exercise not being performed at a high-intensity may be partly responsible for this.

At present, there is no evidence base exploring appetite, EI and eating behaviour responses to weight-bearing HIIE. Should the intensity of exercise be the 'key' in promoting exercise-

induced anorexia, with duration playing a less key role, future work should focus on apparatus-free, weight-bearing modes of HIIE, given its potential superior appetite-suppressing effects as well as the rationale for HIIE that does not require specialised equipment or facilities (see section 2.2).

2.7.4 Assessment of acute energy intake post-exercise

Of note, many of the early studies with immediate suppressions in hunger did not find any translated effects on EI at a test meal, whilst not all studies have demonstrated similar suppressions in hunger and/or reductions in EI (see table 2.1). Furthermore, despite the support provided for 'exercise-induced anorexia', many studies have not considered responses in EE alongside EI, hence being unable to explore the relative effects on energy balance. King, Burley and Blundell (1994) were also the first to consider this, including assessments of not only EI, but also motivation to eat, willingness to start eating, activity-induced EE and relative EI. As any influences of acute high-intensity exercise on subjective appetite appear to be transient, the impact on relative EI (when EE of the exercise was also taken into account) therefore gives a better indication of the effects on energy balance manipulation beyond solely the transient exercise period. Of note, Pomerlau *et al.* (2004) found no differences in subjective appetite, but did see an increased EI following a bout of walking at ~70% VO_{2peak} (mean duration 37.0±4.6 minutes) in active females where, unusually, participants consumed ~127kcal EI more following exercise compared with a resting control condition. Despite this, relative EI was still lower following this exercise condition compared with the resting control, thereby demonstrating the importance of evaluating relative EI as well as absolute EI.

King, Burley and Blundell (1994) also used a battery assessment of the effects of acute exercise on subjective appetite and EI. Not only did these include ratings of hunger and EI at the test meal, but also assessed any delay to the onset of feeding as well as assessing EI across the remainder of the testing day. This offered novel insights into the effects of acute exercise on appetite and eating behaviour, such that a suppression of subjective hunger was found following an acute bout of high-intensity (72±23% VO_{2max}) continuous (26.7±2.5minutes) cycling compared with an acute bout of low-intensity (36±7% VO_{2max}) continuous

(63.2±6.4minutes) cycling matched for EE, but this was short-lived and returned to control levels within around 15 minutes post-exercise (King, Burley and Blundell, 1994). The suppression in subjective hunger that was seen following both acute high-intensity bouts, however, returned to control levels within ~10 minutes post-exercise and therefore was very transient. A delay to the onset of eating was seen following both high-intensity bouts (with no difference between the two durations), although this delay was only 5 minutes. Again, this work provided pivotal support for 'exercise-induced anorexia', albeit in the very transient period following high-intensity exercise. As such, despite suppressions in subjective hunger, King and colleagues (1994) found no effect on the absolute EI at the test meal when this meal was available from 15 minutes following exercise – a finding again in line with those of Reger, Allison and Kuruca (1984) and Thompson, Wolfe and Eikelboom (1988).

In agreement with much of the discussed literature, a recent meta-analysis revealed acute exercise is effective in inducing a short term negative relative EI (and hence an energy deficit), with trivial conclusions on acute exercise having an effect on absolute EI (Schubert *et al.*, 2013). Reasons for a lack of substantive evidence for a reduction of absolute EI may be as Schubert *et al.* (2013) did not focus solely on high-intensity exercise. Given the intensity-dependent relationship between exercise, appetite and eating behaviour responses, findings could be altered with this focus.

2.7.5 Assessment of appetite-associated hormones

Multiple studies have demonstrated anorexigenic changes in appetite-associated hormone concentration with acute high-intensity exercise (Martins *et al.*, 2007; Ueda *et al.*, 2009a, King *et al.*, 2010, Deighton *et al.*, 2013a; Deighton *et al.*, 2013b, Wasse *et al.*, 2013; Sim *et al.*, 2014; Alajmi *et al.*, 2016; Beaulieu *et al.*, 2015; Martins *et al.*, 2015; Panissa *et al.*, 2016; Broom *et al.*, 2017; Hallworth *et al.*, 2017; Holliday and Blannin, 2017a; Larsen *et al.*, 2017; see table 2.1). However, often such suppressions in the appetite hormone profile do not necessarily translate to parallel responses in appetite and/or eating behaviour (Martins *et al.*, 2007; King *et al.*, 2010; Deighton *et al.*, 2013a; Deighton *et al.*, 2013b; Wasse *et al.*, 2013; Sim *et al.*, 2014; Alajmi *et al.*, 2016; Beaulieu *et al.*, 2015; Martins *et al.*, 2015; Panissa *et al.*, 2016; Broom *et al.*, 2017; Holliday and Blannin, 2017a; Larsen *et al.*, 2017; see table 2.1).

Moreover, although parallel changes in subjective and objective measures of appetite are sometimes seen (Burns *et al.*, 2007; Broom *et al.*, 2007; Broom *et al.*, 2009; Ueda *et al.*, 2009b; Wasse *et al.*, 2013; see table 2.1), a lack of statistical correlation between changes in AG (an appetite-stimulating hormone) and subjective feelings of hunger have recently been demonstrated (Broom *et al.*, 2017). This, to some extent, questions the role of episodic hormones such as AG in the assessment of appetite and regulation of energy balance. Given the focus of this thesis is on energy balance regulation and manipulation, further deliberation and exploration of episodic appetite hormones with acute exercise are beyond its scope. The reader is directed to a very comprehensive and in-depth review of potential mechanisms associated specifically with intensity-dependent changes in episodic appetite hormones for further information here (Hazell *et al.*, 2016).

2.7.6 High-intensity intermittent exercise and appetite responses

Despite the body of evidence supporting continuous, high-intensity exercise and its role in manipulating appetite and eating behaviour, the effects of intermittent exercise on appetite, has only recently been further explored. This is despite the work of Reger, Allison and Kurucz (1984) finding a transient suppression of hunger following intermittent high-intensity treadmill running compared with low-intensity, continuous running some thirty five years ago. It is acknowledged that this finding was likely, at least in part, due to the higher intensity of the exercise, yet it could also be argued that the nature of intermittent exercise allows exercisers to reach higher exercise intensities than with continuous high-intensity exercise. Given the intensity-dependent nature of exercise-induced anorexia, reaching greater exercise intensities in this intermittent manner could have important implications for energy balance manipulation.

Deighton *et al.* (2013a) were the first to explore appetite and eating behaviour responses to acute HIIE following the accumulated body of evidence exploring appetite and eating behaviour responses to acute exercise since the late twentieth century (see table 2.1). Authors found significant suppressions in subjective appetite during 6 x 30 seconds of high-intensity cycling as well as significant suppressions in AG immediately following exercise and up to forty five minutes following exercise (Deighton *et al.*, 2013a). Similarly, Holliday and

Blannin (2017a) and Beaulieu *et al.* (2015) found significant reductions in subjective appetite immediately following 4 x 30 seconds of high-intensity cycling and running, respectively. In turn, Holliday and Blannin (2017a) demonstrated significant suppressions in AG up to two hours following exercise. However, neither Holliday and Blannin (2017a) nor Beaulieu *et al.* (2015) found any differences in EI at a meal at one or two hours following exercise, respectively. Furthermore, significant suppressions in subjective appetite were seen immediately following both ~9 minutes and ~18 minutes of high-intensity intermittent cycling, although AG was not suppressed following either condition and there were no differences seen in EI at a test meal two hours following the exercise (Martins *et al.*, 2015). Nonetheless, neither Metcalfe *et al.* (2015) nor Bailey *et al.* (2015) saw any differences in subjective appetite following 2 x 20 seconds and 6 x 3 minutes of high-intensity cycling, respectively.

Given this body of evidence and the barrier of a perceived lack of time to regular PA in inactive individuals (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017), exploring the minimal volume of acute HIIE to induce anorexigenic effects on appetite and eating behaviour is warranted in inactive, overweight and obese individuals. Further, considering the rationale for HIIE to increase PA levels depending on its time efficiency, practicality, affective and enjoyment responses (see section 2.2), further exploration of acute appetite and eating behaviour responses to HIIE is paramount, particularly for an overweight population.

2.7.7 Influence of sex

Studies exploring appetite and eating behaviour responses to acute high-intensity exercise have employed populations with varying levels of activity and bodyweight in both males and females, however few acute studies have compared such responses between, for example, inactive and active participants, overweight/obese and healthy weight participants or males and females (see table 2.1). Those that have, have often found no differences in appetite or eating behaviour responses between males and females (Hagobian *et al.*, 2013; Cadieux *et al.*, 2014; Panissa *et al.*, 2016; Shamlan *et al.*, 2017). Of note, Alajmi *et al.* (2016) did demonstrate that females displayed increased subjective appetite, appetite hormone and EI

following caloric restriction, but these compensatory responses did not occur when the same energy deficit was experienced through acute high-intensity exercise, which has important implications for the context of this thesis.

Those that have noted sex differences in appetite responses suggest that these are unlikely to translate to modulations in eating behaviour or EI and do not always align with subjective appetite responses (Hazell *et al.*, 2017). Hence, there remains little consensus of evidence to suggest that sex influences the appetite or eating behaviour responses to acute exercise (Thackray *et al.*, 2016; Dorling *et al.*, 2018). However, Rocha and colleagues (2015) demonstrated sex differences in a delayed response to one hour of moderate-intensity cycling, whereby EI was reduced on the day following exercise in inactive females compared with active females. However, a contrasting response was displayed in inactive males such that Rocha *et al.* (2013) demonstrated compensatory increases in EI on the remainder of the exercising day following one hour of moderate-intensity cycling in active men while increases in EI occurred three days following exercise in inactive males. Therefore, this suggests that there may be sex differences in appetite and eating behaviour that occur in a delayed manner following acute exercise, of which are worth further exploration particularly if a delayed reduction in EI occurs in an inactive, overweight/obese female population.

Nonetheless, the majority of studies in this area focus on males (see table 2.1). Females are found to be significantly underrepresented in sport and exercise medicine research (Costello, Bieuzen and Bleakley, 2014) and are less active than males (see chapter 1), giving rationale to explore this population further with regard to effective energy balance manipulation with PA and exercise.

One likely reason for a lack of research in females compared with males in this area is due to the consideration and requirement of control of the menstrual cycle in females. It is documented that fluctuations in GLP-1, PYY and insulin are seen at differing stages of the menstrual cycle and that EI can be greater during the luteal phase (days 16-28) of the menstrual cycle (Lissner *et al.*, 1988; Buffenstein *et al.*, 1995; Dye and Blundell, 1997; Brennan *et al.*, 2009) compared with the follicular phase (days 1-14) of the menstrual cycle although there seems to be little influence of menstrual cycle stage on AG (Dafopolous *et al.*, 2009). In turn, GLP-1 and insulin, as well as metabolic rate during sleep, is lower during the follicular phase compared with the luteal phase (Bisdee, James and Shaw, 1989; Brennan *et al.*, 2009).

Hence, it is recommended that studies should assess metabolic and appetite-related measures during the follicular phase of the menstrual cycle, where female sex hormones are lowest (Bruinvels *et al.*, 2017) to avoid stage of the menstrual cycle confounding such measures. With this generates additional difficulty for research studies by limiting the window of time to assess such measures in females, while controlling for variation in cycle length is also challenging (Vlitos and Davies, 1996). Furthermore, recent evidence suggests that utilisation of oral contraceptives has an influence on EI, such that inactive females taking oral contraceptives demonstrated a greater EI compared with inactive females not taking oral contraceptives but this discrepancy was not noted in active females (Rocha *et al.,* 2018). A potential mechanism for this remains unclear but the differing female sex hormone profile with oral contraceptives (see figure 2.5) suggests that utilisation of oral contraceptives could be a confounding factor in inactive females, at least in between-subject designed studies. However, controlling for this only adds to the difficulty in studying metabolic and appetite measures in females. Whether non-oral contraceptive methods also act as a confounding variable to these measures remains to be elucidated but is important to consider when interpreting physiological findings in females.

2.7.8 Influence of weight status

Similarly, appetite and eating behaviour responses to acute exercise have primarily been investigated in lean or healthy weight populations (see table 2.1), which to some extent would make the energy balance and weight management implications redundant. Of the few studies that have assessed responses in those with overweight or obesity, Kissilef *et al.* (1990) observed no change in EI following 'strenuous' (90W) cycling compared with moderate-intensity (30W) cycling, although a reduced EI was seen following 'strenuous' exercise in healthy weight individuals. However, relative intensities or markers of intensity of this exercise bout are unknown. Although few studies have compared overweight or obese individuals with healthy individuals *per se*, reductions in subjective appetite have been seen immediately following 4 x 30 seconds of high-intensity cycling in overweight/obese individuals, although differences in EI were not seen two hours following exercise (Holliday and Blannin, 2017a). However, in contrast, some studies have also shown no change in

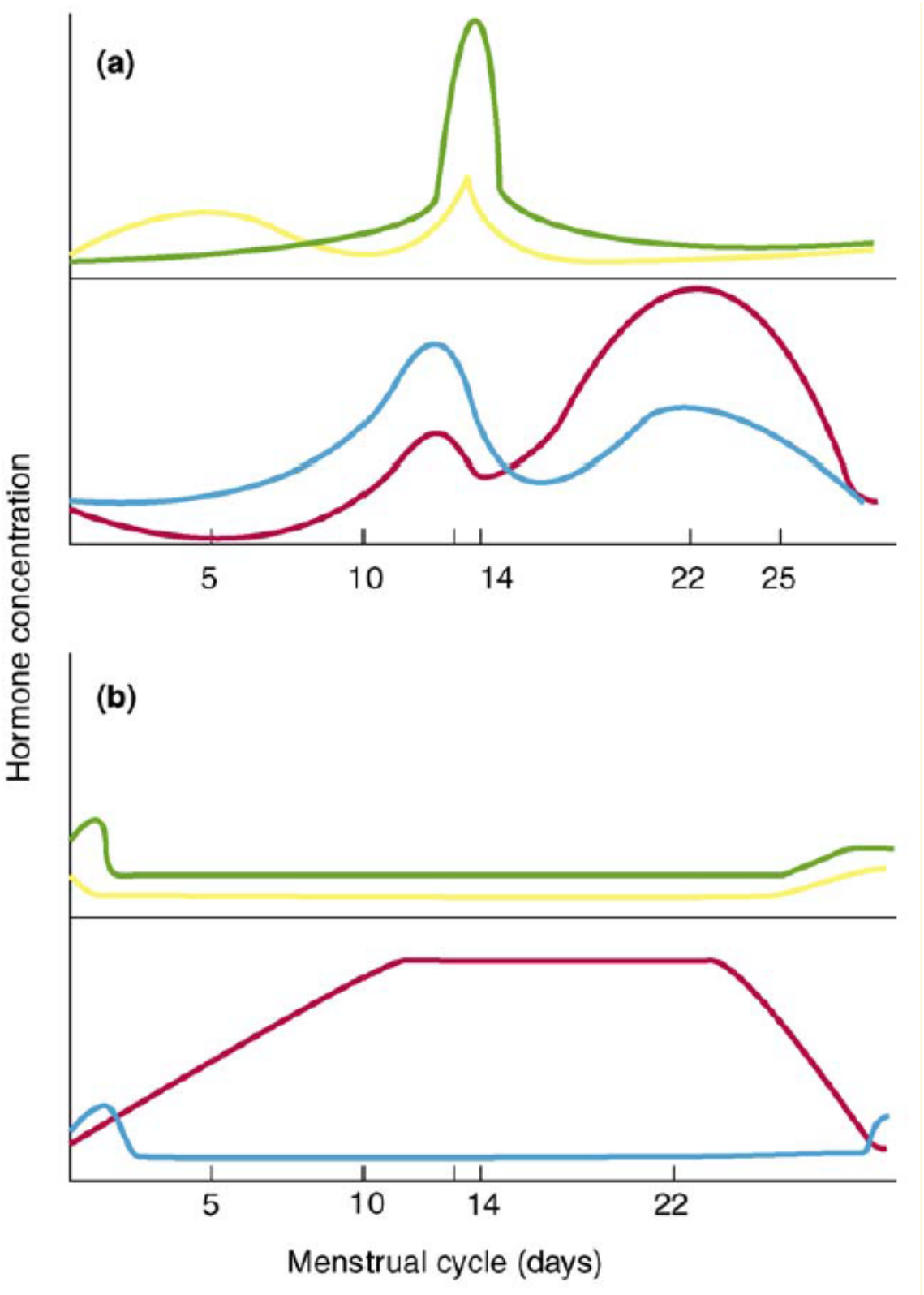

Figure 2.5 Hormonal regulation of the menstrual cycle in women with a normal menstrual cycle (a) and those taking oral contraceptives (b). Red and blue lines demonstrate progesterone and oestrogen concentrations, respectively, while green lines represent luteinising hormone and yellow lines represent follicle-stimulating hormone. From Alvergne and Lummaa (2009)

subjective appetite response following 6 x 1 minute of high-intensity cycling or 24 x 15 seconds of high-intensity cycling in overweight males but did see reductions in EI seventy minutes following exercise (Sim *et al.*, 2014). Although the differences in exercise volumes are acknowledged, a recent review suggests that there are no substantive differences in both subjective or objective appetite and eating behaviour responses between lean or healthy weight and overweight/obese individuals (Dorling *et al.*, 2018). In turn, a recent meta-analysis by Douglas *et al.* (2016) demonstrates that acute exercise in overweight/obese individuals moderately alters AG in an anorexigenic manner and thus may have a role in reducing hunger and energy intake following exercise in an overweight/obese population.

2.7.9 Influence of activity status

Few studies have compared appetite and eating behaviour responses to acute high-intensity exercise in both active and inactive participants. In one such study by Rocha and colleagues (2015), a delayed reduction in EI occurred in inactive females, such that EI was reduced on the day following acute moderate-intensity exercise when compared with active participants. Rocha *et al.* (2013) also demonstrated compensatory increases in EI on the remainder of the exercising day in active men while similar increases in EI occurred three days following one hour of cycling at 50% VO_{2max} in inactive males. It is reasonable to suggest from this that activity level and/or fitness could have an influence on post-exercise appetite and eating behaviour responses but as yet, there is little corroborating evidence to suggest so.

Table 2.1 gives detail on the design and outcome measures (with regard to moderating and mediating factors of acute high-intensity exercise, appetite, eating behaviour and energy balance) of studies exploring the effects of acute high-intensity exercise on appetite, EI and eating behaviour. Studies were included on the basis of assessing the effects of an acute high-intensity (continuous or intermittent, reaching ≥60% VO_{2max} or ≥77% HR_{max}) bout of exercise on measures of appetite (subjective and/or objective measures), eating behaviour and/or EI.

Table 2.1 A review of studies exploring appetite, eating behaviour and energy intake responses to acute high-intensity exercise

Authors	Participants			Exercise protocol			Measures			Outcomes	
	Age (years)	Sex	Activity/ BMI	Duration	Intensity	Mode	Measure	Time point	Method	Markers of intensity reached	Appetite/EI findings
Thompson, Wolfe and Eikelboom (1988)	24±4	M	Active, H (n=16)	Cont., until 4.1kcal·kg^{-1} bodyweight EE reached	35% (LO) v 68% (HI) VO_{2max} v resting control (CO)	Cycling (ergometer)	Subjective hunger EI	Pre and post-test meal Test meal 20 minutes post condition	9-point scale Covert *ad libitum* buffet (drinks, bread, waffles, muffins and spreads)	N/A	↓ hunger post HI v LO and CO ↑ kcal liquid and total CHO intake post LO and HI v CO
Kissilef *et al.*, (1990)	23±5	F	Inactive, H (n=9) and OB (n=9)	Cont., 40 minutes	90W (HI) v 30W (LO) v resting control (CO)	Cycling (ergometer)	EI	Test meal 15 minutes post-condition	Covert *ad libitum* buffet (one-item, yogurt)	N/A	↓ EI post HI v LO in HW females only
King, Burley and Blundell (1994)	a) 21-27	M	a) Active, H (n=11)	a) Cont., ~27 minutes v ~63 minutes b)	a) 70% (HI) v 30% (LO) VO_{2max} v resting control (CO)	Cycling (ergometer)	a) Subjective appetite EI	a) Pre and post- condition and test meal Test meal 15 minutes post – condition and for remainder of testing day	a) VAS Covert *ad libitum* buffet (multiple items) and weighed food diary for remainder of testing day b) VAS	N/A	a) ↓ hunger during and immediately post-HI v CO, but ↔ prior to test meal ↔ EI or macronutrient at the test meal, ↔ EI for 2 days following

	b) 22-31		b) Active, H (n=12)	Cont., ~26 minutes (S) v ~52 minutes (L)	b) 70% VO_{2max} v resting control (CO)		b) Subjective appetite	b) Pre and post-condition and test meal			b) ↓ hunger during and immediately post-L and S v CO, but ↔ by 10 minutes following
							Feeding latency, EI	Test meal 15 minutes post – condition and for remainder of testing day	Covert *ad libitum* buffet (sandwiches, yogurt, fruit, biscuits), weighed food diary for remainder of testing day		Time of test meal request 5 minutes later in L and S v CO, but ↔ EI at test meal or for remainder of day
King *et al.*, (1996)	N/A	F	H (n=13)	Cont. (EX) v resting control (CO)	70% VO_{2max}	Cycling	Subjective appetite	During conditions	VAS	N/A	↔ hunger before test meal
							EI and macronutrient intake	Immed. following condition	High-FAT/low-CHO v low-FAT/high-CHO test meals		↔ EI or macronutrient intake at test meal
Imbeault *et al.*, (1997)	24±3	F	Active, H (n=11)	Cont. until 2050kJ EE	35% (LO) v 75% (HI) VO_{2max} v resting control (CO)	Walking (treadmill)	Subjective appetite	Pre and post-test meal	VAS	N/A	↔ hunger or fullness pre or post-test meal
							EI and macronutrient intake	Test meal "immed. after the shower following exercise"	Covert *ad libitum* buffet (multiple items)		↔ EI or macronutrient intake at test meal, ↓ relative EI post-HI v LO and CO
Pomerlau *et al.*, (2004)	22±2	F	Active, H (n=13)	Cont. until ~350kcal	40% (LO) v 70% (HI) VO_{2max}	Walking (treadmill)	Subjective appetite	Pre-condition, pre and post-test meals	VAS	HR data not shown	↔ subjective appetite
							EI	Test meal 1h (1) and 6.5h (2) post-condition, snack bag	Covert *ad libitum* buffets and snack bag		↑ EI, FAT and PRO at test meal 1 post-HI v CO, ↓ EI at test meal 1 post-HI and LO v CO,

								between, weighed food diary for 3d following	(multiple items)		↔ EI at test meal 2, ↔ for daily relative EI, ↔ EI for 3 days following
Burns *et al.*, (2007)	25±1	F (n=9)and M (n=9)	Active, H	Cont. for 60 minutes	73.5% (EX) VO_{2max} v resting control (CO)	Running (treadmill)	Subjective appetite	Pre, during and post-condition	VAS	Mean HR during EX 173±2 beats ·min^{-1}	↓ hunger during and immediately post-EX v CO up to 60 minutes post
							Appetite/ satiety hormones				↔ AG
Ueda *et al.*, (2009a)	22±4 (HW) 23±3 (OW)	M	Sedentary or active H (n=7) and OB (n=7)	Cont. for 30 minutes	50% (LO) v 69% VO_{2max} (HI) v resting control (CO)	Cycling (ergometer)	Subjective appetite	During and post-condition	VAS	N/A	↓ hunger during and immediately following HI and LO v CO
							Appetite/ satiety hormones		Venous blood samples		↑ GLP-1 post-LO and HI v CO, ↑ PYY at 60 minutes post-HI v LO
							EI	Test meal 60 minutes post-condition	Covert *ad libitum* buffet (one-item, pasta meal)		↓ EI in HI and LO v CO, -ve correlation between GLP-1 and EI
King *et al.*, (2010)	22±1	M	Active, H (n=9)	Cont. for 90 minutes	69% VO_{2max} (EX) v resting control (CO)	Running (treadmill)	Subjective appetite	Pre and post-condition	VAS	Mean HR in EX 173±3 beats·min^{-1}	↓ hunger and PFC during EX v CO until 30 minutes post-condition
							Appetite/ satiety hormones		Venous blood samples		↓ AG during and until 30 minutes post-EX v CO
							EI	Test meals 15, 4.5h and 8h post-condition	Covert *ad libitum* buffets		↔ EI or macronutrient intake

									(multiple items)		
Charlot and Chapelot (2013)	21±2 (HW) and 22±2 (OW)	M	Active/ H (n=9) and inactive/OW (n=9)	Cont. for 60 minutes	70% VO_{2max} (EX) v resting control (CO)	Cycling (ergometer)	Subjective appetite EI	Pre and post-condition and test meal Test meal immed. following condition and for remainder of testing day	VAS Covert *ad libitum* buffet (3 items), food diary for remainder of testing day	65.8±1.5% VO_{2max} achieved in active/HW and 67.4± 1.4% VO_{2max} achieved in inactive/OW	↔ subjective appetite ↔ EI and macronutrient intake at test meal, ↑ EI for remainder of testing day and entire 24h testing period in EX v CO
Deighton *et al.*, (2013a)	23±3	M	Active, H (n=12)	Cont. for 60 minutes (END) v 6x30secs (interspersed with 4 minutes recovery; SIE) v resting control (CO)	68% VO_{2max} (END) v "all-out" (SIE) at 7.5% bodyweight resistance	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones EI	Pre, during and post-condition Test meals at 45 minutes and 4h 15 minutes post-condition	VAS Venous blood samples Covert *ad libitum* buffet (multiple items)	Av. HR ↑ during SIE (167±10 bpm) v END (153±13 bpm) RPE ↑ for SIE (18±1) v END (14±1)	↓ hunger during and immediately (briefly) post-SIE v CO ↓ AG immediately post and 45 minutes post-SIE v CO, ↑ PYY immediately post-END v CO ↔ EI at test meals

Deighton *et al.*, (2013b)	22±3	M	Active, H or OW	Cont. for 60 minutes v 10x4 minutes (interspersed with 2 minutes recovery) v resting control (CO)	59.5% VO_{2max} (END) v 85.8% VO_{2max} (HIIE)	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones EI	Pre and post-condition Test meal 4h post-condition	VAS (composite score) Venous blood samples Covert *ad libitum* buffet (one item)	Av. HR ↑ for HIIE (171±10 bpm) v END (143±8 bpm) RPE ↑ for HIIE (17±1) v END (13±1)	↓ subjective appetite during and immed. post-HIIE v CO ↑ PYY post-HIIE v CO ↔ EI at test meal
Hagobian *et al.*, (2013)	22±2 (M) and 21±2 (F)	F (n= 10) and M (n= 11)	Active, H or OW	Cont. for 60 minutes until 30% daily EE achieved (~82 minutes for M; ~84 minutes for F)	70% VO_{2peak} (EX) v resting control (CO)	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones EI	Pre and post-condition Test meal 40 minutes post-condition	VAS Venous blood samples Covert *ad libitum* buffet (multiple items)	N/A	↔ subjective appetite ↔ AG, PYY or insulin ↔ EI at test meal, but ↓ relative EI post-EX v CO
Wasse *et al.*, (2013)	22±2	M	Active, H (n=11)	Cont. for 60 minutes	70% VO_{2max}	Running (treadmill; RUN) v cycling (ergometer; CYC) v resting control (CO)	Subjective hunger Appetite/ satiety hormones	Pre, during and post-condition and post-standardised meal	VAS Venous blood samples	Mean HR ↑ in RUN (171±10 bpm) v CYC (158±11 bpm)	↔ hunger ↓ AG during RUN v CYC and CO, ↓ AG across RUN and CYC conditions v CO
Alkahtani *et al.*, (2014)	29±4	M	Inactive, OW and OB (n=12)	Intermittent: 30 minutes of alternating intensities (MIIE) v ~18.5	5 minutes at 20% above FAT_{max} interspersed with 5 minutes at 20% below	Cycling (ergo meter)	Subjective appetite	Pre and post-condition	VAS	↓ BLa post-MIIE (1.9±0.1 $mmol \cdot L^{-1}$) v HIIE (3.1±0.3 $mmol \cdot L^{-1}$)	↔ hunger, desire to eat and fullness

				minutes of alternating intensities (HIIE)	FAT_{max} (MIIE) v 15s at 85% VO2max interspersed with 15s recovery (HIIE)					↓ av. HR for MIIE (106±2 bpm) v HIIE (124±3 bpm) ↔ RPE in MIIE (11.5±0.4) v HIIE (12.4±0.5)	
Cadieux *et al.*, (2014)	23±3 (F) and 21±2 (M)	F (n=8) and M (n=8)	Inactive, H	Cont. for 60 minutes	70% VO_{2peak} (EX) v resting control (CO)	Running (tread mill)	Subjective appetite EI	Pre and post-condition and test meal Test meal 75 minutes post-condition and for remainder of testing day and following day	VAS Covert *ad libitum* buffet (multiple items) and snack and meal bags for remainder and following day	N/A	↔ subjective appetite ↔ EI or macronutrient intake
Sim *et al.*, (2014)	30±8	M	Inactive, OW (n=17)	Cont. for 30 minutes (MOD) v intermit.: 5x1 minutes intersperse with 4 minutes recovery (HIIE) v 24x15s intersper sed with 1min recovery (VHIIE)	60% VO_{2peak} (MOD) v 1min at 100% VO_{2peak} interspersed with 4 minutes at 50% VO_{2peak} (HIIE) v 15s at 170% VO_{2peak} interspersed with 1min at 32% VO_{2peak} (VHIIE) v resting control (CO)	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones EI	Pre and post-condition, pre and post-standardised meal and test meal Test meal 70 minutes post-condition (standardised meal at 5 minutes post-condition)	VAS Fingerprick blood samples Covert *ad libitum* buffet (one item)	Av. HR ↑ in HIIE (151±12 bpm) v MOD, ↑ in VHIIE (153±13 bpm) v MOD Av. RPE ↑ in HIIE (14±2) v MOD, ↑ in VHIIE (15±1) v MOD and HIIE ↑ BLa in VHIIE (~8mmol·L^{-1}) v HIIE and MOD; ↑ in HIIE (~5mmol·L^{-1}) v MOD	↔ subjective appetite ↓ AG post-VHIIE v HIIE, MOD and CO ↓ EI post HIIE and VHIIE v CO, ↓ post-VHIIE v MOD

Bailey *et al.*, (2015)	22±2	M	Active, H (n=12)	Cont. for 50 minutes (END) v intermit.: 6x3 minutes intersper sed with 3 minutes recovery (HIIE)	70% VO_{2max} (END) v 3 minutes at 90% VO_{2max} interspersed with 3 minutes at 50% VO_{2max} (HIIE)	Running (treadmill)	Subjective appetite	Pre, during and post-condition	VAS (composite score)	N/A	↔ subjective appetite
							Appetite/ satiety hormones		Venous blood samples		↔ AG, PYY, GLP-1
Beaulieu *et al.*, (2015)	25±3	M	Active, H (n=8)	Intermitt.: 4 x 30s intersperse with 4 minutes recovery	"all out" (HIIE) v resting control (CO)	Running (treadmill)	Subjective appetite	Pre and post-condition and pre and post-test meals	VAS	RPE (1-10) for each 30s bout: 8±2, 9±1, 10±1, 10±1	↓ hunger and motivation to eat post-HIIE, but ↑ hunger and motivation to eat AUC in HIIE v CO
							Appetite/ satiety hormones	Pre and post-condition and next day (fasted)	Venous blood samples		↑ PYY post-HIIE v CO
							EI	Test meals before condition and 1h and 5.5h post-condition	Covert *ad libitum* buffet (multiple items)		↔ EI at any meal, nor total EI
Martins *et al.*, (2015)	33±10	F (n=7) and M (n=5)	Inactive, OW or OB	Cont. until 250kcal EE achieved (~27 minutes END) v intermit. 8s (interspersed with 12s recovery; HIIE) until 250kcal	70% HR_{max} (END) v "all-out" (HIIE and SHIIE) v resting control (CO)	Cycling (ergometer)	Subjective appetite	Pre, during and post-condition	VAS	Av. HR: 133±8bpm, 71±1% HR_{max} (MOD); 162±11bpm, 86±2.5% HR_{max} (HIIE); 160±9	↓ desire to eat during and post-MOD, HIIE and SHIIE v CO
							Appetite/ Satiety hormones	Pre, during and post-condition	Venous blood samples		↔ AG post-conditions, but ↓

				EE achieved (~18 minutes) v 8s (interspersed with 12s recovery; SHIIE) until 125kcal EE achieved (~9 minutes)			EI	Test meal ~1h post-condition	Covert *ad libitum* buffet (multiple items)	bpm, 85±3.5% HR_{max} (SHIIE)	AG AUC in MOD and HIIE v CO ↔ EI at test meal, but ↓ relative EI in MOD, HIIE and SHIIE v CO and MOD v SHIIE
Metcalfe *et al.*, (2015)	21±2	M	Active, H and OW (n=8)	Cont. for 30 minutes (END) v intermit.: 2x20s intersper sed with 10 minutes recovery (HIIE)	50% VO_{2max} (END) v "all out" (HIIE) v resting control (CO)	Cycling (ergometer)	Subjective appetite	Pre and post-condition	VAS	↑ BLa post-END (3.5±0.6 $mmol \cdot L^{-1}$) and HIIE (15.4±1.3 $mmol \cdot L^{-1}$) v CO (0.5±0.1 $mmol \cdot L^{-1}$) and post-HIIE v END	↔ subjective appetite
Alajmi *et al.*, (2016)	a) 22±2	a) F	a) Active, H (n=12)	a) Cont. for 90 minutes	a) 70% VO_{2max} (EX) v resting control (CO)	a) Running (treadmill)	a) Subjective appetite Appetite/ satiety hormones	a) Pre and post-condition	a) VAS (composite score) Venous blood samples	a) N/A	a) ↔ subjective appetite AUC ↓ AG and PYY AUC in EX v CO
							EI	Test meal 6.5h post-condition	Covert *ad libitum* buffet (multiple- items)		↑ EI in EX v CO
	b) 22±3 (F) and 23±4 (M)	b) F (n=10) and M (n=10)	b) Active, H	b) Cont. for 60 minutes	b) 70% VO_{2max} (EX) v resting control (CO)	b) Running (treadmill)	b) Subjective appetite	b) Pre and post-condition	b) VAS (composite score)	b) Mean HR 163±4 $beats \cdot min^{-1}$ (M),	b) ↑ subjective appetite AUC

						Appetite/ satiety hormones		Venous blood samples	174±4 beats·min^{-1} (F) RPE 13±1 (M), 12±0 (F)	↓ AG AUC in EX v CO
						EI	Test meal 4h post-condition	Covert *ad libitum* buffet (multiple items)		↔ EI but ↓ relative EI in END, HIIE and SHIIE v CO
Panissa *et al.*, (2016)		F (n=9) and M (n=11)	Inactive, H and OW	Cont. at 60% maximal load (MOD) v 60x8s interspersed with 12s recovery at 100% maximal load (HIIE) v 60x8s "all out" interspersed with 12s recovery (VHIIE) v resting control (CO)	Cycling (ergometer)	Subjective appetite	Pre and post-Condition	VAS	N/A	↓ hunger post-HIIE and VHIIE v CO
						Appetite/ satiety hormones		Venous blood samples		↓ AG AUC in VHIIE v CO
						EI	Test meal	Covert *ad libitum* buffet (multiple items)		↔ EI, but ↓ relative EI in MOD, HI and HIIE v CO
Broom *et al.*, (2017)	a) 21±2	a) M (n=9)	a) Active, H and OW	a) Cont. until 2510kJ EE achieved	a) 50% VO_{2peak} (LO; ~55 minutes) v 75% VO_{2peak} (HI; ~36 minutes) v resting control (CO)	a) Running (treadmill)	a) Pre and post-condition	a) VAS (hunger only)	a) Mean HR: ↓ in LO (136±15 bpm) v HI (163±19 bpm)	a) ↔ hunger
								Venous blood samples	Mean RPE (6-20): ↑ in HI (14±2) v LO (12±1)	↓ AG immed. post-HI v LO and CO and up to 30 minutes post-HI v CO, ↓ AG AUC in HI v CO
	b) 23±2	b) M (n=9)	b) Active, H	b) Cont. for 45 minutes (SHO) v 90 minutes (LON) v resting control (CO)	b) 70% VO_{2peak} v	b) Running (treadmill)	b) Pre and post-condition	b) VAS (hunger only)	b) Mean HR: ↔ in SHO (169±11	

						resting control (CO)			Venous blood samples	bpm) v LON (169±12bpm) Mean RPE 6-20): ↔ in SHO (13±1) v LON (14±1)	b) ↓ hunger during and post-SHO v CO and during and post-LON v CO, ↓ hunger AUC in SHO and LON v CO ↓ AG AUC in SHO and LON v CO
Hallworth *et al.*, (2017)	31±8	F (n=9)	Active, H	Cont. for 30 minutes (END) v intermit.: 6x30s interspersed with 4 minutes recovery (HIIE)	65% VO_{2max} (END) v "all out" (HIIE) v resting control (CO)	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones	Pre and post-condition	VAS Venous blood samples	Peak HR: ↑ in HIIE (172± 8bpm) v END (153± 15bpm), ↔ av. HR between HIIE (136± 14bpm) and END (137± 15bpm)	↓ hunger 90 minutes post-HIIE v END and CO ↑ GLP-1 immediately post and 90 minutes post-HIIE and MOD v CO
Holliday & Blannin, (2017a)	34±12	F (n=4) and M (n=4)	Inactive, OW	Intermitt.: 4 x 30s interspersed with 3 minutes recovery (HIIE)	"all out" (HIIE) v resting control (CO)	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones	Pre and post-condition	VAS (composite score) Venous blood samples	Av. HR for HIIE: 131±21bpm Av. RPE (6-20) for HIIE: 17±2	↓ subjective appetite immed. post and up to 30 minutes post-HIIE v CO ↓ AG immed. post and up to 2h post-HIIE v CO, ↑

							EI	Test meal 2h post-condition	Covert *ad libitum* buffet (multiple items)		GLP-1 AUC in HIIE v CO ↔ EI or relative EI
Larsen *et al.*, (2017)	48±5	M (n=12)	Inactive, OW	Cont. for 50 minutes (EX)	75% VO_{2peak} (EX) v resting control (CO)	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones	Pre and post-condition	VAS Venous blood samples	N/A	↔ subjective appetite ↓ AG immed. post-EX v CO
Matos *et al.*, (2017)	27±5	M (n=10)	Inactive, OW and OB	Intermitt.: 10 x 1 minute interspersed by 1 minute recovery (HIIE) v cont. for 20 minutes (MOD)	90% HR_{max} (HIIE) v 65% HR_{max} (MOD)	Walking (treadmill)	Subjective appetite	Pre and post-condition	VAS	↑ HR during intervals of HIIE (range 86-93% HR_{max})↑ v MOD (range 61-75% HR_{max}) ↑ RPE during intervals of HIIE (range 15-19 HR_{max}) v MOD (range 8-11)	↔ hunger, satiety or prospective food consumption, ↑ fullness immed. post HIIE v rest (but not MOD)
Shamlan *et al.*, (2017)	23±4 (F) and 24±5 (M)	F (n=19) and M (n=21)	Active, H and OW	Until 950kJ achieved: cont. (LO) v intermitt.: 60x8s interspersed with 12s recovery (HIIE)	50% VO_{2peak} (LO) v 8s at 95% VO_{2peak} (HIIE)	Cycling (ergometer)	Subjective appetite Appetite/ satiety hormones EI	Pre and post-condition Test meal 110 minutes post-	VAS Venous blood samples Covert *ad libitum* buffet (one-item,	N/A	↔ subjective appetite ↔ GLP-1

								condition and for 2d following	pasta meal) and weighed food diary for 2d following		↔ EI or macronutrient intake at test meal or for 2d following
Matos *et al.*, (2019)	28±3	M (n=12)	Inactive, OW and OB	Intermitt.: 10 x 1 minute interspersed by 1 minute recovery (HIIE) v cont. for 20 minutes (MOD) v resting control (CO)	90% HR_{max} (HIIE) v 70% HR_{max} (MOD)	Walking (treadmill)	Subjective appetite	Pre and post-condition	VAS	↑ mean HR during HIIE (83.7±6.8% HR_{max})↑ v MOD (70.5±1.1% HR_{max}) ↑ BLa immed. following HIIE (12.5±2.6mmol·L^{-1} v MOD (4.9±1.4 mmol·L^{-1})	↓ hunger immed. post-HIIE v CO, ↓ prospective food consumption immed. post-HIIE v CO, ↔ satiety, ↔ fullness
							Satiety hormone		GLP-1		↑ GLP-1 1 hour post-MOD v CO and a trend for ↑ GLP-1 1 hour post-HIIE v CO
							EI	Test meal 1 hour post-condition and for remainder of testing day	Covert *ad libitum* buffet (multiple items) and weighed food diary for remainder of testing day to calculate 24-hour EI		↔ EI at test meal and ↔ 24-hour EI

'BLa' denotes 'blood lactate concentration', 'bpm' denotes 'beats per minute', 'Cont.' denotes 'continuous', 'EE' denotes 'energy expenditure', 'EI' denotes 'energy intake', 'F' denotes 'females', 'HR' denotes 'HR', 'H' denotes 'healthy BMI', 'Immed.' denotes 'immediately', 'M' denotes 'males', 'OB' denotes 'obese BMI', 'OW' denotes 'overweight BMI', 'RPE' denotes 'rating of perceived exertion', 'VAS' denotes 'visual analogue scale', '↔' denotes 'no change', '↓' denotes 'significant reduction', '↑' denotes 'significant increase'

2.8 Study design considerations when exploring appetite and eating behaviour responses to acute PA

Given the discrepancies between changes in appetite and actual eating behaviour in studies previously mentioned and those reviewed in table 2.1, it is pertinent to consider the differences in study designs that may be, at least partly, responsible for such discrepancies. Indeed, study design likely plays an important role in better detecting (or not detecting) intensity-dependent changes in appetite and eating behaviour. Knowledge of moderating and mediating factors of acute exercise and its effects on appetite, eating behaviour and energy balance responses also aid in informing and assessing study designs.

The transient nature of appetite suppression with 'exercise-induced anorexia' (King, Burley and Blundell, 1994) is well established. Suppressions in appetite following exercise may be so transient that they are no longer present by the time EI has been assessed in a number of previous studies (see table 2.1). In turn, it could be that study designs exploring the effects of acute exercise on EI have limitations that restrict observing true differences in actual eating behaviour.

Studies that employ a test meal following an acute bout of exercise have done so at predetermined time points ranging from 45 minutes (Deighton *et al.*, 2013a), 70 minutes (Sim *et al.*, 2014; Beaulieu *et al.*, 2015), 90 minutes (Martins *et al.*, 2015), 110 minutes (Shamlan *et al.*, 2017) to 120 minutes following exercise (Panissa *et al.*, 2016; Holliday and Blannin, 2017a). Although Sim *et al.* (2014) did indeed see differences in EI with different intensities of acute exercise, the majority of other studies have failed to see any differences in EI despite seeing suppressions in subjective and/or objective appetite during and following exercise so it is argued that these studies are missing the transient window by assessing EI at these time points (Deighton *et al.*, 2013a; Beaulieu *et al.*, 2015; Martins *et al.*, 2015; Panissa *et al.*, 2016; Holliday and Blannin, 2017a). Meals timed sooner post-exercise (or exercise timed sooner prior to meal times) will likely better detect true differences in eating behaviour and EI and certainly give rationale for further study. Greater hunger ratings and EI have been demonstrated when test meals are administered at 60 minutes after exercise compared with 0, 30 and 120 minutes after exercise (Verger, Lanteaume and Louis-Sylvestre, 1992). Verger, Lanteaume and Louis-Sylvestre (1992) however, employed a moderate-intensity continuous

bout of exercise; while findings are unlikely to reflect similar observations seen with high-intensity exercise and/or HIIE, this does suggest that the timing of exercise and/or the test meal are critical in detecting differences in eating behaviour and EI responses.

King, Wasse and Stensel (2013) were the first to document that studies designed with test meals at predetermined time points also likely restrict freedom of eating behaviour, which limit the translation of findings in previous studies into a free-living setting where, typically, fewer restrictions on eating behaviour exist. In doing so, King, Wasse and Stensel (2013) corroborated the findings of King, Burley and Blundell (1994) whereby onset of eating was delayed following an acute bout of high-intensity (72% VO_{2peak}) continuous (60 minutes) running. King and colleagues (2013) found feeding latency (time from cessation to voluntary eating initiation) was increased (by ~35 minutes) following exercise compared with resting control. Despite this, King, Wasse and Stensel (2013) did not see any differences in EI. However, a voluntary delay in feeding should, in itself, be considered an anorexigenic appetite response as in unrestricted conditions it could lead to a reduction in EI across the post-exercise period that extends beyond the transient window. Thus, it is of interest for studies to be designed so that feeding latency and/or timing of feeding is considered.

At this point, it should also be noted that the type of test meal used also likely influences the measures of eating behaviour and EI detected. Studies range between using one item test meals and multiple-item *ad libitum* buffet test meals. Ueda *et al.* (2009a) utilised a one-item test meal design where a bowl was repeatedly filled with pasta as participants were eating to allow the blinding of participants to the amount eaten such that this could not act as a potential cue to cease eating. Moreover, a one-item meal design focuses on differences in EI without influence from differing food choices and macronutrients, (Blundell *et al.*, 2010). However, one-item test meals may be restricted by boredom (Blundell *et al.*, 2010) compared with composite or multiple-item test meals. Multiple-item test meals can also indeed promote overconsumption if there is great variety, palatability and volume of each food, however offer better external validity and allow detection of differences in macronutrient intakes which could better detect any true differences in eating behaviour (Blundell *et al.*, 2010).

The findings of Sim *et al.* (2014) also highlight the importance of not only exploring the effects on food, energy and macronutrient intake at test meals, but also beyond this time point as

they found free-living EI remained lower for up to 38 hours following 24 x 15s cycling at 170% VO_{2peak} (interspersed with 1 minute at 32% VO_{2peak}) compared with resting control and 30 minutes cycling at 60% VO_{2peak}. In turn, a reduction in EI was seen in inactive females on the day following a bout of moderate-intensity exercise in the work of Rocha *et al.* (2015), with a reduction in EI seen three days following the exercising day in inactive males (Rocha *et al.*, 2013). With regard to manipulating energy balance for weight management strategies, it is imperative to therefore consider the effects on EI (as well as EE) beyond the immediate post-exercise effects and also beyond the laboratory environment in a 'free-living' setting.

It is apparent that many studies, including all of those exploring HIIE, utilise either running or cycling protocols and thus require specific apparatus and/or facilities. Given the barriers and correlates to regular PA, as previously discussed (see section 2.2), there is a strong rationale to explore the appetite and eating behaviour responses to an apparatus-free mode of HIIE. In turn, this would particularly be of interest to an overweight/obese, inactive and/or female population given the apparent sparsity of investigation of this population in this area and the current public health climate. These populations should be the focus for future research in order for implications on energy balance to be meaningful.

It is clear that study design plays an important role in detecting changes in appetite and eating behaviour in response to acute exercise. Studies that assess appetite and EI during the transient period following acute HIIE are warranted, as well as the assessment of feeding latency following exercise. Furthermore, assessments of EI (and EE) responses in the hours and days following acute exercise is clearly of importance. Delayed compensatory responses in EI seen in Sim *et al.* (2014) and Rocha *et al.* (2015) may be of less relevance where exercise is repeated and accumulated across a number of days, as would be recommended in an exercise intervention. In doing so, there should be consideration of a test meal that offers good external validity without promoting opportunity for overconsumption, as well as exploration of both EE and EI in the hours and/or days following acute exercise for greater inference on energy balance implications.

2.9 The role and mechanisms of medium and long-term physical activity on appetite, eating behaviour and energy balance

The relationship between medium and long-term PA levels and EI is one that has been explored in several seminal studies in the field. For the purpose of this review, 'medium-term' PA' encompasses the studies that have explored these measures in observational studies for a duration of up to 2 weeks, while 'long-term' PA includes studies exploring these measures for over 2 weeks. Initially, Edholm and Fletcher (1955) found a positive association between EI and EE over two weeks in a population of 12 army cadets. Of note, EI and EE were only associated when averaged across a weekly or fortnightly period, with no relationship on any single day, demonstrating the variability in day-to-day EI. Soon following this, Mayer, Roy and Mitra (1956) explored the relationship between daily EI and categories of work within mill workers that ranged from 'sedentary' to 'very heavy work' (in doing so, assuming that EE for each of these jobs was related to this category of the physical effort required). Mayer, Roy and Mitra (1956) provided evidence of a J-shaped relationship between EI and physical effort/EE of these workers (see figure 2.6) where the relationship between chronic EE and EI is linear at expenditures of 'light work' or above and a reduced body mass at levels of 'light work' and above. However, in 'sedentary' workers (or at very low levels of activity-induced EE) such a relationship is absent, whereby EI is increased relative to the EE of this category. Hence, Mayer, Roy and Mitra (1956) proposed that a "non-regulated zone" of appetite control exists at such low levels of chronic EE. It is likely that this was not observed by Edholm and Fletcher (1955) due to the typically large activity-induced EE of army cadets. Following this, Edholm and colleagues (1970) replicated a similarly tight coupling between EE and EI in a larger population of 31 army cadets over one week, aligning with the 'J-shaped curve' proposed by Mayer, Roy and Mitra (1956). Again, no relationship within a single day was found, suggesting the absence of a tightly-controlled day-by-day coupling of EI with EE even for those within this "regulated zone".

The "non-regulated zone" at very low levels of activity-induced EE suggests that an energy imbalance (whereby EI is greater than EE) is likely to occur at these levels, leading to weight gain. Given that, globally, one in four males and one in three females self-report not meeting PA guidelines (Guthold *et al.*, 2018), this currently has great relevance to public health. Following a number of decades where these findings and their relevance were somewhat

neglected, Murgatroyd *et al.* (1999) demonstrated the lack of downregulation of EI following an acute period of inactivity, as well as the lack of increase in PA following overfeeding, across two days in inactive males. Although it is acknowledged that this is by no means a chronic period of PA *per se*, again this aligns with previous findings regarding an uncoupled relationship between EE and EI at low levels of chronic PA on the 'J-shaped curve' (Mayer, Roy and Mitra, 1956), when energy balance is challenged.

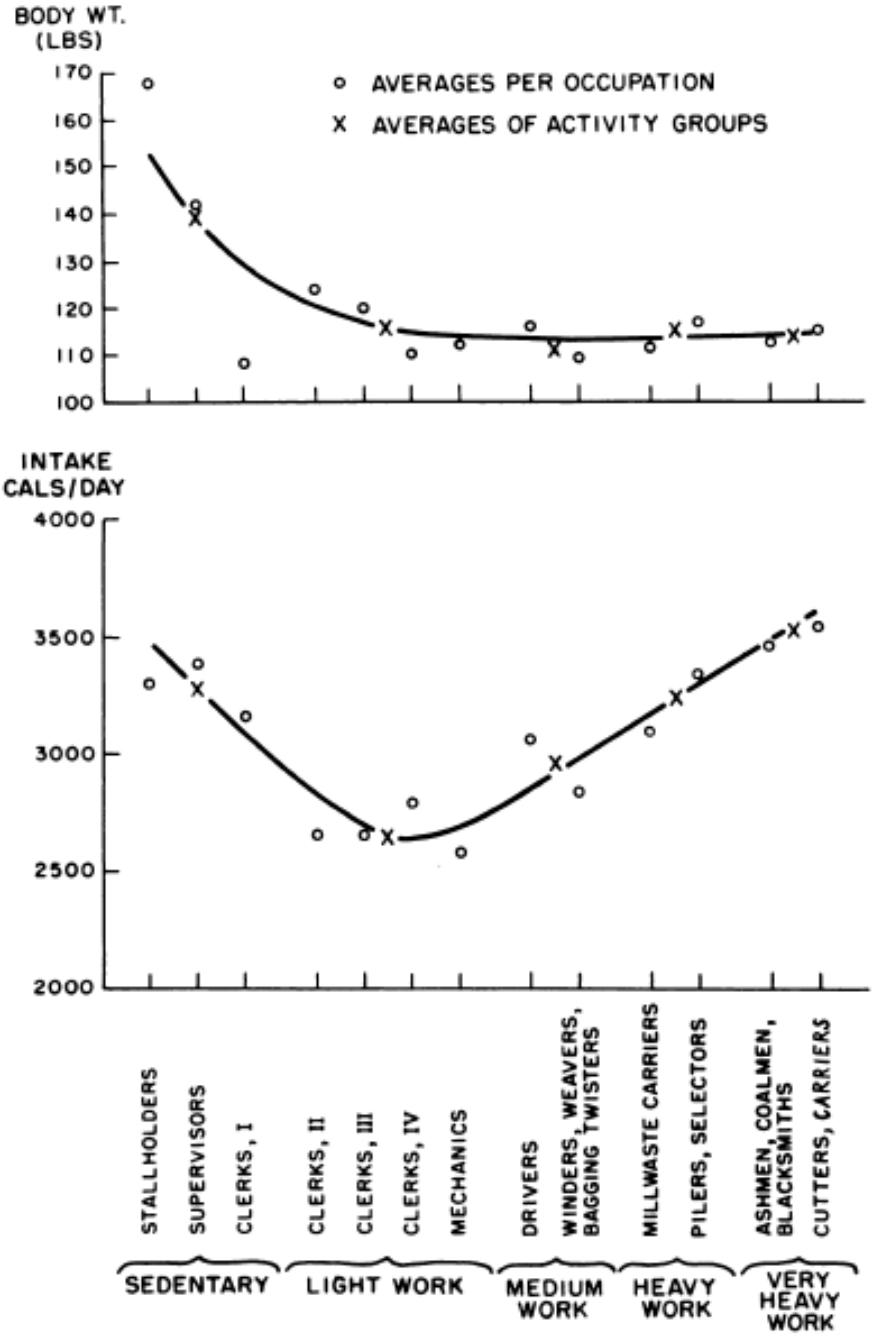

Figure 2.6 The relationship between occupation activity levels and energy intake per day from Mayer, Roy and Mitra (1956)

Similarly, although not a chronic period, a linear relationship between 24-hour EE and daily EI has also been demonstrated in a population of 184 adults over three days (Weise *et al.*, 2014). However, Weise *et al.* (2014) did not record actual levels of PA so it is difficult to ascertain whether this challenges the 'J-shaped curve' or whether this provides support for the linear relationship between daily EE and EI if participants were all somewhat active and not in the 'non-regulated' zone of appetite control.

Of note, when energy deficit is achieved through caloric restriction (reducing EI) at a breakfast meal in active females, appetite following breakfast and EI at a test meal 4 hours following breakfast are both increased (Hubert, King and Blundell, 1998). However, when achieved through exercise (increasing EE), such compensatory responses in appetite and EI are absent (Hubert, King and Blundell, 1998). This is in contrast with both Edholm and Fletcher (1955) and Edholm *et al.* (1970) where no relationship was found between EE and EI within a single day, although discrepancies may be due to the army cadets of Edholm and Fletcher (1955) and Edholm *et al.* (1970) being subject to changes in EE and not subject to caloric restriction (Edholm and Fletcher, 1955). More recently, a cross-sectional study by Beaulieu *et al.* (2017) demonstrated that individuals with greater PA levels (>112 minutes moderate-vigorous-intensity physical activity (MVPA) per day for men and >90 minutes MVPA per day for women) demonstrated a lower EI at a meal following a high energy preload meal compared with a low energy preload meal, whereas this discrepancy was not seen in men nor women who did not meet these thresholds of MVPA. In addition, a suppression of hunger was found in these individuals following the high energy preload meal compared with the low energy preload meal, compared with inactive individuals not meeting these PA thresholds (Beaulieu *et al.*, 2017). These findings also highlight the benefits of exercise and PA with regard to energy balance and weight management, additional to its benefits to metabolic health.

The observational, medium-term findings discussed are certainly of great interest and importance, yet such cross-sectional findings fail to propose any cause and effect, and short term studies cannot be extrapolated to predict longer term responses. Hence, findings which are likely to offer the greatest impact on public health would be those from longer term interventions that investigate modulations in energy balance following manipulation of either side of the energy balance equation (of which increasing activity-induced EE has greater relevance to the purpose of this thesis). Nonetheless, a recent review that incorporates much

of the recent intervention studies demonstrated that, similarly, there is improved coupling of EI and EE with chronic exercise or high habitual PA levels, while inactive individuals have blunted satiety signalling (Drenowatz, do Carmo Greier and Greier, 2019). What remains unknown, is the duration of chronic levels of habitual PA required to see such effects.

Martins, Truby and Morgan (2007) showed EI was reduced in response to a high energy preload meal following six weeks of continuous moderate-intensity exercise (varying modes of exercise), compared with that following a low energy preload meal, in inactive adults. Authors did not see differences in postprandial fullness following either preload after the intervention, therefore suggesting that any modulated sensitivity to episodic signal release did not influence the reductions in EI following the high energy pre-load meals following the intervention, although episodic hormones were not measured. Nonetheless, this supports the phenomenon that increased chronic activity-induced EE levels promote tighter coupling of EI and EE in order to maintain energy balance.

In turn, King *et al.* (2009) found a greater subjective satiety following a standardised breakfast meal in overweight and obese individuals following twelve weeks of moderate-intensity exercise (varying modes of exercise), compared with baseline. However, episodic appetite signals were not assessed in this study and were only speculated to be somewhat responsible for the findings. Authors did not specify the baseline activity levels of the participants but again, these findings align with the original findings of Edholm and Fletcher (1955) and Mayer, Roy and Mitra (1956) that proposed tighter control of EE and EI with increased activity-induced EE over the longer term. Interestingly, King *et al.* (2009) found a paradoxical increase in fasting hunger and hunger across the day, with this being described as a 'dual-process' action of increased PA levels on appetite control.

Martins *et al.* (2010) were the first to replicate this existence of a 'dual-process' response following twelve weeks of moderate-intensity exercise (walking or running) in inactive, overweight/obese adults compared with baseline. Authors demonstrated increased fasted AG and fasted subjective hunger, but also a reduction in postprandial insulin and a tendency for increased postprandial GLP-1 and reduced postprandial AG (Martins *et al.,* 2010). Therefore, this is a hormone profile that reflects the changes seen in fasted hunger and satiety ratings. Increased fasted appetite does appear somewhat paradoxical, yet this seems to be a fairly consistent finding as it was also demonstrated by Caudwell *et al.* (2013a), along with

improved subjective satiety responses to a standardised breakfast meal following a twelve weeks of moderate-intensity exercise (varying modes of exercise) in previously inactive, overweight/obese males and females.

Despite the subjective and objective appetite responses seen in response to a standardised meal, Martins *et al.* (2010) also did not demonstrate any changes in daily EI in either sex as a result of the intervention employed. Arguably, as in the shorter-term, modulations in actual eating behaviour and EI are the most important since these will influence energy balance. Martins, Morgan and Truby (2007) did also not demonstrate any changes in daily EI despite differences in eating behaviour in response to differing preload meals. Nonetheless, both Martins, Morgan and Truby (2007) and Martins *et al.* (2010) did instruct participants to avoid changing their food intake throughout the intervention. Although this control does isolate the exercise intervention, it is also likely at least partly responsible for a lack of observation in EI changes and the question as to whether differences in eating behaviour would have arisen otherwise remains.

Despite the appetite-suppressing effects of acute HIIE (see section 2.7), there has been little exploration of energy balance responses to HIIT interventions. Martins *et al.* (2017) were the first to explore the effects of a HIIT intervention on appetite and eating behaviour in an inactive and obese population. Twelve weeks of intermittent cycling (8s cycling at 85-90% HR_{max} interspersed with 12s recovery) for the duration of either 10 minutes or 20 minutes resulted in similar responses in appetite and eating behaviour responses between the two HIIT interventions, as well as a moderate-intensity cycling (~30 minutes at 70% HR_{max}), each performed three times per week. Following the intervention, ratings of fasted and postprandial hunger increased (with no group differences), while there were no changes in either fasted or postprandial fullness or concentrations of AG, PYY and GLP-1. In turn, there were no differences in EI, although again participants were asked to maintain their habitual diet which is likely the reason for this finding. Martins *et al.* (2017) concluded that the exercise volume (of all groups) was insufficient to induce the changes seen in appetite with previous studies. Previous studies certainly used intervention designs where participants exercised four or five times per week (Martins, Morgan and Truby, 2007; King *et al.*, 2009; Martins *et al.*, 2010; Caudwell *et al.*, 2013a).

A recent systematic review found no consistent evidence to suggest that either acute or chronic (up to 2 weeks) increased PA levels have an effect on energy or macronutrient intake (Donnelly *et al.*, 2014). This finding may, hence, question the potency of such an improved regulation of appetite and satiety responses in free-living settings and the effects on actual eating behaviour over longer durations. Nonetheless, Donnelly *et al.* (2014) highlighted notable methodological shortcomings that lessen the ability to detect any existing relationship including study power, quality and regularity of EI and EE assessment, and a limited focus on overweight/obese individuals and exercise parameters. Future work should look to overcome these in order to have a better insight into the relationship between EE and EI with bettered public health relevance.

Non-homeostatic control of appetite and eating behaviour (food hedonics and behavioural aspects of eating) is also important to consider as Rocha *et al.* (2016) demonstrated a reduction in overall total food cravings, as well as a reduction in food cravings for high-fat foods, fast-food fats and carbohydrates following a moderate-intensity continuous exercise intervention over twelve weeks in inactive males. Yet, similar to previous findings, both eating behaviour and EI remained unchanged following the intervention. Of note, EE also remained unchanged following the exercise intervention which suggests that either compensatory behaviours through non-exercise activity expenditure occurred, or could be due to EE measurement error. Body mass, BMI and waist and hip circumferences were all reduced following the intervention, hence suggesting energy deficit and exercise-induced weight loss did occur in some way with EI and EE. In turn, it therefore cannot be ruled out that the improvements in non-homeostatic appetite control seen are partly due to reductions in body mass and likely improvements in body composition. Nonetheless, longer term exercise interventions should explore the effects on such non-homeostatic appetite control in addition to homeostatic appetite control, eating behaviour and EI. Again, it would also be of interest to explore any changes in these measures both in presence and absence of modulations in body mass and body composition in order to isolate those induced by PA only.

Many longer term free-living measures of eating behaviour and EI are obtained through self-report food diaries. There are various limitations with self-report methods (Hill and Davies, 2001) and it is argued that they should not be used to demonstrate true EI (Subar *et al.*, 2015). Indeed, self-report food diaries were utilised as a measure of EI in both Martins, Morgan and

Truby (2007) and Martins *et al.* (2010) which may also be partly responsible for a lack of change in daily EI seen in these studies. It should also be noted that the limitations associated with self-report food diaries are somewhat eliminated with employing a laboratory-based test meal, which offers better environmental control although reduces external validity. Nonetheless, no differences were seen in daily EI between baseline and at the end of the intervention when measured in a laboratory environment by Caudwell *et al.* (2013a).

Longer term intervention studies suggest bettered appetite control and possibly better matched EI with EE as PA levels increase, however, further research would elucidate mechanisms responsible for this. In turn, if, and to what extent, body weight and body composition play a role within this relationship should be further considered. Given the potential appetite suppressive and/or EI reducing effects of HIIE, further research should explore the role of HIIE within a longer term intervention for regulation of energy balance and weight management.

2.10 The role of bodyweight and body composition within the relationship between chronic PA, appetite, eating behaviour and energy balance

It is noteworthy that many longer-term exercise interventions exploring the relationship between increased PA, appetite regulation and eating behaviour also saw reductions in body mass (which likely derive from modulations in body composition). Given the role of body composition in the regulation of energy balance (figure 2.3), such changes observed in appetite and eating behaviour may be, at least in part, mediated by modulations in body composition induced by the exercise interventions. Notwithstanding the importance of both increasing PA levels and reducing body mass and fat mass in the current public health climate, it is difficult to isolate the cause of changes in appetite control to either body mass loss (or body compositional modulation) or increased PA alone.

Findings from Beaulieu *et al.* (2017) demonstrate a reduced EI at a meal in response to a high energy preload meal compared with a low energy preload meal in those with greater PA levels compared with lower PA levels. However, body mass nor BMI did not differ between these groups suggesting again that PA levels play a role in appetite control and energy balance regulation independent of body mass. Nonetheless, it could be argued that in the Beaulieu *et*

al. (2017) study the 'low' MVPA groups for both males (<112 minutes MVPA per day) and females (<90 minutes MVPA per day) are still fairly high levels of PA relative to the general population and so implications for the current public health climate can be questioned. These findings support those of an earlier cross-sectional study, which showed that those doing regular PA had improved compensation in EI following high and low energy preload meals (Long, Hart and Morgan, 2002). Causal inference cannot be obtained from these findings, yet Martins, Truby and Morgan (2007) went on to demonstrate that a moderate-intensity, continuous exercise intervention in an inactive population improved compensatory EI in response to high and low energy preload meals too, despite not seeing reductions in body mass nor percentage body fat. Furthermore, postprandial GLP-1 was increased following a standardised meal after five days of moderate-intensity continuous exercise, understandably with no loss in body mass or body composition change observed in overweight and obese adolescents (Chanoine *et al.*, 2008). This would suggest increased postprandial subjective satiety however, Chanoine *et al.* (2008) did not find this when measured subjectively. Again, this questions the relationship between episodic hormones and subjective measures of appetite and satiety. Nonetheless, these findings suggest that increasing PA alone still has a role in improving appetite regulation and manipulating energy balance regardless of modulations in body mass.

Reductions in body mass, trunk and leg fat mass, waist and hip circumference were seen in each exercise group in the study of Martins *et al.* (2016) as well as increases in trunk and leg fat free mass (FFM). Yet, Martins *et al.* (2016) did not see any improvements in appetite control which again does question the role of body composition at least being a direct mechanism in this relationship. Martins *et al.* (2013) also concluded that the improved accuracy of compensation for previous EI demonstrated following twelve weeks of moderate-intensity, continuous exercise in inactive, overweight/obese adults was independent of the body mass loss. Ultimately, this is somewhat reasonable given that episodic hormones that control appetite and eating behaviour to some extent are not released from adipose tissue, therefore hormone-induced changes in appetite and eating behaviour are unlikely to be influenced by body mass loss in this manner. Exploring the effects of an exercise intervention on appetite and eating behaviour and episodic appetite hormones in the presence and

absence of body mass loss and/or body compositional modulation would be of great interest to further elucidate the possibility and potency of such mechanisms.

Nonetheless, King *et al.* (2009) found improvements in satiety response to a meal were associated with reductions in body mass (although authors did not assess changes in body composition) following twelve weeks of laboratory-based aerobic exercise. Martins *et al.* (2010) also saw reductions in body mass and fat mass alongside improvements in subjective and objective appetite control while Caudwell *et al.* (2013a) saw reductions in both body mass and fat mass also following twelve weeks of laboratory-based aerobic exercise alongside improved subjective satiety to a standardised breakfast. In turn, Gibbons *et al.* (2017) demonstrated greater suppressions of AG and greater increases in GLP-1 and PYY with greater body mass loss following twelve weeks of moderate-intensity exercise. It is therefore plausible that such improvements in appetite regulation could be somehow mediated by reductions in body and/or fat mass seen in these studies. It is difficult to conclude whether it was the increase in PA levels, or changes in body mass/body composition, that lead to improved appetite control.

Beaulieu and colleagues (2018) also propose that, as well as the weakened satiety response, at low levels of PA there is a greater input from non-homeostatic control of eating behaviour and EI (see figure 2.7). Given the negative association between PA levels and levels of overweight and obesity (Health and Social Care Information Centre, 2015), Beaulieu *et al.* (2018) suggest that this heightened non-homeostatic control of appetite is mediated by a higher body fat in inactive individuals. Negative relationships between levels of PA and disinhibition (or a lack of restraint with regard to food intake) appear to be mediated by body fat and body mass (Shook *et al.*, 2015; Myers *et al.*, 2017), suggesting that body fat and/or body mass (somewhat determined by body fat mass) likely plays a role in this non-homeostatic control of eating behaviour. However, a causal relationship and its potential mechanism between inactivity, body fat and weaker non-homeostatic control of eating behaviour remains to be elucidated.

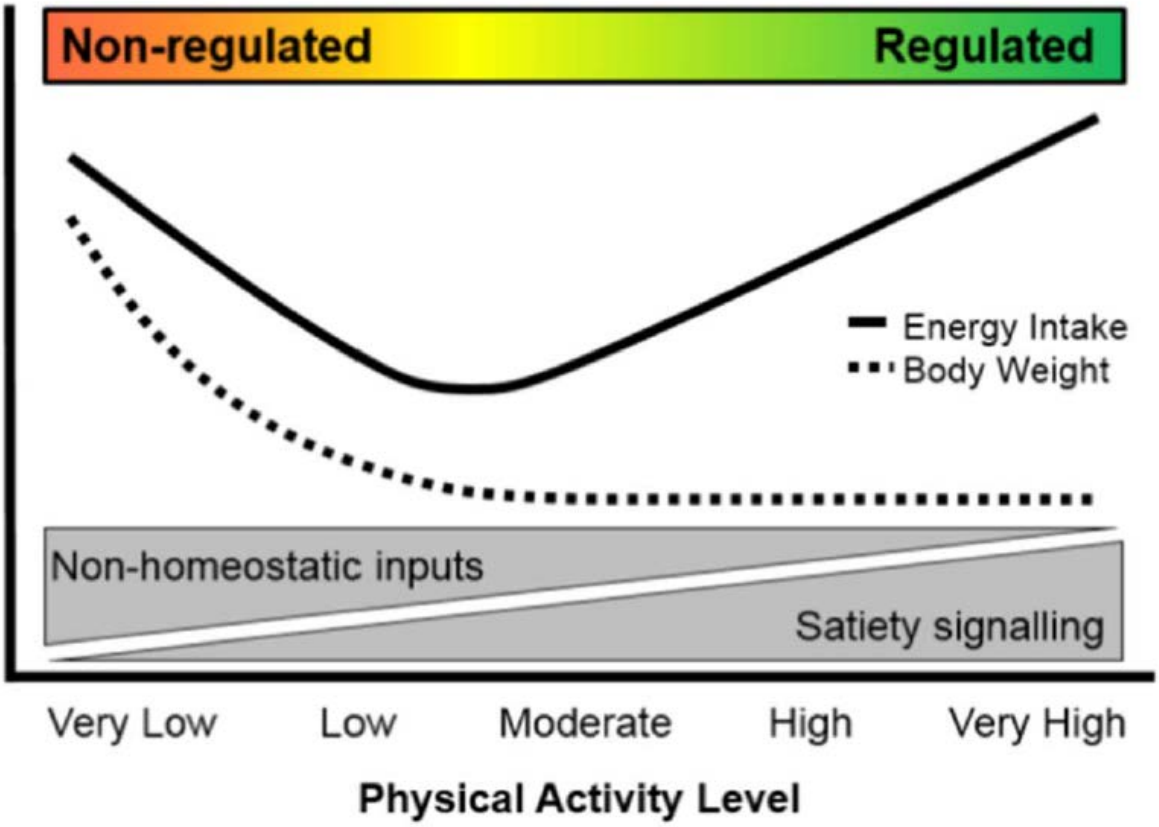

Figure 2.7 The updated J-shaped curve from Beaulieu et al. (2018) depicting appetite control through non-homeostatic and homeostatic control along the continuum of physical activity levels, including the relationship between body weight and physical activity levels

FFM has also been found to be positively associated with daily EI as well as daily protein (PRO), carbohydrate (CHO) and fat intake (FAT; Weise *et al.*, 2014). Hopkins and Blundell (2016) also demonstrated a positive association between FFM and daily EI while both Blundell *et al.* (2012) and Hopkins and Blundell (2016) acknowledged that FFM is a major contributor to BMR. As BMR is also positively correlated with daily EI, BMR likely mediates the relationship between FFM and EI (Blundell *et al.*, 2012; Hopkins and Blundell, 2016). In turn, BMR has also been found to be positively associated not only with EI but also feelings of hunger in overweight and obese individuals (Caudwell *et al.*, 2013b). There appears a negative relationship between fat mass and EI in lean individuals, but this relationship weakens as fat mass increases relative to FFM (Cugini *et al.*, 1998; Cugini *et al.*, 1999). It has been proposed that associations seen between fat mass and EI can indeed be explained by FFM relative to fat mass in overweight and obese individuals (Weise *et al.*, 2014) and that such higher levels

of body fat blunt satiety signals that would otherwise arise from energy stores to inhibit further consumption (Morton *et al.*, 2006). Indeed, Blundell *et al.* (2015) also found a weak relationship between fat mass and EI with increased adipose tissue, suggesting a weakened role of fat mass specifically in overweight and obese individuals. This demonstrates that body fat mass but also FFM likely play a direct role in the control of eating behaviour, giving rationale to promote favourable changes in body composition for improved energy balance regulation.

It is likely that body mass and body composition have roles in modulating appetite, eating behaviour, EI and/or energy balance and should always be considered alongside modulations in energy balance regulation with increased PA levels. However, changes in appetite, eating behaviour, EI and/or energy balance have been seen with increased PA levels, independent of differences in body mass and/or body composition. Therefore, there is certainly rationale to further explore the exact roles of body mass and body compositional change in control of appetite and eating behaviour.

2.11 Study design limitations on exploring appetite and eating behaviour responses to chronic PA

There is a clear role for increased PA levels for improved regulation and control of appetite and eating behaviour (both with and without body mass loss/body compositional change). In turn, these implications are of great relevance given the current public health climate and the prevalence and implications of both physical inactivity and overweight/obesity (as discussed in chapter 1). Nonetheless, despite the appetite-suppressing potential of high-intensity exercise and the specific advantages of apparatus-free HIIE with regard to the barriers and correlates to regular PA in an inactive and overweight/obese population (as discussed in section 2.2), there has been little investigation toward exploring appetite, eating behaviour and energy balance responses to HIIE-based interventions.

Martins *et al.* (2017) were the first and to the author's knowledge, are the only group to have explored a high-intensity exercise intervention in inactive, obese individuals. Despite Martins *et al.* (2017) not finding any meaningful improvements in appetite regulation, further study investigating the longer term effects on appetite, eating behaviour and energy balance of HIIT

interventions is warranted given the potential benefits of HIIE (especially if time efficiency and affective and enjoyment responses are considered; see section 2.2) and evident appetite-suppressing effects at least with acute exercise (see section 2.7). However, given that these appetite-suppressing effects appear transient, it would be of interest to explore a HIIT intervention where individual HIIE bouts are undertaken within this transient window prior to a meal time. Accumulation of any reductions in EI at the meal following HIIE bouts in this way would be of great interest to explore in a free-living setting, given the promising effects of laboratory-based, acute bouts of high-intensity exercise. In turn, investigating how this may influence longer term homeostatic and non-homeostatic appetite control is also warranted.

Most long term studies exploring the effects of increased chronic PA levels on appetite, eating behaviour and energy balance do so in an inactive and/or overweight or obese population. This gives greater weight to implications that can be inferred from findings to the current public health climate. However, all protocols that have so far done this require specialist apparatus (such as a treadmill, cycle ergometer, rowing machine) and/or facilities and, as a result, are typically always laboratory based and supervised. Given that access to specialist apparatus and/or requirement of particular facilities are both negative correlates of regular PA (Trost *et al.*, 2002; Cerin *et al.*, 2010) and especially so in an overweight, inactive population (Cerin *et al.*, 2010), this gives rationale to explore a free-living intervention where access to apparatus nor facilities is not required. In turn, affective and enjoyment responses should be monitored across a longer term exercise intervention, given the strength of these responses (particularly to high-intensity exercise) in promoting or deterring PA adherence (Trost *et al.*, 2002; Kwan and Bryan, 2010a; Kwan and Bryan, 2010b; Hardcastle *et al.*, 2014; Biddle and Batterham, 2015).

Given the body compositional and metabolic health benefits of exercise and PA, and particularly high-intensity exercise, there is a clear rationale for further study exploring the longer term effects of a HIIT intervention. In turn, it would be of interest to explore HIIE bouts being timed prior to meals such that EI is undertaken and assessed during the transient window of 'exercise-induced anorexia'. In addition, as previously discussed, such an intervention should also align with the knowledge on barriers and correlates to regular PA and should be time efficient, induce positive (or prevent negative) affective and enjoyment responses and not require any specialist apparatus nor facilities.

2.13 Aims of objectives of the thesis

The aims and objectives of the programme of research as part of this thesis are as follows:

Aim 1: To assess physiological, affective and enjoyment responses as well as appetite, eating behaviour and acute energy balance responses to apparatus-free HIIE in inactive, overweight and/or obese females.

> **Objective 1 (Study 1, chapter 4):** Identify an appropriate mode of apparatus-free HIIE that can replicate the physiological responses, as well as better the affective and enjoyment responses, seen with traditional apparatus-based HIIE in inactive, overweight and/or obese females.
>
> **Objective 2 (Study 2, chapter 5):** Determine dose responses to the apparatus-free mode of HIIE of varying intervals (4x30 seconds vs. 2x30 seconds vs. rest), in order to identify the minimum number of intervals required to elicit a meaningful effect on appetite feeding latency, EI and energy balance in inactive, overweight and/or obese females.

Aim 2: To explore the effective utilisation of apparatus-free HIIE bouts within a HIIT intervention for inactive, overweight and/or obese females.

> **Objective 3 (Study 3, chapters 6 and 7):** Incorporate findings from achieving objectives 1 and 2 to develop an innovative approach to increasing PA levels in overweight and obese, inactive females in order to promote meaningful improvements in metabolic health markers, appetite, eating behaviour and energy balance, as well as tackle specific barriers faced by this population, through the incorporation of time-efficient exercise. Objective 3 is split across chapter 6 (focusing on measures of EI, EE, resulting energy balance as well as fasting and postprandial subjective appetite and plasma levels of appetite and satiety-related hormones following a standardised meal) and chapter 7 (focusing on measures of VO_{2peak}, fasted insulin, glucose and lipid profiles as well as markers of affect, enjoyment and social cognitions of the intervention).

Chapter 3

General methods

This chapter will critically justify the methods that are common to the experimental chapters of this thesis (Chapters 4, 5, 6 and 7). Specific protocols and measures are described in individual chapters, including any deviations from the general methods expressed below.

3.1 Study designs

A within-subject, counterbalanced, crossover study design was used for studies 1 and 2 (Chapters 4 and 5), where participants were randomly assigned to each condition to minimise any order effect. The first experimental trial for studies 1 and 2 took place a minimum of five days following familiarisation and a minimum of seven days following familiarisation for study 3 (Chapters 6 and 7). A between-subject design was used for study 3, where participants were randomly assigned to either exercise group (1:1 ratio).

3.2 Ethical approval

For study 1 (chapter 4), ethical approval was obtained from the ethics committee of the Institute of Sport and Exercise Sciences at the University of Worcester, and the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. For studies 2 and 3 (chapters 5, 6 and 7), ethical approval was obtained from the ethics committee of the Institute of Sport and Exercise Sciences at the University of Worcester only.

3.3 Participants and recruitment

Participants were recruited from within and around the areas of Worcester and Birmingham by email, poster, social media and word of mouth.

To ensure applicability of findings to a relevant population as well as suitability for inclusion that would not compromise participant safety nor the validity of findings, participants were only recruited if they met the following criteria:

- Female;
- Self-reporting <150 minutes MVPA·week^{-1} confirmed by <150 minutes leisure time MVPA·week^{-1} on the International Physical Activity Questionnaire (IPAQ; Craig *et al.*, 2003; appendix 1);
- Non-smoking;
- Premenopausal;
- Not pregnant or breastfeeding;
- Not dieting, no intent to diet and not attempting weight loss;
- Resting blood pressure <140/90mmHg;
- No known cardiac arrhythmias;
- No medication known to influence appetite or lipid metabolism;
- No known musculoskeletal metabolic or cardiovascular disorder;
- No pain and/or swelling in hips or knees or disability that precluded ability to undertake cycling, squatting or star jumps.

For study 1, participants were required to have a BMI within the range of 18-35kg·m^{-2} and to be aged between 18 and 50 years. For studies 2 and 3, participants were required to have a BMI within the range of 25-35kg·m^{-2} and to be aged between 18 and 45 years due to the requirements of the ACSM Guidelines for Exercise Testing and Prescription (2013). An additional inclusion criterion of a score of ≤3.5 on the restraint scale on the Dutch Eating Behaviour Questionnaire (DEBQ; van Strien *et al.*, 1986a; appendix 2) was also required for entry into studies 2 and 3.

For all studies, participants arrived following an overnight fast (minimum of 10 hours; water was permitted). Experimental trials took place during the follicular phase of the menstrual cycle (days 1 – 14) for eumenorrheic females and 28±2 days apart for females taking contraceptive medication preventing menstruation. It has been demonstrated that in females taking oral contraceptive pills, phase of the menstrual cycle does not influence the concentration of AG at rest nor during or following exercise (Alajmi, 2014, pp. 80 – 104).

However, phase of the menstrual cycle did influence aspects of subjective appetite such that hunger and prospective food consumption were increased in the follicular phase, while fullness and satisfaction were decreased in the follicular phase, compared with the luteal phase (Alajmi, 2014, pp. 80 – 104). Given this, along with the known effects of menstrual cycle phase on other measures used in this thesis, such as BMR (Solomon, Kurzer and Calloway, 1982), studies of this thesis aimed for all experimental trials to occur during the follicular phase of the menstrual cycle to ensure phase of the menstrual cycle was controlled as best as possible for all regularly menstruating participants.

3.4 Participant enrolment and screening

A single familiarisation visit preceded the first experimental trial in all studies. Familiarisation trials preceded experimental trials by at least five days for studies 1 and 2 and by at least seven days for study 3. For study 3, this was to allow sufficient time for the 5-day EI and EE measurement at baseline prior to the baseline testing day (which was not required for studies 1 and 2). For study 1, participants attended the Human Performance Laboratory at either the University of Worcester or the University of Birmingham while for studies 2 and 3, all participants attended the Human Performance Laboratory at the University of Worcester.

Upon familiarisation, a hard copy of the study information sheet (study 1 – appendix 3, study 2 – appendix 4, study 3 – appendix 5) was given to all potential study participants which explained the purpose and processes of the study, the experimental procedures and the risks and benefits of participation. Participants were offered the opportunity to ask any questions, before signing informed consent (study 1 – appendix 6, study 2 – appendix 7, study 3 – appendix 8). Following this, participants completed a health screening questionnaire (study 1 – appendix 9, study 2 – appendix 10, study 3 – appendix 11) to confirm study eligibility. Participants also completed a blood analysis screening questionnaire (appendix 12) and the IPAQ (appendix 1) for all studies, while the DEBQ (appendix 2) was also completed for studies 2 and 3 to assess restrained eating. Participants were familiarised with all study procedures and measures, described in more detail below, and underwent a submaximal exercise test during familiarisation for study 2 and a familiarisation of a maximal exercise test during familiarisation for study 3.

3.5 Pre-testing day standardisation

For all studies, participants were given food diary instructions (appendix 13) and a weighed food diary record (appendix 14) in which they were asked to record 24-hour dietary (food and drink) intake on the pre-testing day (day prior to their first experimental trial). This was to limit between test variability, as lack of standardisation of food intake on this day has been demonstrated to influence appetite and eating behaviour as well as appetite and satiety hormone concentrations on the testing day that follows (Chandarana, 2009). Participants were instructed that this intake was to be replicated across the day prior to the remaining experimental trials. Participants were also instructed to arrive on the testing day having fasted for at least 10 hours to, similarly, ensure standardised feeding on testing days.

Due to the potential confounding effects on measures and exercise performance, participants were also asked to refrain from caffeine (Astorino and Roberson, 2010; Pesta *et al.*, 2013), alcohol (Pesta *et al.*, 2013) and strenuous exercise on the pre-testing day. Experimental trials occurred at the same time of day within each participant to reduce the influence of circadian rhythm on variables (Consoli *et al.*, 1981).

3.6 Measures

3.6.1 Anthropometry

Measurement of height, body mass and BMI were made in all studies as below:

- Height: measured using a stadiometer (Seca, Birmingham, UK) to the nearest 0.1cm, with no footwear and participants stood flat footed facing directly ahead;
- Body mass: measured using digital scales (Seca, Birmingham, UK for studies 1 and 2; Sartorius, Goettingen, Germany for study 3) to the nearest 0.1kg, with no footwear and single layer clothing;
- BMI: calculated by dividing weight (in kilograms; kg) by the square of height (in metres).

For study 3, waist and hip circumferences were made as below:

- Waist circumference: measured at the narrowest part of the torso (above the level of the umbilicus and below the xiphoid process) to the nearest 0.1cm;
- Hip circumference: measured at the maximal circumference of the buttocks to the nearest 0.1cm;
- Waist-to-hip ratio: waist circumference was divided by hip circumference.

All anthropometric measurements were assessed by the same main female researcher. Waist and hip circumferences were measured in triplicate and a mean value was recorded.

3.6.2 Blood pressure

Blood pressure was assessed as a screening measure during familiarisation trials for all studies and as an experimental measure during trials for study 3. Blood pressure was measured in triplicate (with the mean value recorded) using an automated blood pressure monitor (Omron, Milton Keynes, UK) after a rest period of at least fifteen minutes, with participants seated, relaxed and refraining from talking during the measurements. Additionally, when assessed in experimental trials during study 3, measures were taken when participants were fasted and had refrained from caffeine for at least 10 hours prior to the measure.

3.6.3 Ratings of perceived exertion

During experimental trials for studies 1 and 2, as well as during the free-living intervention of study 3, ratings of perceived exertion (RPE) were assessed for participants' by asking them to rate their '*current perceived level of exertion*' (which was described as how much physical exertion they felt under at that time) using the Borg scale (Borg, 1982; appendix 15) which ranges from 6 (no exertion at all) to 20 (maximal exertion). The RPE scale has previously been shown to act as a reliable and valid tool for monitoring and prescribing exercise intensity as it has demonstrated strong positive correlations ($r=0.74$ and $r=0.83$, respectively, both $p<0.05$) with heart rate (HR) and blood lactate measures in young and middle-aged males and females (Scherr *et al.*, 2013).

3.6.4 Blood glucose and lactate concentration

During experimental trials for studies 1 and 2, capillary blood samples were taken from the fingertip and concentrations of blood glucose and lactate concentration were assessed using a desktop analyser (Biosen C_Line, EKF Diagnostics, Cardiff, UK). Manufacturer-reported coefficient of variation (CV) for the device is ≤1.5%. Validity and reliability of the device has been demonstrated over a range of glucose and lactate values from resting to maximal exercise (~14mmol·L^{-1}; Davison *et al.*, 2000). Coefficients of determination of 0.9675 and 0.9061 were demonstrated between samples assessed using both the Biosen C_Line and the Yellow Springs Instruments (YSI) 2300 (YSI Incorporated, Yellow Springs, Ohio, USA), as well as with the Kodak Ektachem DT II (Eastman Kodak, Rochester, New York), respectively, thus demonstrating high validity of the Biosen C_Line device (Davison *et al.*, 2000). Furthermore, a CV of 1.4% has been demonstrated for test-retest reliability on the Biosen C_Line, with inter-investigator reliability ranging from 1.3 – 3% (Davison *et al.*, 2000).

All whole blood glucose and lactate concentration measures were analysed in duplicate, with the mean value taken, unless the discrepancy between values was >2% in which case a third measure was taken. Calculated CVs for duplicate measurements of blood glucose concentration and BLa were 0.48% and 0.68% for study 1 and 0.92% and 0.76% for study 2, respectively.

Differences in glucose and lactate concentrations between whole blood and plasma should be noted, such that whole blood glucose concentrations are demonstrated to be ~11% lower than plasma glucose concentrations when haematocrit is normal (Kim, 2016). Similarly, although most literature uses whole capillary blood for lactate concentration assessment, this typically only equates to 60-70% of lactate concentration in blood plasma (Boning, 2001). Such differences should be considered when interpreting the findings of studies 1 and 2.

3.6.5 Plasma volume

During experimental trials for studies 1 and 2, at each time point, capillary blood samples were obtained in a 60µL heparinised capillary tube and a 10µl capillary slide for the measurement of haematocrit and haemoglobin concentration, respectively. The 60µL tubes

were centrifuged for a period of 5 minutes at 12000 revolutions per minute (RPM) and haematocrit was manually calculated using a Hawksley reader (Hawksley and Sons Ltd, Sussex, UK). Haemoglobin was measured using a HemoCue Hb 201+ System (HemoCue AB, Ängelholm, Sweden). From this, change in plasma volume from rest (ΔPV) was determined manually using the equations of Dill and Costill (Dill and Costill, 1974). All haematocrit and haemoglobin measures were analysed in duplicate, such that the mean value for each time point was taken when both measures were within ≤5.0% of each other. If the discrepancy between values was greater than >5%, a third measure for haemoglobin was taken, while the outlying haematocrit values were replaced using multiple imputations. Calculated CVs for duplicate measurements of haemoglobin and haematocrit were 0.42% and 0.83% for study 1 and 0.33% and 0.71% for study 2, respectively.

3.6.6 Affective and enjoyment responses

For all studies, affective responses were measured using the Feeling Scale (FS), which is an 11-point scale that ranges from -5 (very bad) to +5 (very good; Hardy and Rejeski, 1989; appendix 16). Participants were asked to rate their 'current feeling' following the words '*while participating in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasant. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. Scientists have developed this scale to measure such responses.*' The FS is a widely used measure of affective valence during and following exercise which is demonstrated to be related to other measures of affective valence including the Felt Arousal Scale (FAS; Svebak and Murgatroyd, 1985), with correlations ranging from 0.51 – 0.88 (Lang, 1980) and 0.41 – 0.59 with alternative affective valence measurement scales (Russell, Weiss and Mendelsohn, 1989). Furthermore, the FS has been demonstrated one of the most commonly utilised measure of affect in HIIE (Stork *et al.*, 2017).

An additional measure of positive and negative affect was assessed in studies 1 and 2 using another widely-used measure of affect: the Positive and Negative Affect Scale (PANAS; Watson, Clark and Tellegen, 1988; appendix 17). This is a 20-item scale formed of one subscale consisting of 10 positive activated affect-related adjectives and one subscale consisting of 10

negative activated affect-related adjectives. Participants were asked to rate how they are feeling 'right now' on a Likert scale of 1 ('very slightly or not at all') to 5 ('extremely') with all scores totalled to determine a total score for both positive activated affect and negative activated affect, each ranging from 10 – 50, in accordance with Watson, Clark and Tellegen (1988; appendix 17). Reliability of the PANAS has been demonstrated, with Cronbach's alpha coefficients ranging from 0.86 – 0.90 and 0.84 – 0.87 for the positive and negative affect subscales, respectively, thus suggesting good reliability of the PANAS (Watson, Clark and Tellegen, 1988; appendix 17).

A measure of enjoyment of the exercise condition was obtained using the Physical Activity Enjoyment Scale (PACES) (Kendzierski and DeCarlo, 1991; appendix 18), which again is shown to be the most commonly used measure of exercise enjoyment in HIIE (Stork *et al.*, 2017). The PACES consists of 18 bipolar items relating to exercise enjoyment (e.g. 'I enjoy it … I hate it') which anchor ends of a 7-point Likert scale, thus participants must rate their attitudes to each item on a 1-7 Likert scale in response to the phrase '*please rate how you feel at the moment about the physical activity you have been doing*' in accordance with Kendzierski and DeCarlo (1991; appendix 18). Of the 18 items, 11 of the items are reverse-scored before the score for each item is totalled which can range from a minimum of 18 to a maximum of 126. Slight adaptations to the wording of the questionnaire were utilised for study 3 (compared with studies 1 and 2), such that the phrase participants were asked to answer the questionnaire in response to was '*please rate how you feel at the moment about the physical activity you have been doing this week*' as a weekly rating of enjoyment was required. Similar to other studies that have used the PACES to assess PA enjoyment each week throughout an intervention (Heisz *et al.*, 2016), this was a preferred method as opposed to taking a measure of enjoyment after all exercise sessions or one singular rating at the end of the intervention. Item-total correlations of the PACES ranged from r=0.35 – 0.89 and r=0.45 – 0.87 in separate studies (Kendzierski and DeCarlo, 1991), while the internal consistency of the PACES was 0.93 when assessed by Cronbach's alpha suggesting good reliability (Kendzierski and DeCarlo, 1991).

3.6.7 Collection and analysis of expired gas

Samples of expired gas were collected and analysed during the familiarisation and experimental trials of studies 2 and 3 using an online gas calorimetry system in the breath-by-breath mode (MetaLyzer 3B, Cortex Medical, Peipzig, Germany). This system has previously been demonstrated to be reliable for exercise testing, such that intraclass reliability coefficients for volume of oxygen (O_2) utilisation (VO_2), volume of carbon dioxide (CO_2) output (VCO_2) and minute ventilation were 0.969, 0.964 and 0.953, respectively, while variability in measurements was slightly reduced in the MetaLyzer 3B device compared with the MetaMax I device (Meyer *et al.*, 2001). Prior to data collection for each test, a three-point calibration was undertaken using a flow sensor, a calibration gas of mixed, and known concentrations of O_2 (14.99%) and CO_2 (5.04%) (Cranlea & Company, Birmingham, UK) while volume was calibrated using a 3L syringe.

Participants wore and breathed through a face mask attached with a head strap (Hans Rudolph, Shawnee, USA) during BMR and exercise tests, where a unit was fitted into the front of the mask consisting of a sample line, a turbine and a volume transducer. For the assessment of BMR, a face mask was utilised instead of a hood system due to practicality and availability, however no differences in BMR, following an overnight fast, have been demonstrated when measured using both hood- and mask-based measurements (Mcanena *et al.*, 1986; Isbell *et al.*, 1991). Expired gas was collected in the sample line and analysed for O_2 and CO_2 volumes using O_2 and CO_2 sensors, respectively.

3.6.8 Exercise tests

Preliminary submaximal exercise test

For study 2, participants undertook a continuous, submaximal graded exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, Netherlands) for the purpose of calibrating the Actiheart activity monitors only (described in more detail below). As assessment of VO_{2peak} was not required for study 2, participants only undertook a submaximal exercise test at study familiarisation. Following recommendations by the ACSM

Guidelines for Exercise Testing and Prescription (2013), this test required participants to cycle at an initial workload of 25W at a constant cadence of 60-70RPM for 3 minutes, before workload was increased in 25W increments every 3 minutes until 80% of age-predicted HR was reached (ACSM, 2013), using the Karvonen formula (Karvonen, Kentala and Mustala, 1957). If this criterion was met mid-stage, participants were instructed to complete the whole stage before cessation of the test. HR (Polar H7, Polar Electro OY, Kempele, Finland) and expired gases were measured throughout the test.

Maximal exercise test

For study 3, as assessment of VO_{2peak} was required, participants undertook a ramped maximal exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, Netherlands). The secondary purpose of this test was to collect submaximal expired gas analysis for the purpose of calibrating the Actiheart activity monitors (see section 3.6.10). An alternative exercise test protocol to that of study 2 was therefore sought to minimise the number of exercise tests and laboratory trials for participants. A test that has previously been utilised in an overweight/obese, inactive population to assess VO_{2peak} as well as calibrate Actiheart activity monitors (Gillen *et al.*, 2013; Gillen *et al.*, 2014) was utilised and consisted of participants pedalling at 50W for 2 minutes before workload was increased by 1W every 2 seconds thereafter, maintaining a cadence of 60-70RPM until volitional exhaustion was reached or the pedal cadence consistently fell below 50RPM. HR (Polar H7, Polar Electro OY, Kempele, Finland) and expired gases were measured throughout the test.

3.6.9 Basal metabolic rate assessment

During the initial experimental trial of study 2 as well as both experimental trials of study 3, BMR was assessed. Participants rested, motionless, in a supine position on a physically comfortable plinth (Compher *et al.*, 2006) in a darkened, quiet room whilst awake wearing a face mask (Mcanena *et al.*, 1986; Isbell *et al.*, 1991). Pulmonary gas exchange was continually measured throughout the duration of the assessment which lasted ≥20 minutes. In order to discount any effect of any PA prior to a measure of BMR, ≥20 minutes rest is required before

assessment (Compher *et al.*, 2006). Moreover, a BMR measurement of 10 minutes duration where the first 5 minutes of assessment is discarded has been demonstrated to give accurate assessments of BMR with a CV <10% (Compher *et al.*, 2006). Therefore, for the final 10 minutes of the total resting time (20 minutes), each VO_2 measurement was multiplied by the respiratory exchange ratio (RER)-specific caloric equivalent of oxygen (appendix 19) to calculate the EE in kilocalories per minute (Frayn, 1983). All values were then averaged to estimate BMR and converted into a value of kcal per day by multiplying the mean value by 1440 (Frayn, 1983). Participants refrained from eating and avoided strenuous PA prior to measurement (Compher *et al.*, 2006).

3.6.10 Energy expenditure estimation

In studies 2 and 3, TEE and AEE were assessed objectively using an Actiheart monitor (CamNtech, Cambridge, UK) which is a device worn on the chest attached either by a chest strap or by ECG pads (Whitesensor WS, Ambu, Ballerup, Denmark), depending on participant preferences to ensure participant comfort and adherence to wear time. Using either attachment methods, the central electrode is placed at the fourth intercostals, while the second electrode is then placed approximately 10cm to the left of the central electrode such that the device remains as horizontal as possible for maximum accuracy. This wearing position is recommended for clean HR data (Brage *et al.*, 2005) and instructions detailing this were provided to each participant for studies 2 and 3 (appendix 20).

The Actiheart device contains both an accelerometer and a HR monitor and uses simultaneous recordings from both to determine AEE. Using accelerometry alone for the measurement of AEE is vulnerable to error due to monitor placement during PA (Yngve *et al.*, 2003), as well as picking up background noise during free-living activities (Steele *et al.*, 2003). As using HR monitoring alone to estimate free-living AEE can also be subject to error (if HR is raised due to factors other than PA, such as stress) using both HR monitoring and accelerometry synchronously negates such disadvantages of either method alone. With the Actiheart device, both HR and accelerometer measures are inputted into a validated branched-model calculation (Brage *et al.*, 2004), then used to determine AEE and this combination of accelerometry and HR has been demonstrated to accurate estimate daily living TEE (Brage *et*

al., 2015), daily living AEE (Assah *et al.*, 2011) as well as AEE specifically during low-moderate PA (Thompson *et al.*, 2006) and walking and running (Brage *et al.*, 2007). Moreover, precision of EE estimations is increased further if HR is individually calibrated (Brage *et al.*, 2015), as described in more detail below. In this thesis, such individual HR-VO_2 calibration curves were determined through an individual assessment of HR and VO_2 during an exercise test (as described in section 3.6.8 and described in further detail in chapters 5 and 6) and the 'Group Cal JAP2007' energy model available in the Actiheart software (version 4.0.116, CamNtech, Cambridge, UK) was used.

For both study 2 and 3, recordings during wear time of the Actiheart device were made in 15 second epochs (time in between recordings). Data was downloaded, processed and analysed within the Actiheart software which outputted both AEE (as described above) and TEE which was calculated as the sum of BMR, AEE and an assumed 10% daily EI value representing diet-induced thermogenesis (Westerterp, 2004). Total and mean daily METs were calculated, while to calculate % time spent in sedentary, light, moderate, vigorous and very vigorous-intensity METs, arbitrary cut-off points were used as per recommendations from the ACSM (Garber *et al.*, 2011; see table 3.1). Minimum wear time was set at 80% of the 24-hour period for each day (19.2 hours).

Table 3.1 Cut-off points used for categorising metabolic equivalents of task from activity monitors

Intensity	METs cut-off point
Sedentary	<1.5
Light	≥1.5 and <3.0
Moderate	≥3.0 and <6.0
Vigorous	≥6.0 and <8.8
Very vigorous	≥8.8

Actiheart activity monitor calibration

For study 2, measures of RER and VO_2 were averaged during the final minute of each 3-minute stage during the submaximal exercise test. Mean VO_2 values for each test stage were

multiplied with the RER-specific caloric equivalent of oxygen (appendix 19) to calculate estimated EE (Frayn, 1983). For study 3, measures of VO_2 for every breath-by-breath sample of expired gas were multiplied with the RER-specific caloric equivalent of oxygen (appendix 19) to calculate estimated EE (Frayn, 1983) across each participant's range of VO_2 values throughout the maximal exercise test. For study 3, HR was continually recorded throughout the maximal test. For study 2, HR was averaged for the final minute of each stage of the submaximal test. For both study 2 and 3, recorded HR and corresponding EE estimates were utilised to predict a regression equation to establish the individual relationship between HR and VO_2 from resting to maximal HR. Alongside these values, measured BMR (see chapters 5 and 6 for further detail), as well as maximum HR (calculated by the Actiheart software as 220 – (0.8 x age)) and sleeping HR (measured during monitor wear time) were all additionally inputted into individual profiles on the Actiheart software to improve EE estimations before EE analysis (Assah *et al.*, 2011).

3.6.11 Energy and macronutrient intake

For study 2, two differing methods of energy and macronutrient intake assessment were utilised. Participants were offered an *ad libitum* lunch buffet on the trial testing day (see section 5.2.6 for further detail on instructions provided to participants as well as table 5.1 for the foods that made up the buffet) where participants' energy and macronutrient intake were assessed using manufacturer nutritional information. The buffet was presented identically before each trial and participants utilised the same crockery throughout trials, while all items were displayed in excess of expected consumption. This buffet-style meal was chosen to avoid boredom or limited food choice that can be seen with single-item *ad libitum* buffet meals (Blundell *et al.*, 2010) which could induce premature cessation of feeding. Energy and macronutrient intakes are highly reproducible at *ad libitum* buffets (Arvantini, Richard and Tremblay, 2000) and such additional information on macronutrient intake, as well as EI and food choice, is of interest and something that single-item buffets cannot achieve. The buffet used in study 2 consisted of typical Western diet items consumed at lunch, whilst the number of items was still limited so as not to present opportunity for overconsumption due to increased food choice leading to the "cafeteria diet" effect which has previously been

demonstrated in both males as well as females assessed during the follicular phase of the menstrual cycle (Larson *et al.*, 1995a; Larson *et al.*, 1995b). For study 2, relative energy intake at the *ad libitum* buffet following either the resting condition or the 2x30 or 4x30 condition was calculated as absolute EI minus the total EE of the condition period.

In study 2, energy and macronutrient intake were also assessed for the remainder of the testing day as well as the three days following the testing day using self-report weighed food diaries. In study 3, energy and macronutrient intake was also assessed using self-reported weighed food diaries for five days at baseline, week 4 and week 8 of the exercise intervention. It is acknowledged that self-report food diaries in this way require high compliance from participants. Hence, although self-report EI assessment is highly reproducible and valid (Hu *et al.*, 1999), due to the reliance upon participant compliance it is known that inaccurate reporting due to bias and inaccurate reporting is, unfortunately, common (Bingham, 1987; Borrelli, 1990).

Self-reported weighed food diaries can therefore be subject to a number of biases including social desirability bias, reactivity bias and memory-based bias such that these can often lead to under-reporting of dietary intake. For example, it is acknowledged that the task of recording a weighed food diary itself can influence actual eating behaviour (Marr, 1971). Moreover, typically around 30% of individuals are found to under-report their dietary intake while under-reporting itself can reach a magnitude of 15% (Poslusna *et al.*, 2009); thus, a self-reported 2000kcal daily EI could therefore be underreported by ~300kcal, although it is of course plausible that the extent of inaccurate reporting depends on the individual. It is also important to note that such under-reporting is more common for people with overweight/obesity (Prentice *et al.*, 1986; Schoeller, Bandini and Dietz, 1990; Macdiarmid and Blundell, 1998; Wehling and Lusher, 2017), low socio-economic status (Morgan *et al.*, 1987) as well as being female (Hebert *et al.*, 1997; Macdiarmid and Blundell, 1998) meaning that under-reporting in studies of this thesis is plausible and should be considered when interpreting findings of studies 2 and 3 in this thesis.

Participants were provided with detailed instructions attached to the food diaries (appendix 13) in order to complete the self-report food diary (appendix 14) as accurately as possible, along with digital kitchen scales (Salter Disc Electronic Digital Kitchen Scales, Kent, UK) where required. Participants were asked to weigh intakes where possible and where required and

to estimate as accurately as possible where weighing was not possible, as well as detail brands and preparations where possible. All self-reported food diaries were analysed using the dietary analysis software Nutritics (version 5; Nutritics, Ireland). Where individual items were not available on the database within the programme, nutritional information was inputted manually using manufacturer information.

It is also important to note that there is not currently a "gold-standard" approach to assessing energy and macronutrient intake in free-living conditions that would have been feasible or practical for the present studies. However, the common inaccuracies in reporting and systematic under-reporting seen in previous literature should be considered when interpreting the data from these tools in studies 2 and 3. Best efforts were made to ensure that the days of the week upon which energy and macronutrient intake were identical across testing periods within each participant in studies 2 and 3, to minimise within-subject day-to-day variation such as between weekend days and week days (Borrelli, 1990). Although it is acknowledged that a three day (study 2) or a five day (study 3) period may not be reflective of habitual dietary intake and eating behaviour, this was deemed a suitable amount of time to ensure sufficient level of detail on daily energy macronutrient intake while not being too much of a burden for participants to complete accurately as validity of energy and macronutrient intakes seems to decline by five days of consecutive recording (Gersovitz, Madden and Smicklas-Wright, 1978).

3.6.12 Subjective appetite assessment

For studies 2 and 3, subjective appetite was measured using the typical VAS method adapted from the original 6 questions of Hill and Blundell (1982) to four questions assessing: 'hunger', 'fullness', 'desire to eat' and 'expected food intake' on separate 150mm scales (appendix 21). Scales were anchored at either end by 'not hungry at all' and 'the hungriest I have ever been' for the assessment of 'hunger', by 'not full at all, empty' and 'very, very full' for the assessment of 'fullness', 'very weak' and 'very strong' for the assessment of 'desire to eat' and by 'nothing' and 'lots' for the assessment of 'expected food intake'. Participants were asked to mark along the horizontal scale of 150mm length with a small vertical cross at the point that best represents their responses to each question. For studies 2 and 3, it was

ensured that participants were not able to see their previous VAS responses, to ensure this did not influence their response on the subsequent scales, although when previously assessed this did not seem to occur (Douglas and Leidy, 2019).

Assessing appetite in this way has been demonstrated to correlate with (although does not always reliably predict) EI and is a method with good within-subject reliability and validity with regard to predict meal initiation and food intake, while being sensitive to experimental manipulation (Stubbs *et al.*, 2000). Nonetheless, the original six question components of the VAS (Hill and Blundell, 1982) are found to co-vary extensively (Stubbs *et al.*, 2000). In turn, participants' responses to either the 'hunger' or 'fullness' questions are generally equal to the mean of the remaining components (Reid *et al.*, 1998). Therefore, throughout this thesis, as informed by previous studies such as Holliday and Blannin (2017a), VAS scores were obtained by measuring the distance (in millimetres) from the extreme left end of each scale and a composite VAS score was generated through the following formula:

$$\frac{[\text{hunger score} + \text{desire to eat score} + \text{expected food intake score} + (150 - \text{fullness score})]}{4}$$

Additionally, for study 2, 'nausea' was also assessed on a VAS scale (appendix 21) to assess any feelings of nausea related to the exercise conditions. This scale was anchored by 'not nauseous at all' and 'extremely nauseous' and analysed separate to the composite VAS score. Although VAS component scores in this thesis were based on 150mm scales and original VAS component scores were based on 100mm scales (Hill and Blundell, 1982), scores obtained from both 150mm and 100mm scales have been shown to be highly reproducible both before a meal and following a meal (Chaput *et al.*, 2010). Thus, this suggests scores from 150mm scales expressed as a percentage can be used to compare scores across studies that use 100mm length VAS scales.

3.6.13 Dietary restraint assessment

Dietary restraint was assessed for studies 2 and 3 as an inclusion criterion, such that participants were required to not be restrained eaters (≤3.5 on the restraint scale of the DEBQ; van Strien *et al.*, 1986a; appendix 2). Moreover, the measure of dietary restraint was

also used as a measure during experimental trials of study 3. The DEBQ was the chosen assessment of dietary restraint for these studies given its preferable homogeneity with food intake measures and stability across weight categories, as well as its internal consistency (α=0.95) and similar test-retest reliability (r=0.92) compared with the Three Factor Eating Questionnaire (TFEQ; r=0.91; Stunkard and Messick, 1985) and the Revised Restraint Scale (r=0.95) Herman and Polivy, 1980) (Allison, Kalinsky and Gorman, 1992). Furthermore, the DEBQ has demonstrated good predictive validity for eating restraint by assessing actual EI and desired EI as a proxy measure of eating restraint (van Strien *et al.*, 1986b). Nonetheless, it has been demonstrated that no current assessments of self-reported eating restraint are predictive of actual EI and energy balance when assessed over 6 months (Williamson *et al.*, 2007), which should be considered for the findings of study 3 in this thesis.

3.6.14 Standardised meals

For all studies, participants were provided with a standardised breakfast which consisted of: porridge made with semi-skimmed milk, brown sugar and a glass of orange juice. This meal provided approximately 20% of predicted daily energy requirements for a sedentary day for each individual, as UK breakfast consumption should (and tends to) provide ~20% of daily energy requirements in adults (Gaal *et al.*, 2018). For each participant, this was estimated using the Mifflin *et al.* (1990) equation based on each individual's age, height and weight measured upon familiarisation and then multiplied by a factor of 1.4 (for a sedentary day). The equation established by Mifflin *et al.* (1990) has been demonstrated to be superior in providing accurate estimates of actual BMR in both nonobese and obese individuals, compared with other established equations including the Harris-Benedict equation (Frankenfield *et al.*, 2003). For the breakfast meal provided in all studies in this thesis, approximately 58% of the energy content of this meal was from CHO, 24% from fat and 18% from PRO.

3.6.15 Environmental temperature and humidity

For all studies, laboratory temperature was automatically maintained (and monitored by the

primary researcher) at 21˚C through an automatic air conditioning system, while humidity ranged from 29 to 56% across studies when assessed using a hygrometer (see individual study chapters for ranges for each study).

3.6.16 Venous blood

Sampling

For study 3, venous blood samples obtained through a cannula by the primary researcher in the antecubital vein of the arm were collected in appropriate collection tubes. Potassium/ethylenediaminetetraacetic (EDTA)-treated tubes were used for the collection of venous blood for analysis of fasted and postprandial GLP-1 and PYY, while serum tubes were used for the collection and analysis of fasted and postprandial insulin and fasted TAG as well as fasted high-density lipoprotein cholesterol (HDL-C), fasted low-density lipoprotein cholesterol (LDL-C) and fasted total cholesterol (TC). Blood collected in tubes treated with sodium fluoride and potassium oxalate was used for the analysis of fasted and postprandial glucose. Venous blood was also collected into pre-treated potassium/EDTA-treated monovettes for the analysis of fasted and postprandial AG. A 30µL solution containing potassium phosphate buffer, p-hydroxymercuribenzoic acid and sodium hydroxide was added to 2.7mL potassium/EDTA-treated monovettes (Sarstedt, Leicester, UK) prior to collection of venous blood to prevent degradation of AG. All collection tubes were centrifuged at 3000RPM for 10 minutes at 4˚C (Sorvall ST 8R, Thermo Fisher Scientific, Massachusetts, United States). The plasma supernatant was then aliquoted into 2mL Eppendorf tubes which were stored at -80˚C for later analysis. 50µL 1M hydrochloric acid was added to Eppendorf tubes containing 500µL plasma for the analysis of AG, to protect AG from degradation.

3.7 Pilot work ahead of study 1

Extensive pilot work was undertaken prior to the final design of study 1, whereby 30 second intervals of high-intensity cycling, star jumping and squatting of differing volumes were undertaken by a population of inactive females (n=5). This was undertaken in order to

ascertain both the feasibility of the high-intensity exercises for this population as well as to determine suitable recovery periods in between intervals of 30 seconds. The purpose of this pilot testing was also to determine if 4 x 30 seconds of each proposed exercise mode for study 1 would likely suffice in reaching a high-intensity. Heart rate and BLa responses were assessed and indeed demonstrated that a sufficiently high-intensity could be reached with these exercise modes in this population. Participants also expressed feelings of RPE to suggest that they were exercising at a high-intensity, whilst the exercises were still deemed feasible for this population given that participants were able to achieve up to 4 x 30 seconds of each exercise mode and did not report any adverse feelings such as pain, nausea or light headedness. Therefore, 4 x 30 seconds of each exercise mode were taken forward into study 1, with no changes to the exercise modes made.

3.8 Statistical analysis

Throughout this thesis, data are presented as mean±standard deviation in text and tables and mean±standard error in figures. All values are presented to one decimal place (1 d.p.), unless otherwise stated. All statistical analysis was conducted using the software 'Statistical Package for Social Sciences' (SPSS; version 23.0, SPSS inc., Chicago, Illinois, USA). Specific statistical analyses will be described in each chapter, but prior to all analyses, normality of data was assessed using the Shapiro-Wilk test. Extreme outliers (>3x interquartile range of the dataset) were also removed from the dataset before statistical analysis. Throughout, statistical significance was accepted at the level of $p<0.05$, with a trend for significant differences accepted within the range of $p\geq0.05$ to $p<0.1$.

For each analysis of variance (ANOVA), effect size was calculated using partial eta squared (η^2_p) where $\eta^2_p>0.01$ was considered small, $\eta^2_p>0.06$ was considered medium and $\eta^2_p>0.14$ was considered large (Cohen, 1988). For resultant *post-hoc* pairwise comparisons and t-tests, Cohen's d, with 95% confidence intervals (95% CI), was used to determine effect size. For pairwise comparisons, a Cohen's d of 0.2 or greater was considered a small effect size, while 0.5 or greater considered medium and 0.8 or greater considered large (Sawilowsky, 2009). Missing data analysis using the multiple imputations technique was used for missing data points with specific chapters provide further detail on when this technique was used.

Chapter 4

Study 1 - Acute physiological, affective and enjoyment responses to apparatus-free high-intensity intermittent exercise in inactive females

Abstract

The effectiveness of HIIE as a viable physical activity strategy is contentious due to factors including affective and enjoyment responses as well as the apparatus required. The purpose of this study was to determine the acute physiological, affective and enjoyment responses to apparatus-free and cycle ergometer-based HIIE. Eighteen inactive females (BMI 25.5±4.2 $kg \cdot m^{-2}$, age 35±12 years, leisure time MVPA 74±54 $minutes \cdot week^{-1}$) completed three conditions of 4 x 30 seconds of high-intensity exercise: cycling (CYCLE); squatting (SQUAT, squats with a 5kg weighted arm extension) or star jumps (JUMP). Peak HR, BLa and RPE were measured at rest, during exercise and post-exercise. Change in plasma volume (ΔPV), affective and enjoyment responses were also measured post-exercise. HR_{peak} was similar following CYCLE (164±12$beats \cdot min^{-1}$) and JUMP (162±13$beats \cdot min^{-1}$; $p>0.05$). BLa was significantly greater following CYCLE (11.1±1.6$mmol \cdot L^{-1}$) versus JUMP (7.3±2.4$mmol \cdot L^{-1}$; $p<0.01$) and SQUAT (5.0±1.8$mmol \cdot L^{-1}$; $p<0.001$). Post-exercise ΔPV was significantly reduced in SQUAT (-6.90±8.51%; $p=0.003$), JUMP (-6.91±10.25%; $p=0.011$) and CYCLE (-7.60±8.08%; $p=0.001$). Affective valence was significantly greater following SQUAT and JUMP versus CYCLE ($p<0.01$ and $p=0.026$, respectively). SQUAT was perceived significantly more enjoyable ($p=0.002$), and there was a trend for JUMP to be more enjoyable, than CYCLE. In conclusion, star jumping can elicit important physiological responses with positive affective and enjoyment responses, indicating the potential effectiveness of apparatus-free HIIE.

4.1 Introduction

Low volume HIIE interventions (a total of ≤30 minutes of brief, repeated bursts of high-intensity exercise, interspersed with periods of rest or low-intensity exercise; Gillen and Gibala, 2014) has been shown to elicit improvements in metabolic health in the longer term similar to those seen with longer duration, continuous, moderate-intensity exercise interventions in inactive populations. These include improvements in cardiovascular fitness, insulin sensitivity and glycaemic control (Cocks *et al.*, 2016; Gillen *et al.*, 2016; Babraj *et al.*, 2009; Freese *et al.*, 2015). In addition, as HIIE has an inherent reduced time commitment, it can negate the perceived lack of time as a consistently cited barrier to regular PA (Salmon *et al.*, 2003; Cerin, *et al.*, 2010). Furthermore, this barrier is particularly reported in females (Salmon *et al.*, 2003), who are typically less active than males (Hallal *et al.*, 2012).

While most low volume HIIT interventions employ high-intensity cycling against a set resistance (Gillen and Gibala, 2014), such reliance on a cycle ergometer poses a barrier to compliance given that poor access to PA apparatus and facilities, as well as limited access to PA apparatus at home, are also barriers to regular PA (Trost *et al.*, 2002). Apparatus-free protocols are therefore warranted (Gray *et al.*, 2016).

McRae *et al.* (2012) previously observed an 8% improvement in VO_{2peak} following an intervention of four weeks duration that consisted of aerobic-resistance bodyweight-based HIIT, while Blackwell *et al.* (2017) also demonstrated an ~8% improvement in cardiorespiratory fitness with a bodyweight-based HIIT intervention of four weeks duration. High-intensity stair climbing for six weeks elicited a 12% improvement in cardiorespiratory fitness in inactive females (Allison *et al.*, 2017). Similarly, 4 x 4 minutes of high-intensity walking or jogging elicited a cardiorespiratory fitness improvement of ~7% over twelve weeks in inactive, overweight/obese adults compared with a walking control group, although 4-6 x 30 seconds of "all-out" walking or jogging did not induce improvements in cardiorespiratory fitness in this population (Lunt *et al.*, 2014). Therefore, it appears plausible that apparatus-free HIIT interventions can induce important improvements in metabolic health, but further research is required to optimise such interventions. Determining the acute physiological responses to apparatus-free HIIE is of interest to ascertain the intensity reached before incorporating it into a HIIT intervention. Allison *et al.* (2017) demonstrated that an acute bout

of high-intensity stair climbing resulted in similar HR, BLa and RPE responses to cycle ergometer-based HIIE. Furthermore, Gist, Freese and Cureton (2014) similarly showed that 4 x 30 seconds of "burpees" elicited similar HR responses to 4 x 30 seconds of high-intensity cycling. Necessary physiological responses may, therefore, be achieved in more practical, apparatus-free protocols of low volume HIIE in an inactive population.

It is also recognised that cycle ergometer-based HIIE may not be tolerable or appealing to all (Gibala *et al.*, 2012) as it requires a high amount of effort (Gillen and Gibala, 2014), which is negatively correlated to regular PA (Trost *et al.*, 2002). As aversive affective responses to cycle ergometer-based HIIE likely leads to a reduction in adherence (Biddle and Batterham, 2015; Hardcastle *et al.*, 2014), a more positive affective response to PA is desirable from HIIE. In turn, a positive affective response is associated with increased participation and adherence (Kwan and Bryan, 2010a), as well as an increase in exercise motivation (Kwan and Bryan, 2010b). Greene, Greenlee and Petruzzello (2018), however, demonstrated a reduction in affective valence following bodyweight-based HIIE compared with moderate-intensity, continuous exercise although no differences in enjoyment were found. Whether a more positive affective response can be obtained with apparatus-free HIIE without compromising physiological responses remains to be elucidated.

No study has yet explored the affective and enjoyment responses, as well as the physiological responses, of differing modes of apparatus-free low volume HIIE. Such responses are important to optimise the effectiveness of long-term HIIT interventions. Therefore, the aim of this study was to determine the acute physiological, affective and enjoyment responses to two apparatus-free low volume HIIE protocols versus a cycle ergometer-based HIIE protocol, in inactive females.

4.2 Methods

4.2.1 Study design

A within-subject, counterbalanced, crossover design was utilised, with participants randomly assigned to each of the three conditions: high-intensity squats (SQUAT), high-intensity star jumps (jumping jacks; JUMP) and high-intensity cycling (CYCLE). Data was collected across two

sites: at the University of Worcester and the University of Birmingham. Data collection at both sites followed an identical protocol and used identical apparatus, analyses and equipment.

4.2.2 Participants

Twenty four inactive females were recruited for the study, while 18 completed the study (BMI 25.5±4.2 $kg \cdot m^{-2}$, age 35±12 years, leisure time MVPA 74±54 $minutes \cdot week^{-1}$). Inclusion and exclusion criteria as well as ethical approval are as described in section 3.2. Participants were split into either the 'healthy BMI' category (n=9; BMI 22.2±1.8 $kg \cdot m^{-2}$, age 28±10 years, leisure time MVPA 48±56 minutes $MVPA \cdot week^{-1}$) or the 'overweight/obese BMI' category (n=9; BMI 28.85±3.0 $kg \cdot m^{-2}$, age 41±9 years, leisure time MVPA 22±34 minutes $MVPA \cdot week^{-1}$). BMI was statistically significant between groups ($p<0.001$). Leisure time MVPA was not statistically significantly different between groups ($p=0.258$), however the mean age of the 'overweight/obese BMI' category was significantly greater than that of the 'healthy BMI' category ($p=0.01$). Of the n=18 total participants, 15 were of Caucasian ethnicity and 3 were of Asian ethnicity.

4.2.3 Preliminary visit and familiarisation

Prior to starting the study protocol, participants reported to either the University of Worcester (n=13) or the University of Birmingham (n=5), where they received written (appendix 3) and verbal information about the study, before written informed consent (appendix 6) was obtained. The Physical Activity Readiness Questionnaire (PAR-Q; Thomas, Reading and Shephard, 1992; appendix 9) and the IPAQ (Craig *et al.*, 2003; appendix 1) were then administered for health screening and assessing current PA levels, respectively.

Height was measured using a stadiometer and body mass was measured using scales (Seca, Birmingham, UK). Resting blood pressure was measured using an automatic blood pressure monitor (Omron, Milton Keynes, UK). Participants then undertook 30 seconds of each exercise condition to ensure feasibility and familiarisation and had explained to them the measures that would be taken during the experimental trials. Following this, participants

were given food diary instructions (appendix 13) and a weighed food diary record (appendix 14) and instructed to record 24-hour food intake prior to their first experimental trial; this intake was to be replicated twenty four hours prior to the remaining two trials.

4.2.4 Experimental trials

Participants returned to the laboratory between 07:30 and 09:30, after an overnight fast (minimum of 10 hours; water was permitted) for three experimental trials. Eumenorrheic participants undertook each experimental trial at least five days apart during the follicular phase of the menstrual cycle (days 1-14). Participants taking contraceptive medication preventing menstruation undertook experimental trials approximately 28 days apart. Participants' adherence to minimising strenuous physical activity on the pre-testing day was checked verbally by the researcher upon participant arrival to each experimental trial. Adherence to the pre-testing day food diary was also checked upon arrival to each experimental trial (pre-testing day energy and macronutrient intakes all $p>0.05$ between conditions).

Room temperature was maintained by an air conditioning system at an ambient temperature of ~21°C while humidity ranged from 29-55% when assessed using a hygrometer.

4.2.5 Protocol

Within 15 minutes of arrival during all experimental trials, participants were given a standardised breakfast which consisted of: porridge made with oats, milk and brown sugar and a glass of orange juice. The breakfast meal provided approximately 20% of the estimated daily energy needs for a sedentary day for each individual, based on their age, height and weight (Mifflin *et al.*, 1990). Following breakfast, participants had a 2-hour rest period seated at a desk, where they were allowed to read, watch television or work quietly. Following this rest period, a finger prick capillary blood sample was taken for the measurement of resting BLa, as well as resting HR, positive and negative affect using the PANAS (Watson, Clark and Tellegen, 1988) and affective valence using the FS (Hardy and Rejeski, 1989; appendix 16).

Additional capillary blood samples for measures of haematocrit and haemoglobin were also obtained at this point.

A warm-up period then commenced (one minute of cycling at 30W on an electronically braked cycle ergometer (Lode BV, Groningen, The Netherlands) in CYCLE and one minute of walking on the spot on flat ground at a self-selected pace in SQUAT and JUMP). Following this, either CYCLE, SQUAT or JUMP commenced.

CYCLE consisted of 4 x 30 seconds of "all out" (maximal effort) cycling against a resistance (5% body mass). Participants pedalled as fast as possible ~5 seconds before the resistance was applied automatically and continued to cycle as fast as possible throughout each 30 seconds

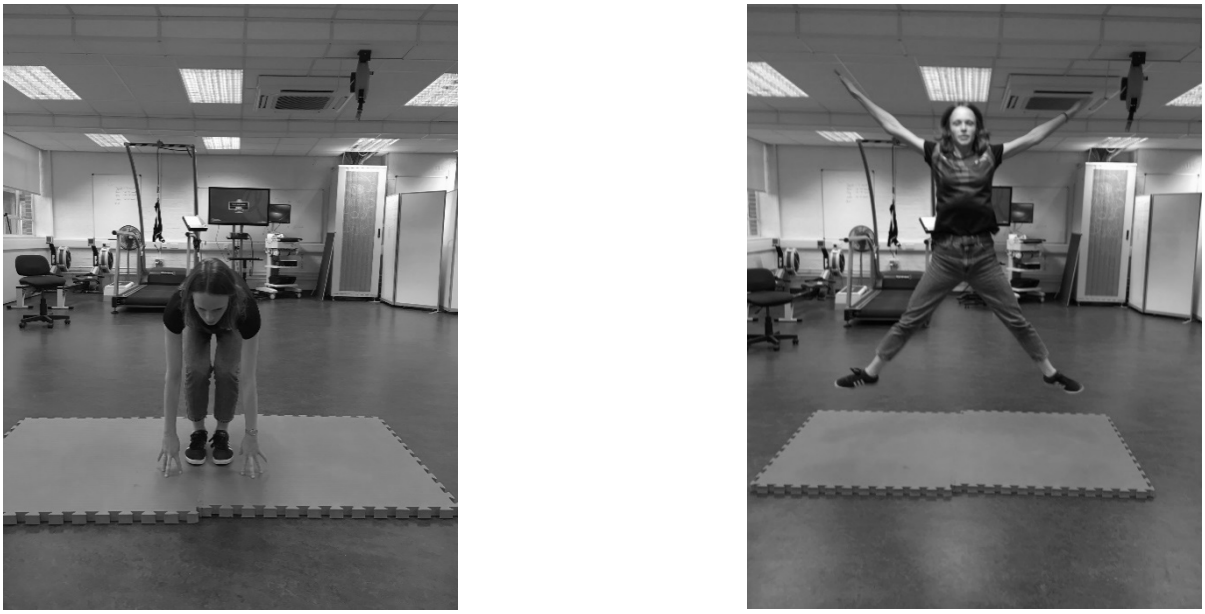

Figure 4.1 A demonstration of the JUMP exercise condition

interval. A 3-minute recovery period was employed between each 30 seconds interval in CYCLE, during which participants were encouraged to undertake light cycling at 30W. The JUMP and SQUAT conditions consisted of 4 x 30 seconds of star jumps and squats, respectively. In the JUMP condition, participants were instructed to bend at the knees to touch the floor with their hands and then jump vertically, stretching out both arms and legs as laterally as possible (see figure 4.1). Participants performed as many jumps as possible in each 30 seconds, i.e. "maximal effort" jumping.

In the SQUAT condition, participants were instructed to undertake a squat movement from standing, to the height of a chair (47cm), before returning to standing again (see figure 4.2). Participants were performed as many as possible during each 30 seconds (whilst maintaining a safe technique). Whilst squatting, participants held a 5kg hand weight at chest height which was lifted above their head upon standing. Between 30 seconds intervals in the JUMP and SQUAT condition, participants had a 90 seconds recovery period where they were encouraged to undertake walking on the spot (without holding the hand weight) at a self-selected pace.

Figure 4.2 A demonstration of the SQUAT exercise condition

At the end of the first (I1), second (I2) and fourth interval (I4/immediately post-exercise), measures of peak HR (HR_{peak}), rating of perceived exertion (RPE) and BLa were obtained. Immediately post-exercise, additional measures of haematocrit, haemoglobin and measures of positive and negative affect as well as affective valence were also recorded. Participants were then allowed a 30 minutes recovery period where they were encouraged to remain seated. A measure of positive and negative affect, affective valence and a self-reported measure of enjoyment of the exercise bout was taken at the end of this recovery period. Figure 4.3 shows a summary of the experimental design.

4.2.6 Measures

HR_{peak}

HR_{peak} was measured using a HR monitor attached to a chest strap (Polar FT4, Polar Electro OY, Kempele, Finland).

RPE

RPE using the 6-20 Borg Scale (Borg, 1982; appendix 15). Participants were asked to rate their '*current perceived level of exertion',* as described in section 3.6.3.

Affective responses

Affective valence was measured using the FS (Hardy and Rejeski, 1989; appendix 16), while positive and negative affect were measured using the PANAS (Watson, Clark and Tellegen, 1988; appendix 17).

Enjoyment

A measure of enjoyment of the exercise condition was obtained using the PACES (Kendzierski and DeCarlo, 1991; appendix 18).

Blood lactate concentration

A 10µL capillary blood sample was obtained in a capillary tube for the analysis of BLa using a desktop analyser (Biosen C_Line, EKF Diagnostics, Cardiff, UK). All measures were analysed in duplicate with a third measure obtained if disagreement between the first two measures was >2%.

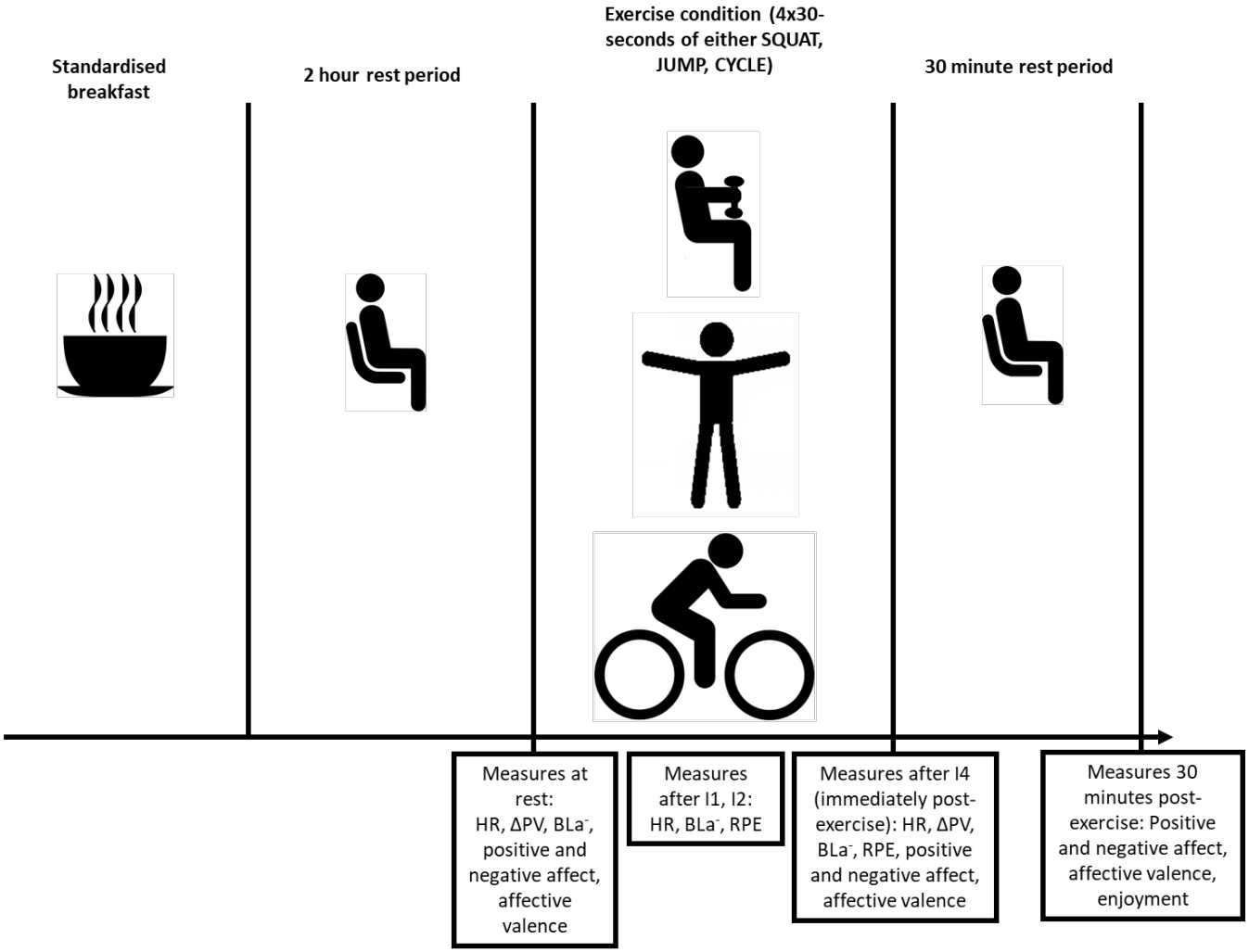

Figure 4.3 Summary of experimental design; 'HR' denotes 'heart rate', 'ΔPV' denotes 'change in plasma volume from rest', 'BLa' denotes 'blood lactate concentration', 'RPE' denotes 'rating of perceived exertion', 'I1', 'I2' and 'I4' denote 'interval 1', 'interval 2' and 'interval 3', respectively

Plasma volume

Further capillary blood samples were obtained in an additional 60µL heparinised capillary tube and a 10µl capillary slide for the measurement of haematocrit and haemoglobin concentration, respectively. The 60µL tubes were centrifuged for a period of 5 minutes at 12000RPM (Sorvall ST 8R, Thermo Fisher Scientific, Massachusetts, United States) and haematocrit was manually calculated using a Hawksley reader (Hawksley and Sons Ltd, Sussex, UK). Haemoglobin was measured using a HemoCue Hb 201+ System (HemoCue AB,

Ängelholm, Sweden). From this, ΔPV was determined manually using the equations of Dill and Costill (Dill and Costill, 1974). All measures were analysed in duplicate with a third measure obtained if disagreement between the first two measures was >5%. Both corrected (for immediately post-exercise) and uncorrected responses (for all time points) of BLa were measured. Uncorrected values are presented and used for ease of comparison with previous literature. Statistical analysis was repeated with corrected values to ensure any effects observed were not due to changes in haemodilation

4.2.7 Statistical analysis

A one-way ANOVA confirmed no differences between groups at rest in any measure. A 3x4 (condition x time) repeated measures ANOVA was used to assess differences in HR and uncorrected BLa at rest and after intervals 1, 2 and 4. A 3x3 (condition x time) repeated measures ANOVA was used to test for significant differences in the measure of RPE, positive and negative affect and affective valence at rest, immediately post-exercise and thirty minutes post-exercise.

For ΔPV, a one-sample t-test was used to assess change from rest in each condition, with a one-way repeated measures ANOVA used to test for significant differences between conditions. A one-way repeated measures ANOVA was also used to assess differences in enjoyment between conditions. All ANOVAs were repeated as mixed design repeated measures ANOVAs with an additional factor of 'BMI category' (<25kg·m^{-2}: 'healthy BMI' category; >25kg·m^{-2}: 'overweight/obese BMI' category) was inputted as the between-subjects

factor. For all ANOVAs, the Greenhouse-Geisser correction was used if the assumption of sphericity was violated. Significant interaction and main effects of ANOVA were investigated further by conducting *post-hoc* pairwise analyses using Bonferroni tests.

Due to one participant experiencing light-headedness after the second interval of CYCLE, the remainder of the protocol was not completed and so missing data analysis using the multiple imputations technique was used for missing data points for HR (2 cases), BLa (1 case), RPE (1 case), affective valence (1 case), negative affect (1 case) and positive affect (1 case), whilst researcher error during analysis also meant that missing data analysis using the multiple imputations technique was used for missing data points for haemoglobin (5 cases) and haematocrit (7 cases) in the calculation of ΔPV. Extreme outliers (>3x interquartile range of the dataset) were removed from the dataset before statistical analysis. All statistical analysis was undertaken using the software SPSS (SPSS version 23.0, SPSS inc., Chicago, Illinois, USA).

4.3 Results

4.3.1 Physiological responses

Table 4.1 (page 96) shows peak HR (HR_{peak}; absolute HR and $\%HR_{max}$), BLa and RPE responses across all conditions throughout resting and exercise time points.

HR_{peak}

There were no differences in resting HR between conditions. A significant condition x time interaction effect was found for HR_{peak} (see table 4.1; values to nearest integer): $F(6, 102)=4.188$, $p=0.001$, $\eta^2_p=0.198$. HR_{peak} increased significantly with each interval in all conditions (all $p<0.001$, differences not shown in table 4.1). Following interval 1, HR_{peak} was significantly greater in CYCLE (149±14 beats·min^{-1}) versus SQUAT (139±17 beats·min^{-1}; $p=0.001$, $d=0.608$, 95% CI=-14.759 - -4.241). Following interval 2, HR_{peak} was also significantly greater in CYCLE (159±12 beats·min^{-1}) versus SQUAT (147±17 beats·min^{-1}; $p<0.001$, $d=0.840$, 95% CI=-18.073 - -6.594) as well as in JUMP (155±12 beats·min^{-1}) versus SQUAT ($p=0.037$,

d=0.587, 95% CI=-16.879 - -0.454). No significant differences were seen between conditions following interval 4 (immediately post-exercise). There was a significant main effect of condition: F(2, 34)=6.216, p=0.005, η^2_p=0.268 as well as a significant main effect of time: F(1.479, 25.145)=500.189, p<0.001, η^2_p=0.967. Across all time points, HR_{peak} was significantly greater in CYCLE (136±9 beats·min^{-1}) compared with SQUAT (128±13 beats·min^{-1}; p=0.002, d=0.74, 95% CI 2.907 – 12.538). Across all conditions, HR_{peak} increased with each subsequent time point (all p<0.05, effects not shown in table 4.1). No effects of BMI category were found for differences in HR (all p>0.05).

Blood lactate concentration

There was no difference in BLa at rest between conditions. One extreme outlier was removed from the dataset (one for SQUAT after interval 2 and one for CYCLE post-exercise), resulting in n=17 for this measure. A significant condition x time interaction was found for uncorrected BLa: F(3.048, 48.77)=47.382, p<0.001, η^2_p=0.748. BLa increased significantly with every increasing interval in each condition (all p<0.001, differences not shown in table 3). Following interval 2, BLa was significantly lower in SQUAT (3.4±0.7mmol·L^{-1}) versus CYCLE (6.0±1.5mmol·L^{-1}; p<0.001, d=2.27, 95% CI=1.705 – 3.645) and JUMP (4.6±1.4mmol·L^{-1}; p=0.001, d=1.11, 95% CI=0.492 – 1.884). BLa was also significantly greater in CYCLE versus JUMP at this time point (p=0.001, d=1.03, 95% CI=0.662 – 2.311). Following interval 4, BLa was also significantly lower in SQUAT (5.0±1.8mmol·L^{-1}) versus CYCLE (11.1±1.6mmol·L^{-1}; p<0.001, d=3.55, 95% CI=-7.443 - -4.718) and JUMP (7.3±2.4mmol·L^{-1}; p=0.003, d=1.05, 95% CI=-3.739 - -0.77). BLa was also significantly greater in CYCLE versus JUMP following interval 4 (11.1±1.6mmol·L^{-1} vs. 7.3±2.4mmol·L^{-1}; p<0.001, d=1.85, 95% CI=2.369 – 5.282). There was also a significant main effect of condition: F(2, 32)=54.472, p<0.001, η^2_p=0.773 and a significant main effect of time: F(1.507, 24.109)=220.094, p<0.001, η^2_p=0.932. Across all time points, BLa was greater in CYCLE (5.4±0.8mmol·L^{-1}) compared with SQUAT (3.1±0.7mmol·L^{-1}; p<0.001, d=3.18, 95% CI 1.711 – 2.885) and JUMP (3.9±1.1mmol·L^{-1}; p<0.001, d=1.55, 95% CI 0.911 – 2.048). BLa was also greater in JUMP compared with SQUAT at this time point (p=0.01, d=0.91, 95% CI 0.186 – 1.451). Across all conditions, BLa increased with each subsequent time point (all p<0.05, effects not shown in table 4.1).

For uncorrected blood lactate values, there was a significant condition x time x BMI category interaction: F(6, 60)=3.213, p=0.007, η^2_p=0.176. Within JUMP, at rest the 'healthy BMI' group had a significantly greater BLa (1.5±0.2mmol·L^{-1}) than the 'overweight/obese' group (1.2±0.2 mmol·L^{-1}; p=0.009, d=1.45, 95% CI=0.098 – 0.589). After interval 2, the 'overweight/obese' group had a significantly greater BLa (5.3±1.3mmol·L^{-1}) than the 'healthy BMI' group (3.9±1.1mmol·L^{-1}; p=0.036, d=1.12, 95% CI=0.098 – 2.586) in JUMP. Immediately post-exercise in JUMP, there was a trend for BLa to be significantly greater in the 'overweight/obese' group (8.5±2.1mmol·L^{-1}) compared with the 'healthy BMI' group (6.2±2.3mmol·L^{-1}; p=0.054). Significant effects of BMI category within conditions or time points are not shown.

When corrected for ΔPV following interval 4, the significant condition x time interaction remained: F(2, 34)=66.416, p<0.001, η^2_p=0.796 as did the significant main effects of condition: F(2, 34)=74.271, p<0.001, η^2_p=0.814 and time: F(1, 17)=204.080, p<0.001, η^2_p=0.923. For corrected blood lactate values, there was a trend for a condition x time x BMI category interaction: F(2, 32)=2.546, p=0.094, η^2_p=0.137.

Plasma volume

ΔPV immediately post-exercise were each significantly lower than rest in SQUAT (-6.90±8.28%; t(17)=-3.437, p=0.003, 95% CI=-11.130 - -2.663), JUMP (-6.9±10.3%; t(17)=-2.861, p=0.011, 95% CI=-12.010 - -1.815) and CYCLE (-7.6±8.0%; t(17)=-3.988, p=0.001, 95% CI=-11.620 - -3.579). No significant differences were found between conditions for ΔPV immediately post-exercise: F(2, 34)=0.044, p=0.957, η^2_p=0.003. No effects of BMI category were found for differences in ΔPV.

RPE

Ten extreme outliers were removed from the dataset (four for SQUAT at rest, four for JUMP at rest and two for CYCLE at rest), resulting in n=11 for this measure. There was no difference in RPE at rest between conditions. A significant condition x time interaction was found for

RPE: $F(6, 60)=7.615$, $p<0.001$, $\eta^2_p=0.432$ (see table 4.1). RPE increased significantly with every increasing interval within each condition (all $p<0.001$, differences not shown in table 4.1). Following interval 1, RPE was significantly greater in CYCLE (14.1±2.8) versus SQUAT (11.7±2.9; p=0.008, d=0.84, 95% CI=0.666 – 4.243). Following interval 2, RPE was also significantly greater in CYCLE (15.5±2.6) versus SQUAT (12.9±2.8; p=0.005, d=0.96, 95% CI=0.851 – 4.421). Following interval 4, RPE was significantly greater in CYCLE (17.5±2.2) versus SQUAT (13.6±3.4; p=0.001, d=1.36, 95% CI=1.809 – 6.009) and JUMP (15.6±3.4; p=0.036, d=0.66, 95% CI=0.117 – 3.702).

RPE was also significantly greater in JUMP (15.6±3.4) compared with SQUAT at this time point (13.6±3.4; p=0.034, d=0.59, 95% CI=0.144 – 3.856). There was a significant main effect of condition: $F(2, 34)=18.062$, $p<0.001$, $\eta^2_p=0.515$, whereby across all time points, RPE was significantly greater in CYCLE (13.3±1.8) compared with SQUAT (11.1±2.2, $p<0.001$, d=1.09, 95% CI 1.117 – 2.855) as well as with JUMP (12.1±2.2; p=0.018, d=0.60, 95% CI 0.153 – 1.847).

RPE was also significantly greater in JUMP (12.1±2.2) across all time points compared with SQUAT (11.1±2.2; p=0.032, d=0.45, 95% CI 0.072 – 1.901). There was also a significant main effect of time: $F(1.604, 27.260)=214.862$, $p<0.001$, $\eta^2_p=0.927$, whereby across all conditions, RPE increased with each subsequent time point (all $p<0.05$, effects not shown in table 4.1).

No effects of BMI category were found for differences in RPE.

4.3.2 Affective and enjoyment responses

Affective valence

For affective valence responses, see figure 4.4. There was no difference in rating of affective valence at rest between conditions. A significant condition x time interaction was found for rating of affective valence: $F(4, 68)=13.238$, $p<0.001$, $\eta^2_p=0.438$. Affective valence was significantly greater immediately post-exercise in SQUAT (3.3±1.6) versus both JUMP (2.1±2.2; p=0.005, d=0.5, 95% CI=0.328 – 2.005) and CYCLE (0.6±2.1; $p<0.001$, d=1.0, 95% CI=1.415 – 3.918), as well as in JUMP compared with CYCLE (p=0.026, d=0.5, 95% CI=0.155 –

Table 4.1 Physiological responses to each exercise condition at each time point

	SQUAT				JUMP				CYCLE			
	R	**I1**	**I2**	**I4**	**R**	**I1**	**I2**	**I4**	**R**	**I1**	**I2**	**I4**
HR(beats·min^{-1}; values to nearest integer)	70±9	139 ±17	147 ±17	156 ±18	71 ±10	145 ±16	155 ±12*	162 ±13	71±9	149 ±14*	159 ±12*	164 ±12
%HR$_{max}$		75±9	79±8	84±9		78±7	84±5	87±5		80±7	86±6	88±5
BLa (mmol·L^{-1})	1.4±0.4	2.6 ±0.5	3.4 ±0.7	5.0 ±1.8	1.4±0.3	2.5 ±1.0	4.6 ±1.4*	7.3 ±2.4*	1.5 ±0.4	3.0 ±0.8	6.0 ±1.5$^{*\#}$	11.1 ±1.6$^{*\#}$
RPE	6±0	12±3	13±3	14±3	6±0	13±3	14±3*	16±3*	6±0	14±3*	16±3*	18±2$^{*\#}$

* denotes significantly different to SQUAT at that time point (p<0.05), $^{\#}$ denotes significantly different to JUMP at that time point (p<0.05; (only between condition differences represented), 'HR' denotes 'HR', 'BLa' denotes 'blood lactate concentration', 'RPE' denotes 'rating of perceived exertion', 'R' denotes 'rest', 'I1', 'I2' and 'I4' denote 'Interval 1', 'Interval 2' and 'Interval 4 (immediately post-exercise)', respectively

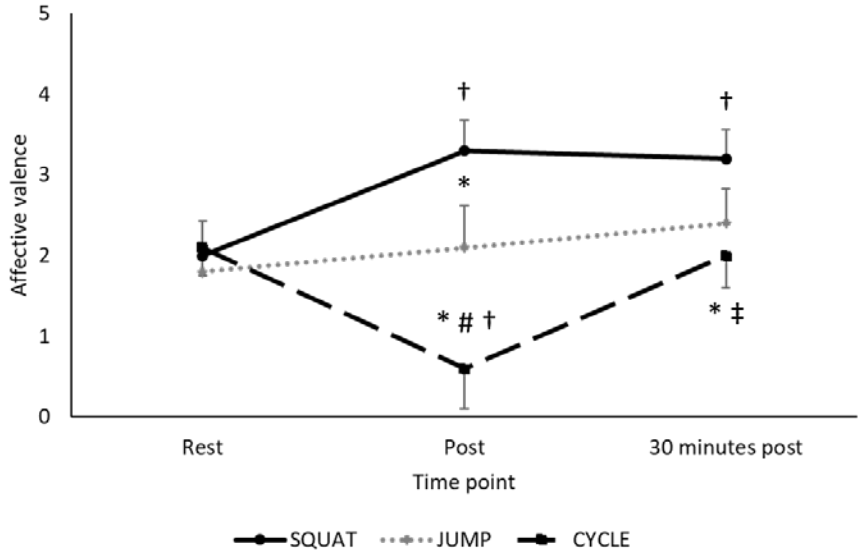

Figure 4.4 Affective valence responses for each exercise condition at each time point; * denotes significantly different to SQUAT at that time point (p<0.05), $^{\#}$ denotes significantly different to JUMP at that time point (p<0.05), † denotes significantly different to rest ('rest') in that condition (p<0.05), ‡ denotes significantly different to immediately post-exercise ('post') in that condition (p<0.05)

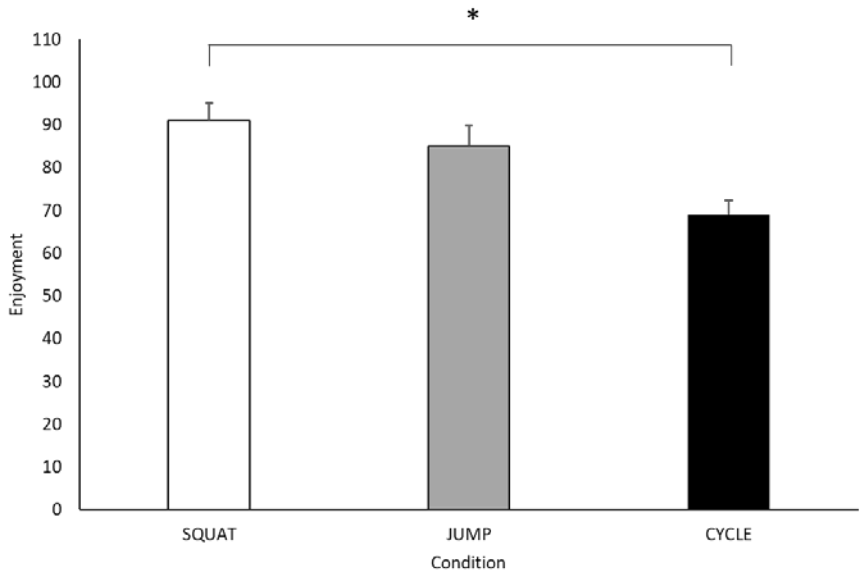

Figure 4.5 Enjoyment responses for each exercise condition; * denotes significantly different to SQUAT (p<0.05)

2.845). Thirty minutes post exercise, affective valence was significantly greater in SQUAT (3.2±1.5) versus CYCLE (2.0±1.7; p=0.005, d=0.5, 95% CI=0.328 – 2.005) as well as a trend for it also being greater than JUMP (2.4±1.8) at this time point (p=0.084). Within SQUAT, affective valence was significantly greater immediately post-exercise (3.2±1.5) versus rest (2.0±1.8; p=0.005, d=0.5, 95% CI=0.372 – 2.184) as well as thirty minutes post-exercise (3.2±1.5) versus rest (2.0±1.8; p=0.007; d=0.5, 95% CI=0.301 – 2.032). Within CYCLE, affective valence was significantly lower immediately post-exercise (0.6±2.1) versus rest (2.1±1.5; p=0.031, d=0.63, 95% CI=0.121 – 2.879) and significantly lower immediately post-exercise versus thirty minutes post-exercise (2.0±1.7; p=0.002, d=0.5, 95% CI=0.527 – 2.251).

There was also a significant main effect of condition: F(2, 34)=8.326, p=0.001, η^2_p=0.329, but no significant main effect of time: F(1.335, 22.694)=2.208, p=0.146, η^2_p=0.115. Across all time points, affective valence was significantly greater in SQUAT (2.8±1.5) compared with JUMP (2.1±1.6; p=0.047, d=0.45, 95% CI 0.008 – 1.362) and CYCLE (1.6±1.5; p=0.001, d=0.8, 95% CI 0.544 – 1.937). No effects of BMI category were found for differences in RPE, while significant effects of BMI category within conditions or time points are not shown.

Positive affect

For positive affect, no significant differences were found between conditions at rest. A trend for a significant condition x time interaction was found for positive affect: F(2.397, 40.744)=2.774, p=0.065, η^2_p=0.140. There was also a significant main effect of condition: F(2, 34)=3.574, p=0.039, η^2_p=0.174, however, there were no significant *post-hoc* effects. There was a significant main effect of time: F(2, 34)=3.862, p=0.031, η^2_p=0.185, whereby across all conditions, positive affect was significantly greater immediately post-exercise (25.3±9.4) compared with thirty minutes post-exercise (22.8±9.0; p=0.008, d=0.22, 95% CI = 0.605 – 4.321). No effects of BMI category were found for differences in positive affect, while significant effects of BMI category within conditions or time points are not shown.

Negative affect

For negative affect, twelve extreme outliers were removed (three for SQUAT at 30 minutes post, two for JUMP immediately-post condition, four for JUMP at 30 minutes post, one for CYCLE at rest and two for CYCLE at 30 minutes post), resulting in n=12 for this measure. No significant differences were found between conditions at rest. There was no significant condition x time interaction effect: $F(4, 44)=1.818$, $p=0.142$, $\eta^2_p=0.142$ and no significant main effect of condition: $F(2, 22)=0.222$, $p=0.803$, $\eta^2_p=0.02$. There was a trend for a significant main effect of time: $F(1.23, 2.66)=3.606$, $p=0.073$, $\eta^2_p=0.247$. No effects of BMI category were found for differences in negative affect, while significant effects of BMI category within conditions or time points are not shown.

Enjoyment

For enjoyment responses, see figure 4.5. One extreme outlier was removed from the dataset (for CYCLE), resulting in n=17 for this measure. A significant effect of condition was found for enjoyment: $F(2, 32)=8.789$, $p=0.001$, $\eta^2_p=0.355$ (Figure 10). Enjoyment of SQUAT (90.7±17.2) was significantly greater than enjoyment of CYCLE (68.5±14.3; $p=0.002$, $d=1.41$, 95% CI=8.041 – 36.312). In addition, there was a trend for enjoyment of JUMP (84.7±20.0) to be significantly greater than enjoyment of CYCLE (68.5±14.3; $p=0.088$). No significant effects of BMI category were found.

4.4 Discussion

The main finding of this study is that 4 x 30 seconds of "all out" star jumping elicited physiological responses indicative of a high intensity workload. Such responses are likely important stimuli for long term adaptations and were comparable with those achieved in cycle ergometer-based HIIE. HR_{peak} during exercise did not differ between JUMP and CYCLE, with mean values in both exceeding 85% of predicted HR_{max}. From interval 2 of JUMP and CYCLE, and thereafter, BLa exceeded levels associated with the lactate threshold (~4mmol·L^{-1}); this likely denotes an increased contribution of anaerobic metabolism and

infers a high-intensity work rate is likely achieved (Skinner and McLellan, 1980). Furthermore, plasma volume decreased similarly in all three conditions, indicating marked metabolic stress in working skeletal muscle post-exercise.

The findings are similar to those of Gist, Freese and Cureton (2014), where 4 x 30 seconds of "burpees" elicited similar HR responses to 4 x 30 seconds of high-intensity cycling. The uncorrected BLa reported by Gist, Freese and Cureton (2014) following the "burpees" (of 8.2±3.3mmol·L^{-1}), are comparable with the uncorrected values observed after JUMP in the present study (7.5±2.6mmol·L^{-1}). Likewise, Allison *et al.* (2017) established that both 3 x 20 seconds of high-intensity cycling and 3 x 20 seconds of high-intensity stair climbing elicited similar HR and BLa responses. HR and uncorrected blood lactate values were comparable with those obtained in the present study for JUMP and CYCLE, which is of note as the stair climbing and cycling protocols elicited improvements in cardiorespiratory fitness in inactive populations when employed in HIIT interventions over six weeks (Allison *et al.*, 2017; Gillen *et al.*, 2014).

Considering the similar HR responses achieved between JUMP and CYCLE, the greater BLa seen in CYCLE could be due to a greater percentage of type II muscle fibres recruited (Skinner and McLellan, 1980) and reliance on a smaller mass of muscle during cycling. In turn, cycling has also been shown to induce greater peripheral fatigue, compared with running and calisthenic (bodyweight) exercise (and hence likely greater blood lactate accumulation; Fitzsimons *et al.*, 1993). Similarly, running has been shown to elicit lower lactate levels at the same relative intensity to cycling (Matsui, Kitamura and Miyamura, 1978). Hence, lower BLa in JUMP should not diverge from the potential efficacy of this protocol to illicit adaptations if employed within a longer-term intervention as such physiological responses are likely exercise mode-specific. Indeed, McRae *et al.* (2012) demonstrated improvements in VO_{2peak} with a calisthenic-based HIIT intervention lasting four weeks.

This is the first study to assess changes in plasma volume with apparatus-free HIIE. Metcalfe *et al.* (2015) found significant and pronounced reductions in plasma volume and muscle glycogen with an acute cycle-ergometer HIIE protocol. It is proposed that the accumulation of metabolites in the muscle cells due to glycogen breakdown leads to an osmotic influx into the muscle environment from the blood, which thereby causes a reduction in blood plasma volume (Raja *et al.*, 2006). Although the present study was unable to measure glycogen

breakdown and the absolute reductions in plasma volume seen are smaller than those of Metcalfe *et al.* (2015), it is reasonable to suggest that the reduction in plasma volume seen immediately post-exercise in all three HIIE conditions in the present study is a marker of glycogen breakdown. The fuel sensor, 5'-AMP-activated protein kinase (AMPK), is activated during high-intensity exercise but is allosterically inhibited by the presence of muscle glycogen (McBride *et al.*, 2009). Hence, the breakdown of glycogen (for which a reduction in plasma volume is a marker) that is associated with acute HIIE, releases this allosteric inhibition, enabling AMPK phosphorylation and the upregulation of the AMPK-signalling pathway (McBride *et al.*, 2009). In turn, AMPK activation has been implicated in mediating important health-related benefits of HIIT interventions, which also certainly warrant further study of the HIIE protocols explored in the present study (Gibala *et al.*, 2009).

The acute physiological responses seen with JUMP and CYCLE are consistent with previous HIIE literature (Allison *et al.*, 2017; Gist, Freese and Cureton, 2014). Such responses were achieved with an exercise period lasting just 7.5 minutes in JUMP (including warm-up). Pilot testing established that a shorter recovery period was required in JUMP, compared with CYCLE, to ensure sufficient recovery prior to the following 30 seconds interval. Thus, without the requirement for apparatus, as well as reduced recovery intervals, 4 x 30 seconds of "all out" star jumping appears a more time efficient low volume HIIE protocol that provides a sufficient physiological and metabolic stimulus.

Moreover, the present study found a greater BLa following interval 2, as well as a trend for a greater BLa immediately post-exercise, within the 'overweight/obese' BMI category in JUMP compared with the 'healthy BMI' category. This would imply a greater level of peripheral fatigue accumulated in this group during this protocol and, in addition, reflects the concentrations reached following the stair climbing protocol in Allison *et al.* (2017) to an even greater extent. Notably, and importantly, this finding was not associated with an increase in RPE nor reduction in affect or enjoyment, which may have been otherwise expected. In turn, this warrants further study of the JUMP protocol in an inactive overweight/obese female population in particular given the posed aversive responses with HIIE (Biddle and Batterham, 2015).

Affective responses are important to consider as aversive responses to HIIE will likely lead to a reduction in intensity and/or adherence in a previously inactive population (Biddle and

Batterham, 2015). Of note, Olney *et al.* (2018) recently demonstrated that affective responses to differing protocols of HIIE were considered more aversive than response to moderate-intensity continuous exercise, however both protocols were considered equally as enjoyable. Yet, it is noted that although participants in Olney *et al.* (2018) were unaccustomed to HIIE itself, they were already recreationally active. Therefore, findings could differ for a previously inactive population. Nonetheless, our findings suggest improved affective responses to apparatus-free HIIE compared with cycle ergometer-based HIIE, as affective valence was greater immediately following SQUAT and JUMP versus CYCLE. The 'dual-mode' theory posits that, above the ventilatory threshold, there is greater influence of interoceptive factors on affective responses (Ekkekakis, 2003). Indeed, exercise above the ventilatory threshold has been shown to induce a negative affective response during and immediately post-exercise (Decker and Ekkekakis, 2017; Ekkekakis, Lind and Vazou, 2009; Sheppard and Parfitt, 2008) and hence the lower affective valence observed following CYCLE may be explained by the higher BLa seen. However, the BLa at the end of JUMP reached 7.3±2.4mmol·L^{-1}, yet affective valence did not decrease from resting values (in fact, a non-significant increase was observed). These data suggest further research is warranted regarding application of the 'dual-mode' theory to affective responses to HIIE.

A positive affective response is associated with increased adherence to PA (Kwan and Bryan, 2010a), as well as increased exercise motivation (Kwan and Bryan, 2010b). Additionally, the importance of the affective and enjoyment responses when considering HIIE as a viable PA option have recently been highlighted (Jung, Bourne and Little, 2014). Enjoyment of PA has also been positively correlated with increased participation (Trost *et al.*, 2002) and in the present study, there was a trend for JUMP to be considered more enjoyable than CYCLE. Conversely, a higher perceived effort of exercise is a negative correlate to regular PA (Trost *et al.*, 2002). A lower RPE was observed in JUMP compared with CYCLE, despite similar HR, with the mean RPE value obtained immediately following JUMP similar to that of a high-intensity stair climbing intervention that saw improvements in cardiorespiratory fitness and a tendency for improvements in insulin sensitivity (Allison *et al.*, 2017). As such, the affective, enjoyment and perceived exertion responses to JUMP infer that this and other forms of energetic, apparatus-free, callisthenic or bodyweight-based exercise based on this model are more likely

to be adhered to than a cycle ergometer-based exercise intervention, particularly in inactive females. Long-term adherence in free-living settings should therefore be explored.

In addition, the present study found that affective valence was, overall, significantly lower in the 'overweight/obese' BMI category compared with the 'healthy BMI' category at rest, prior to exercise across all conditions. These findings are in contrast to those by Ekkekakis, Lind and Vazou (2009), where there were no differences between a group of obese females, a group of overweight and a group of 'normal' weight females at rest prior to an incremental exercise test. In addition, Ekkekakis, Lind and Vazou (2009) found affective valence ratings were, overall, significantly lower in the group of obese females compared with the other groups pre, post and during all points of the incremental exercise test. Discrepancies between the findings of Ekkekakis, Lind and Vazou (2009) and the present study could be due to the differing modes of exercise (continuous vs. intermittent high-intensity exercise as well as apparatus-free vs. cycle ergometer-based exercise). It is also acknowledged that both overweight and obese individuals were combined into the 'overweight/obese' BMI category in the present study, which should be considered when comparing with the findings of Ekkekakis, Lind and Vazou (2009) who split participants into 'healthy weight', 'overweight' and 'obese' categories. Nonetheless, in the 'overweight/obese' BMI category in the present study, overall, affective valence significantly increased from rest to 30 minutes post-exercise, whereas affective valence in the 'healthy BMI' category was significantly lower immediately post-exercise compared with 30 minutes post-exercise across all conditions. Reasons for these discrepancies in direction of change of affective valence between these groups in the present study are unclear, but warrants further investigation and strengthens the rationale for exploring HIIE protocols further in an overweight/obese, as well as an inactive, female population.

Greene, Greenlee and Petruzzello (2018) found that a high-intensity intermittent bodyweight exercise group induced levels of perceived exertion and HR responses immediately following an acute exercise bout that were similar to JUMP in the present study. Their findings showed that affective valence declined during the HIIE condition. Interestingly, these findings are in contrast to those of pre and post the JUMP condition in the present study. Moreover, Greene, Greenlee and Petruzzello (2018) demonstrated that ratings of affective valence increased throughout a moderate-intensity continuous exercise condition to levels similar to those seen

immediately post-exercise in JUMP in the present study. This is perhaps a surprising finding, especially considering that Greene and colleagues (2018) used an active population accustomed to regularly exercising at a high-intensity, but may be due to differences in exercise protocols, and nonetheless still offers positive implications for the JUMP protocol of the present study. Greene, Greenlee and Petruzzello (2018), however, found that in-task affect, but not post-task affect, predicted enjoyment responses to the exercise bouts in this study. Greene and colleagues (2018) also, however, acknowledge that in task affect being the better predictor of future exercise behaviour is not necessarily be the case with HIIE and still remains a novel aspect within HIIE research. Hence, the lack of affective response measures during exercise in the present should not take away from those obtained and interpreted post-exercise.

It is acknowledged that affective responses were not measured during task in the present study. However, it was suspected that such affective responses during a HIIE protocol would be very transient in relation to the work and rest periods, as demonstrated previously (Decker and Ekkekakis, 2017). It was deemed, therefore, that a sole measure post-exercise was more appropriate, particularly as greater post-exercise positive affect has been associated with greater frequency of exercise at three months follow-up (Kwan and Bryan, 2010a). Nonetheless, heterogeneity in the timing and methods of affective measures with exercise has been noted (Stork *et al.*, 2017) and further research should explore affective responses during and after intermittent high-intensity exercise to examine whether these parameters remain in predicting future exercise behaviour in HIIE specifically and whether one does so better than another. It is acknowledged that Stork, Gibala and Ginis (2018) pose that HIIE may be more challenging and stimulating than continuous exercise and that the availability of rest periods in between short work intervals could be responsible for the similar levels of enjoyment shown in the present study as well as others, as discussed.

Nonetheless, with the lack of homogeneity across exercise parameters of HIIE studies and great variance in work to recover/rest ratios, exercise mode, relative intensity and duration of protocols, it is inherently difficult to identify or determine a protocol for an effective HIIE protocol or effective HIIT intervention for an inactive population. It should be said that affective responses to high-intensity exercise will also likely depend on the prior activity levels of the participant group and that studies should, hence, focus on utilising inactive populations

unaccustomed to high-intensity exercise in order to make inferences regarding the potential adherence of HIIE in this population regarding the affective and enjoyment responses.

The present study investigated acute responses to a single session of low volume HIIE in controlled laboratory settings. Hence, ecological validity is limited. Long term adaptations and health benefits can therefore only be hypothesised and should be explored in subsequent studies. Of note, recent work by Blackwell *et al.* (2017) demonstrated that an unsupervised, home-based intervention of apparatus-free HIIE (including star jumps) can lead to ~8% improvements in cardiorespiratory fitness in inactive adults. However, it should be noted that this improvement was of a smaller magnitude than the ~17% improvement in cardiorespiratory fitness achieved in the laboratory-based HIIT intervention in the same study. This could be due to reduced intervention adherence and/or attenuated physiological responses achieved with apparatus-free HIIE. Therefore, initial investigation into acute physiological, affective and enjoyment responses to apparatus-free HIIE is warranted in order to optimise longer-term exercise programmes.

4.5 Conclusion

Comparable HR_{peak} and plasma volume responses were achieved by JUMP and CYCLE, with a lower RPE in JUMP compared with CYCLE immediately post-exercise. Additionally, JUMP achieved BLa similar to previous literature reporting efficacious HIIE protocols and interventions. A more positive affective response was observed with JUMP, which also had a tendency to be more enjoyable than CYCLE. High-intensity intermittent star jumping can therefore achieve important physiological responses, whilst achieving more positive affective and possible preferable enjoyment responses than cycle ergometer-based HIIE in inactive females. These findings are of paramount importance to the design and implementations of effective low volume HIIT interventions as a viable PA strategy for improved metabolic health in similar populations.

Chapter 5

Study 2 – Acute appetite and eating behaviour responses to apparatus-free high-intensity intermittent exercise in overweight, inactive females

Abstract

Appetite can be reduced following HIIE, yet, exploring eating behaviour responses at pre-determined time points post-exercise restricts the ecological validity and may mask true differences in EI. In addition, the requirement of specialised apparatus questions the effectiveness of many HIIE protocols for public health interventions. Therefore, the purpose of this study was to investigate appetite and participant-determined eating behaviour responses in response to a previously studied protocol, of 2 x 30 seconds or 4 x 30 seconds of apparatus-free HIIE ("all out" star jumps). Twelve inactive, overweight/obese females (BMI 29.2±2.9 $kg \cdot m^{-2}$, age 38±7 years, leisure time MVPA 28±39 $minutes \cdot week^{-1}$) completed three conditions in a randomised, counterbalanced manner. Following a standardised breakfast, participants rested for 2.5hours, before undertaking rest (NoEx); 2 x 30 seconds (2x30) or 4 x 30 seconds (4x30) of high-intensity intermittent star jumping. Time of request (feeding latency) of, and EI at, an *ad libitum* buffet available following each condition were measured. Feeding latency was similar across conditions (30±32min, 31±25min and 28±22min for 4x30, 2x30 and NoEx, respectively, all $p>0.05$). There were no differences in subjective appetite or absolute EI (all $p>0.05$), but a trend for r a condition effect with a small effect size on relative energy intake was found ($p=0.064$; $n^2_p=0.221$) such that there was a reduction in relative energy intake with a medium effect size of 121kcal following 4x30 (630±181kcal) compared with NoEx (751±278kcal; $p=0.086$; $d=0.52$). 4 x 30 seconds of "all-out" star jumping induces a meaningful daily relative EI of 121kcal. Given the commonly-reported barriers to regular PA in this population, future research should explore the role of such apparatus-free HIIE timed in close proximity to meals to further elucidate daily energy balance responses which can inform effective weight management strategies.

5.1 Introduction

An acute bout of HIIE can transiently reduce subjective appetite, yet reductions in post-exercise *ad libitum* EI are rarely observed (Reger, Allison and Kuruca, 1984; Thompson, Wolfe and Eikelboom, 1988; King, Burley and Blundell, 1994; Deighton *et al.*, 2013a; Beaulieu *et al.*, 2015; Holliday and Blannin, 2017a). This is perhaps unsurprising given that the majority of acute exercise studies utilise an *ad libitum* test meal at a pre-determined time point. For example, an *ad libitum* buffet was used to assess post-exercise EI at 45 minutes (Deighton *et al.*, 2013a), 70 minutes (Sim *et al.*, 2014; Beaulieu *et al.*, 2015), 90 minutes (Martins *et al.*, 2015), 110 minutes (Shamlan *et al.*, 2017) and 120 minutes post-exercise (Panissa *et al.*, 2016; Holliday and Blannin, 2017a). At these points, the transient suppression of appetite may have subsided and, hence, will miss any effects of a transient suppression of appetite on eating behaviour. In addition, the assessment of other eating behaviours (including eating initiation) is not possible using this design.

Lessening the restriction on timing of eating would likely better reflect free-living EI responses to exercise. King, Wasse and Stensel (2013) did assess post-exercise feeding latency and a significant delay in voluntary feeding of ~35 minutes compared with a resting condition was shown. Of note, this was independent of an effect on EI. However, despite an unaffected EI, such a response can still be considered anorexigenic and in unrestricted conditions it could delay feeding post-exercise and/or lead to a reduction in EI across the post-exercise period that extends beyond the transient window. Therefore, a delay in the onset of eating is likely efficacious for manipulating eating initiation as a specific characteristic of eating behaviour.

For appetite, eating behaviour and energy balance to be meaningfully influenced by an acute bout of HIIE in the context of weight management, HIIE must be effective for its successful implementation in a free-living setting. Typically, HIIE protocols and interventions are apparatus-based and those that have seen transient suppressions in appetite and/or reductions in EI require specialised apparatus such as a cycle ergometer (Holliday and Blannin, 2017a) or a treadmill (Beaulieu *et al.*, 2015). However, reliance on specialised apparatus is likely to pose a barrier to compliance given that poor access to apparatus and facilities, as well as limited access to apparatus at home, have previously been highlighted as negative correlates of regular PA (Trost *et al.*, 2002). Moreover, the true time-efficiency of such HIIE

has recently been of contention (Hardcastle *et al.* 2014), while access to specialist apparatus is unlikely to aid the time-efficiency potential of HIIE, as a perceived lack of time is a commonly-reported barrier to regular PA (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017). It has also recently been argued that effective HIIE protocols and interventions should focus on being low cost and accessible in order to achieve more translational findings from the laboratory to public health policy (Gray *et al.*, 2016).

Furthermore, findings from study 1 (Chapter 4) of this thesis suggest that an acute bout of apparatus-free HIIE (4 x 30 seconds "all-out" star jumps) can elicit physiological responses indicative of a high intensity workload. This included reaching a similar HR to that during an acute bout of 4 x 30 seconds "all-out" cycling, which has previously demonstrated suppressions in subjective appetite post-exercise (Holliday and Blannin, 2017a). As such, this apparatus-free mode of HIIE that replicates the physiological stimuli of cycle-based HIIE shown to transiently suppress appetite could feasibly be implemented in close proximity to meals, in a free-living setting, to manipulate energy balance towards a deficit. If such a bout of exercise can offer an effective acute perturbation in energy balance, via an increase in EE and a decrease in EI, this would give rise to effective exercise protocols for not only metabolic health benefits (as described in section 2.1), but also weight management.

The findings of study 1 (Chapter 4) of this thesis demonstrated that both 2 x 30 and 4 x 30 seconds of "all out" star jumping induced physiological responses akin to a high-intensity. Therefore the aim of this study was to the aim of this study was to elucidate responses of appetite and eating behaviour to an apparatus-free HIIE protocol (star jumps) of varying intervals (4 x 30 seconds vs. 2 x 30 seconds vs. resting control) in inactive, overweight females, potentially determining the minimum number of repetitions required to successfully suppress acute appetite, reduce EI and influence eating initiation.

5.2 Methods

5.2.1 Study design

A within-subject, counterbalanced, crossover study design was utilised, with participants

randomly assigned to each of the three conditions: resting control (NoEx), 2x30 seconds of star jumps (2x30) and 4 x 30 seconds of star jumps (4x30).

5.2.2 Participants

An *a priori* power calculation was conducted using the G*Power software (G*Power, Düsseldorf, Germany). Given the effect sizes seen for the change in subjective appetite (as a proxy measure) following a similar HIIE protocol in previous research (Holliday and Blannin, 2017a) and for eating latency in the study of King, Wasse and Stensel (2013) and based on an expected large effect (f=0.4), alpha level of 0.05 and a statistical power of 0.8, a sample size of 12 participants was deemed sufficient to detect a meaningful change in subjective appetite and hence a likely meaningful change in feeding latency (actual power = 0.82).

Fifteen inactive, overweight/obese females were recruited for the study, while 12 started and completed the study (BMI 29.2±2.9 $kg \cdot m^{-2}$, age 38±7 years, leisure time MVPA 28±39 $minutes \cdot week^{-1}$). Inclusion and exclusion criteria, as well as ethical approval, were as described in section 3.2. Of the n=12 total participants, 10 were of Caucasian ethnicity, 1 was of Asian ethnicity and 1 was of Hispanic ethnicity.

5.2.3 Preliminary visit and familiarisation

Before starting the study, participants received written (appendix 4) and verbal information about the study, before written informed consent (appendix 7) was obtained. A pre-participation health questionnaire, based on ACSM pre-screening guidelines (American College of Sports Medicine, 2013; appendix 10), was completed by participants for health screening purposes. Height was measured using a stadiometer and body mass was measured using scales (Seca, Birmingham, UK). Resting blood pressure was measured using an automatic blood pressure monitor (Omron, Milton Keynes, UK). The IPAQ (Craig *et al.*, 2003; appendix 1) was completed to assess current PA levels. The DEBQ (Van Strien *et al.*, 1986a; appendix 2) was then completed by participants to assess eating restraint. Following this, participants received a demonstration of the required star jump technique and completed

one 30 seconds bout. Participants were also familiarised with the experimental protocols, including the measures to be administered during the experimental trials. Participants were then given food diary instructions (appendix 13) and a weighed food diary record (appendix 14) in which they were asked to record 24-hour food intake prior to their first experimental trial. Participants were instructed that this intake was to be replicated twenty four hours prior to the remaining trials.

Participants then undertook a continuous, submaximal graded exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, Netherlands) to determine a prediction of VO_{2peak}. This was required for the calibration of the Actiheart activity monitors (CamNtech, Cambridge, UK) and hence the measures of TEE and AEE assessed during and following testing days. The exercise test consisted of cycling at an initial workload of 20W and a constant cadence of 60-70RPM. Workload was increased in 20W increments every 3 minutes until 80% of age-predicted HR was reached, using the Karvonen formula (Karvonen, Kentala and Mustala, 1957). If this criterion was met mid-stage, participants were instructed to complete the whole stage before cessation of the test. HR (Polar H7, Polar Electro OY, Kempele, Finland) was recorded and expired gases were measured throughout using an online gas calorimetry system (MetaLyzer 3B, Cortex Medical, Peipzig, Germany). Measures of HR, RER and rate of oxygen utilisation (VO_2) were averaged during the final minute of each 3-minute stage. This mean value was then used to calculate the predicted EE by multiplying the VO_2 with the RER-specific caloric equivalent of oxygen (appendix 19; Frayn, 1983). The relationship between HR and EE was calculated for each participant by regression analysis. Measured BMR (assessed upon the first experimental visit as described in section 3.6.9) as well as predicted VO_{2max}, HR_{max} and EE from resting to maximal HRs were then inputted to individually calibrate the activity monitors using the Actiheart software (CamNtech, Cambridge, UK) to ensure calibrated recording and analysis of EE when wearing the monitors.

Finally, participants were presented with a familiarisation *ad libitum* buffet meal at the end of the preliminary visit. A hedonic scale was completed after consumption of the meal to ensure that no hedonic bias was associated with this buffet meal. This was done using a standard 9-point hedonic scale (Peryam and Pilgrim, 1957; appendix 20) and any items rated 1 (“dislike extremely”) or 9 (“like extremely”) were not included in the buffet during experimental trials (Sim *et al.* 2014) to prevent possible hedonic bias.

5.2.4 Experimental trials

Participants returned to the laboratory at 08:00am on their first experimental trial and at 08:30am on the remaining experimental trials. Eumenorrheic participants undertook each experimental trial at least five days apart during the follicular phase of the menstrual cycle (days 1-14; mean day 8±3, 9±5 and 6±3 for 4x30, 2x30 and NoEx, respectively). Participants taking contraceptive medication preventing menstruation undertook each experimental trial 28±2 days apart. However, due to unavoidable and practical reasons, a total of 6 visits fell outside of these windows. Participants' adherence to minimising strenuous physical activity on the pre-testing day was checked verbally by the researcher upon participant arrival to each experimental trial. Adherence to the pre-testing day food diary was also checked upon arrival to each experimental trial ($p>0.05$ for all pre-testing day energy and macronutrient intakes when adhered and documented).

Room temperature was maintained by an air conditioning system at an ambient temperature of ~21°C while humidity ranged from 32-56% when assessed using a hygrometer.

5.2.5 Protocol

Figure 5.1 depicts the design of each experimental trial. Participants were instructed to be fasted and to minimise PA prior to all experimental trials, including arriving at the laboratory by car where possible. On the first experimental trial, participants first underwent a measurement of BMR using indirect calorimetry and an online gas calorimetry system (MetaLyzer 3B, Cortex Medical) for the purpose of calibrating the activity monitors. Participants rested, motionless, in a supine position in a darkened, quiet room in as close proximity to waking as possible for 25 minutes, with continuous measurement of pulmonary gas exchange. VO_2 measurements were averaged during the final 10 minutes of the measurement period and utilised to calculate the predicted EE by multiplying the VO_2 with the caloric equivalent of RER (appendix 19), which were then averaged to estimate BMR (Frayn, 1983). At 9:00am on all experimental trials, participants were provided with a standardised breakfast which consisted of: porridge made with oats, milk and brown sugar and a glass of orange juice. The breakfast meal provided approximately 20% of the estimated

daily energy needs for a sedentary day for each individual (417±34kcal; for macronutrient contributions see section 3.6.14), based on their age, height and weight (Mifflin *et al.*, 1990).

Following breakfast consumption, participants began a rest period where they were invited to quietly read, watch television or work at a desk in the laboratory to minimise environmental food cues. During this period an Actiheart activity monitor was applied, with recording set to begin at 12:00pm. At 11:45am (2.75 hours following breakfast consumption), a finger prick blood sample was obtained through the insertion of a sterile lancet into the fingertip for the measures of blood lactate and concentrations and plasma volume measures. Measures of affective valence as well as positive and negative affect and subjective appetite were recorded at this point.

Each exercise/resting control condition commenced at ~12:00pm (~3 hours following breakfast consumption). For the resting (NoEx) condition, participants were instructed to continue reading, watching television or working at a desk in a seated position. Each exercise condition started with a warm-up period of one minute of walking on the spot on flat ground at a self-selected pace, following that of study 1. Participants then began either 2 x 30 seconds (2x30) or 4 x 30 seconds (4x30) "all-out" bouts of star jumps. The adopted technique consisted of bending at the knees to touch the floor and then jump vertically, stretching out both arms and legs as laterally as possible, as quickly as possible for the duration of each 30 seconds (see section 4.2.5 and figure 4.1 for further detail). Between intervals, participants had a 90 seconds recovery period where they were encouraged to undertake stepping on the spot, similar to the warm-up exercise, to allow for active recovery. The total duration of time for each exercise bout was 9 minutes for 4x30 and 5 minutes for 2x30.

Measures of HR, RPE, affective valence, positive and negative affect and subjective appetite were again assessed following the cessation of each condition (~12:10pm) and a further finger prick blood sample was also taken at this time point, as previously described. At 12:15pm (~3.25 hours following breakfast consumption) in each condition, participants were made aware that "a buffet style lunch is available at any time between now and the final measure at 2:15pm, for the next 2 hours". Upon request of the meal, measures of affective valence, positive and negative affect and subjective appetite were repeated and an additional finger prick blood sample was taken, as previously described. Participants were told to "eat until

comfortably full" and consumed the buffet in a room away from the laboratory, devoid of other people or additional food cues.

Participants were asked to return to and remain in the laboratory following their meal. To ensure that participants did not leave the laboratory before requesting the meal or request the meal sooner in order to leave the laboratory sooner (thus allowing for true request of feeding latency), a final measure of BMR was recorded at 2 hours post cessation of each condition (2:15pm). Following this, participants were free to leave the laboratory, but instructed to continue wearing the Actiheart monitor for the remainder of the day as well as the following 3 consecutive days. Participants were also provided with food diary instructions (appendix 13) and a weighed food diary (appendix 14) and were instructed to record all food and drink consumption for the remainder of the day and the following 3 days.

During the first experimental trial, the drinking of water was permitted and offered *ad libitum* until all measurements had been obtained. During this trial, water intake was measured pre and post-conditions. This amount was provided on all subsequent experimental trials and the participant was encouraged to consume the whole amount.

5.2.6 Measures

HR_{peak}

HR_{peak} was recorded through the use of a recordable HR monitor attached to a chest strap (Polar RS800CX, Polar Electro OY, Kempele, Finland).

RPE

RPE was measured using the 6-20 Borg Scale (Borg, 1982; appendix 15).

Affective responses

Affective valence was measured using the FS (Hardy and Rejeski, 1989; appendix 16) while

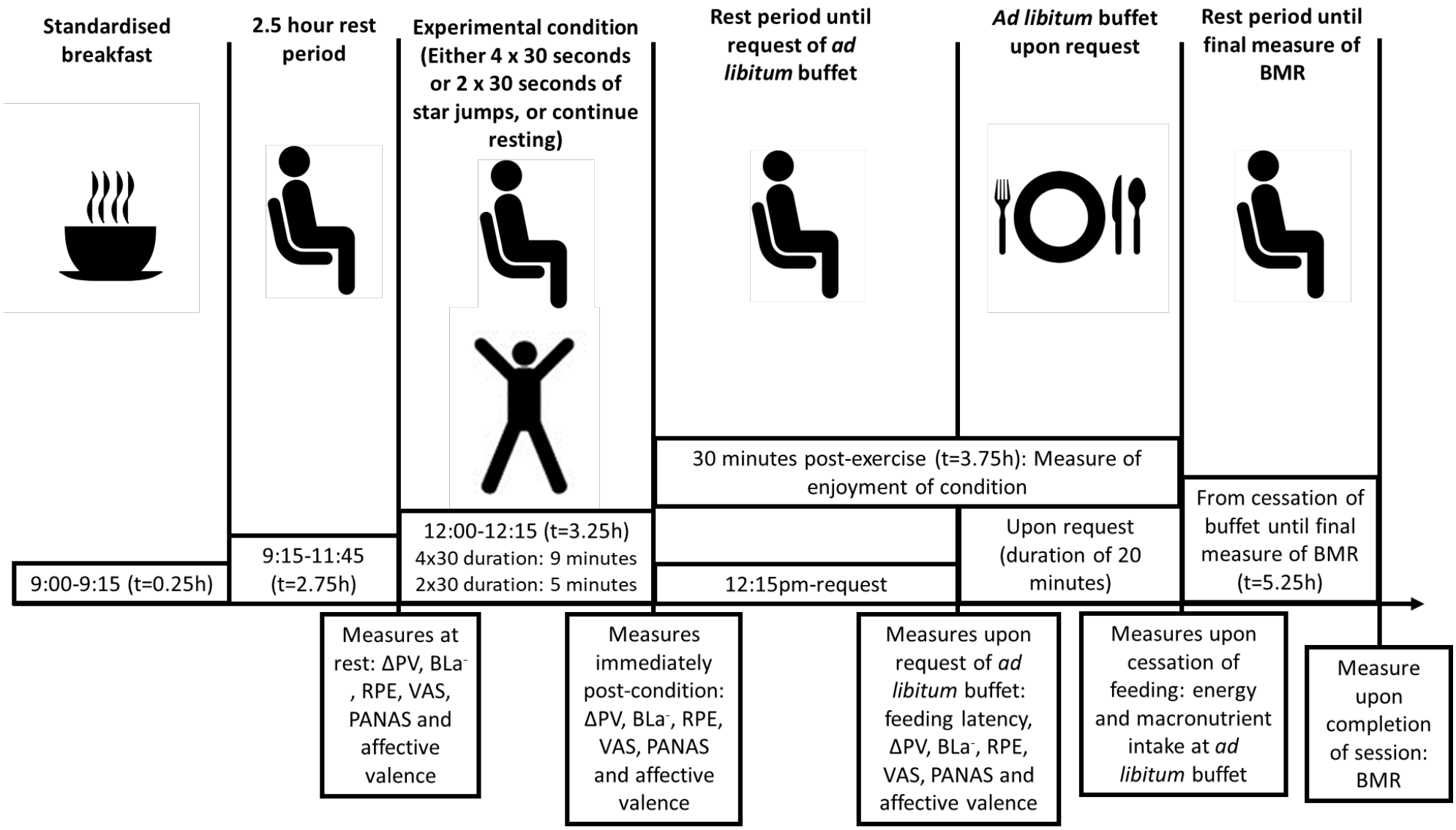

Figure 5.1 Summary of experimental design; 't' denotes 'time', 'h' denotes 'hours from start of experimental trial'; 'BMR' denotes 'basal metabolic rate', 'ΔPV' denotes 'change in plasma volume from rest', 'BLa' denotes 'blood lactate concentration', 'RPE' denotes 'rating of perceived exertion', 'VAS' denotes 'Visual Analogue Scale', 'PANAS' denotes 'Positive and Negative Affect Scale', 'BMR' denotes 'basal metabolic rate'

the PANAS assessed positive and negative affect (Watson, Clark and Tellegen, 1988; appendix 17).

Enjoyment

A measure of enjoyment of the bout of exercise was obtained (for 2x30 and 4x30) using the PACES (Kendzierski and DeCarlo, 1991; appendix 18).

Subjective appetite

At the time points when subjective appetite was assessed, participants completed the VAS (Hill and Blundell, 1982; appendix 22) which assessed subjective feelings of hunger, fullness, desire to eat and prospective food consumption using 150mm VAS. A composite appetite score was then generated as the mean of these four values after fullness was reverse-scored (Holliday and Blannin, 2017a). A fifth 150mm VAS was used to assess feelings of nausea.

Feeding latency and energy intake

Participants were not made explicitly aware that eating behaviour was being assessed and instead were told that the primary outcome of the study was to investigate the physiological and psychological responses of differing volumes of HIIE. Feeding latency was measured as the time (in minutes) that participants verbally requested the buffet from cessation of condition (12:15pm) until request of the buffet. The buffet was presented identically before each trial and items were displayed in excess of expected consumption. Items offered, along with nutritional information (obtained from food labels), are shown in table 5.1.

Sandwiches were offered in quarters to remove familiar cognitive or visual cues that may influence self-regulated food intake (Stubbs *et al.*, 1998). At the meal, participants were not given a specific amount of time to finish eating but were told the researcher would return in 20 minutes and were instructed to “eat until they are comfortably full”. Participants chose

and ate the items in isolation in a room near to, but not visible from, the laboratory where there were no social interactions. Energy and macronutrient values of items consumed were calculated covertly, according to manufacturer information.

Table 5.1 Food items, with nutritional information, of the ad libitum lunch buffet meal

Food	Energy density (kcal·100g^{-1})	CHO (grams·100g^{-1})	FAT (grams·100g^{-1})	PRO (grams·100g^{-1})
Bread	232	42.5	1.7	9.2
Margarine	708	0.9	78	0.5
Cheese and onion sandwich filler	320	5.1	28	11
Egg mayonnaise sandwich filler	197	3.8	16	10
Tuna mayonnaise sandwich filler	190	11	12	8.6
Ready salted crisps	134	12	8.4	1.7
Salt and vinegar crisps	132	13	7.9	1.5
Cheese and onion crisps	132	13	7.9	1.8
Chocolate cereal bar	112	16	3.2	2.2
Cranberry cereal bar	108	16	2.9	1.9
Banana	103	23.2	0.5	1.2
Apple	47	12	0.5	0.5
Orange	40	7.8	0.5	0.8

'CHO' denotes 'carbohydrate', 'FAT' denotes 'fat', 'PRO' denotes 'protein'

Total EI and macronutrient intake were calculated for the remainder of the testing day as well as each of the three days following the testing day through the use of a self-report weighed food diary (appendix 14). Mean daily EI and mean daily macronutrient intake were calculated as well as a mean value for each over the three full days using the nutritional analysis software Nutritics (Nutritics Ltd, Ireland).

Energy expenditure

Participants were asked to wear the activity monitor (attached to the chest strap at chest height with instructions provided; appendix 21) for the remainder of the testing day as well as each of the three days following the testing day. Total and activity EE, as well as METs, were derived for the remainder of the testing day and each of the three days following each testing day. MET values were calculated according to the cut-off points described in table 3.1.

Blood lactate and glucose concentration

At the time of each finger prick blood sample, a 10µL capillary blood sample was obtained in a capillary tube for the analysis of blood lactate and glucose concentrations using a desktop analyser (Biosen C_Line, EKF Diagnostics, Cardiff, UK). All measures were analysed in duplicate with a third measure obtained if disagreement between the first two measures was >2%.

Plasma volume

Further capillary blood samples were obtained before and immediately following each condition in an additional 60µL heparinised capillary tube and a 10µl capillary slide for the measurement of haematocrit and haemoglobin concentration, respectively. The 60µL tubes were centrifuged for a period of 5 minutes at 12000RPM (Sorvall ST 8R, Thermo Fisher Scientific, Massachusetts, United States) and haematocrit was manually calculated using a Hawksley reader (Hawksley & Sons Ltd, Sussex, UK). Haemoglobin was measured using a HemoCue Hb 201+ System (HemoCue AB, Ängelholm, Sweden). From this, ΔPV was determined manually using the equations of Dill and Costill (Dill and Costill, 1974).

All measures were analysed in duplicate with a third measure obtained if disagreement between the first two measures was >5%. Both corrected and uncorrected responses of blood glucose and BLa were measured. Uncorrected values are presented and used for ease of comparison with previous literature. Statistical analysis was repeated with corrected values to ensure any effects observed were not due to changes in haemodilation.

5.2.7 Statistical analysis

A 3x3 repeated measures ANOVA (condition x time) was used to assess differences for VAS (composite score) and blood glucose and lactate concentrations (uncorrected and corrected for ΔPV), positive affect, negative affect and affective valence at rest, immediately following each condition and upon buffet request. A 3x3 repeated measures ANOVA (condition x time) was also used to assess daily EI, daily percentage of kilocalories (%kcal) from CHO, PRO and FAT as well as daily TEE and AEE on each of the three days following the testing day (days 1, 2 and 3). A one-way repeated measures ANOVA was used to assess for differences between conditions in feeding latency, VAS AUC (composite score), absolute and relative EI and %kcal from CHO, PRO and FAT at the buffet meal. A one-way repeated measures ANOVA was also used to assess for any order effect in feeding latency, absolute EI and relative EI as well as differences between conditions in TEE and AEE, MET categories of EE, EI and %kcal from CHO, PRO and FAT for the remainder of the testing day. Differences in TEE and AEE for each test condition was also assessed using a one-way repeated measures ANOVA. A 2x3 repeated measures ANOVA (condition x time) was used to assess differences for RPE as well as ΔPV at rest and immediately following each condition. ΔPV between conditions was assessed using individual one sample t-tests, while enjoyment of exercise and HR_{peak} of 4x30 and 2x30 were assessed using a paired samples t-test. For all ANOVAs, the Greenhouse-Geisser correction was used if the assumption of sphericity was violated. Significant interaction and main effects of ANOVA were investigated further by conducting *post-hoc* pairwise analyses using Bonferroni tests.

Due to analysis error, missing data analysis using the multiple imputations technique was used for missing data points for blood glucose concentration (2 cases), BLa (2 cases), ΔPV (2 cases) and affective valence (1 case). Due to the activity monitor malfunctioning on a total of 6 days following the testing day and one remainder of the testing day, as well as participants not meeting the minimum requirement of 80% wear time on a total of 5 days following the testing day and one remainder of the testing day and one participant being unwell on one day following the testing day, missing data analysis was used for these missing data points for total (14 cases) and activity EE (14 cases), percentage time spent in sedentary (14 cases), light (14 cases), moderate (14 cases) and vigorous-intensity activity (14 cases). Due to one

participant failing to complete the food diary for one day following the testing day and one participant being unwell for one day following the testing day, missing data analysis was also used for daily EI on the three days following testing day (2 cases) and %kcal from CHO (2 cases), FAT (2 cases) and PRO (2 cases). Extreme outliers (>3x interquartile range of the dataset) were removed from the dataset before statistical analysis. All statistical analysis was undertaken using the software SPSS (SPSS version 23.0, SPSS inc., Chicago, Illinois, USA).

5.3 Results

5.3.1 Appetite, energy intake and energy expenditure responses

Feeding latency

No significant main effect was found for feeding latency: $F(2, 22)=0.071$, $p=0.931$, $\eta^2_p=0.006$. Feeding latency was 30±32min, 31±25min and 28±22min for 4x30, 2x30 and NoEx, respectively. No order effect was detected for feeding latency ($p=0.696$).

Subjective appetite

For subjective appetite responses, see figure 5.2. No significant condition x time interaction effect was found for VAS (composite): $F(2.114, 23.256)=1.888$, $p=0.172$, $\eta^2_p=0.146$, but a trend for a main effect of condition was found: $F(2, 22)=3.357$, $p=0.053$, $\eta^2_p=0.234$. There was also a significant main effect of time: $F(2, 22)=15.762$, $p<0.001$, $\eta^2_p=0.589$). Across all conditions, composite VAS score upon buffet request (93.5±32.1mm) was significantly greater than immediately post-condition (66.4±36.9mm, $p=0.001$, $d=0.78$, 95% CI 13.126 – 41.043) as well as upon buffet request compared with rest (67.7±28.0mm, $p=0.007$, $d=0.86$, 95% CI 7.405 – 44.109). For VAS AUC (composite), there was a significant main effect of condition: $F(1.31, 14.413)=5.144$, $p=0.031$, $\eta^2_p=0.319$. There were no significant *post-hoc* differences, although a trend was seen for VAS AUC (composite) to be greater in NoEx (164±49mm) compared with 2x30 (130±28mm; $p=0.097$).

For VAS (nausea), five extreme outliers were removed from the dataset (two for NoEx rest, one for 4x30 post and two for 4x30 request), resulting in n=9 for this measure. There was no significant condition x time interaction effect: $F(1.697, 13.574)=1.524$, $p=0.251$, $\eta^2_p=0.160$. There was also no significant main effect of time: $F(2, 16)=0.605$, $p=0.558$, $\eta^2_p=0.07$ but there was a trend for a significant main effect of condition: $F(1.132, 9.056)=3.637$, $p=0.086$, $\eta^2_p=0.313$. Extreme outliers that were removed consisted of a small number of participants expressing some feelings of nausea, which were outside of 3x interquartile range as the remainder of participants expressed very little or no feelings of nausea. However, inclusion of these extreme outliers did not change the lack of condition x time interaction effect for VAS (nausea): $F(4, 44)=1.684$, $p=0.171$, $\eta^2_p=0.133$, nor the lack of a main effect of time: $F(2, 22)=0.051$, $p=0.950$, $\eta^2_p=0.005$ nor the lack of a main effect of condition: $F(2, 22)=0.923$, $p=0.412$, $\eta^2_p=0.077$).

Energy and macronutrient intake

There was no significant main effect of condition for absolute EI at the *ad libitum* buffet: $F(2, 22)=1.280$, $p=0.298$, $\eta^2_p=0.104$. However, there was a trend for a main effect of condition for relative EI at the buffet (values to the nearest integer): $F(2, 22)=3.13$, $p=0.064$, $\eta^2_p=0.221$, whereby there was a trend for relative EI to be lower in 4x30 (630±181kcal) compared with NoEx (751±278kcal; $p=0.086$; see figure 5.3). For %kcal from CHO at the buffet meal, one extreme outlier was removed from the dataset (for 4x30). There was no significant main effect of condition: $F(2, 20)=0.399$, $p=0.676$, $\eta^2_p=0.038$. For %kcal from FAT at the buffet meal, there was also no significant main effect of condition: $F(2, 22)=0.1$, $p=0.905$, $\eta^2_p=0.009$. There was also no significant main effect of condition for %kcal from PRO at the buffet meal: $F(2, 22)=0.707$, $p=0.504$, $\eta^2_p=0.06$. No order effect was detected for either absolute or relative energy intake at the *ad libitum* buffet ($p=0.830$ and $p=0.855$, respectively).

For EI responses on the remainder of the testing day and the days following the testing day, see table 5.3. There was no significant main effect of condition for the remainder of the testing day: $F(2, 22)=0.199$, $p=0.821$, $\eta^2_p=0.018$. For EI on days following the testing day, one

Table 5.2 Uncorrected blood lactate and glucose concentrations and change in plasma volume from rest across time points and conditions

	4x30			2x30			NoEx		
	Rest	**Post**	**Request**	**Rest**	**Post**	**Request**	**Rest**	**Post**	**Request**
BLa (mmol·L^{-1})	1.3±0.4	6.4±1.6$^{*\#}$	4.1±2.8	1.3±0.4	4.0±1.1*	2.4±1.4	1.2±0.4	1.1±0.4	1.2±0.4
Glucose (mmol·L^{-1})	4.0±0.3	4.3±0.4*	4.1±0.5	4.0±0.3	4.1±0.3	4.0±0.4	4.0±0.3	3.9±0.3	3.9±0.3
ΔPV (%)		-9.3±6.6*	0.7±6.3		-10.7±9.0*	-1.0±7.5		-0.4±6.5	-0.7±5.4

* denotes significantly different to NoEx at that time point (p<0.05); $^{\#}$ denotes significantly different to 2x30 at that time point (p<0.05), 'post' denotes 'immediately post-condition' (p<0.05)

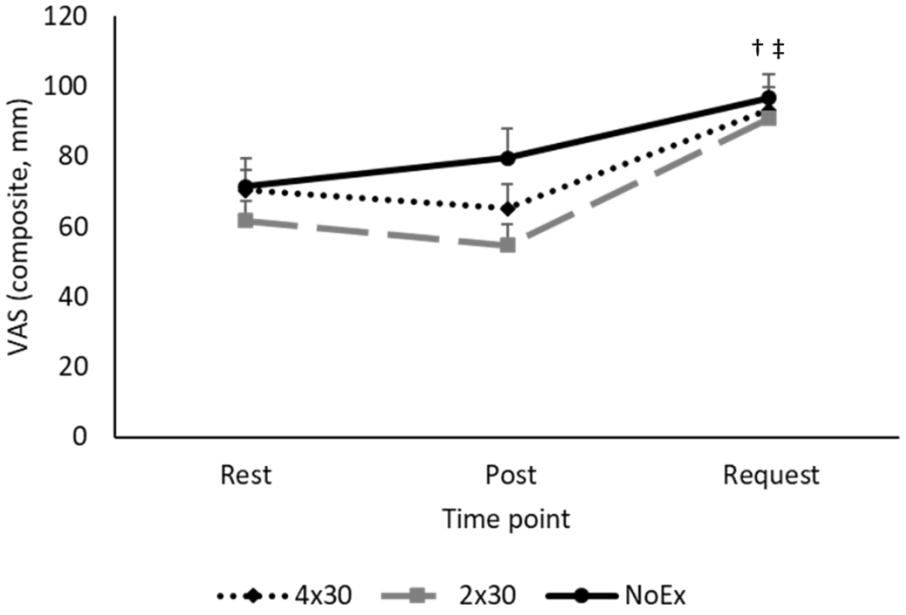

Figure 5.2 VAS (composite) scores for each condition at each time point; † denotes significant difference upon request compared with 'rest' (p<0.05), ‡ denotes significant difference upon request compared with 'post'(p<0.05), 'VAS' denotes 'visual analogue scale'

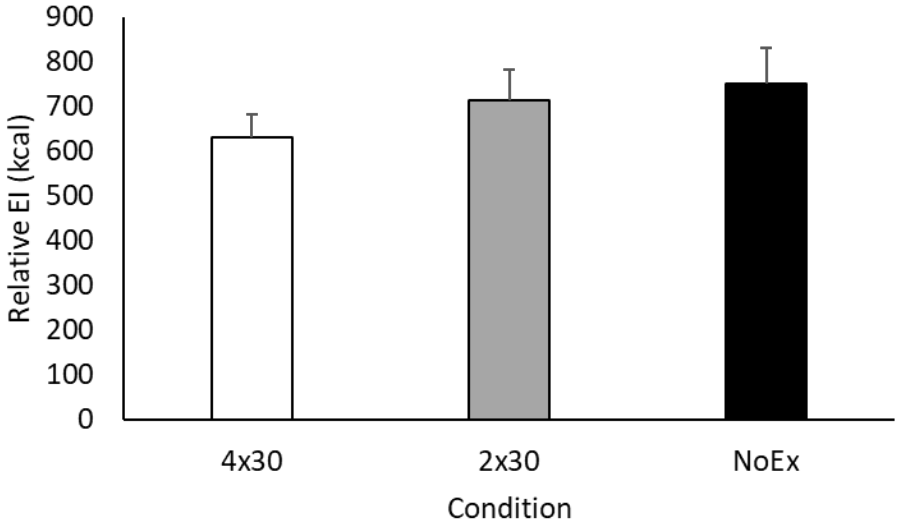

Figure 5.3 Relative energy intake for each condition at the ad libitum buffet

extreme outlier was removed from the dataset (for 2x30 day 2), resulting in n=11 for this measure. For the days following the testing day, there was no condition x day interaction effect for EI: F(4, 40)=0.313, p=0.868, η^2_p=0.03. There was also no main effect of day: F(1.316, 13.158)=0.220, p=0.713, η^2_p=0.021 but there was a trend for a main effect of condition: F(2, 20)=2.776, p=0.086, η^2_p=0.217.

For %kcal from CHO, one extreme outlier was removed from the dataset (for 4x30 day 3), resulting in n=11 for this measure. For remainder of the testing day, there was a significant main effect of condition: F(2, 22)=4.372, p=0.025, η^2_p=0.284, whereby there was a trend for %kcal from CHO across the remainder of the testing day to be greater in 2x30 (45.6±13.6%) compared with 4x30 (34.3±8.8%; p=0.055). For the three days following the testing day for %kcal from CHO, there was no significant condition x day interaction effect: F(4, 40)=1.01, p=0.414, η^2_p=0.092. There was also no significant main effect of day: F(2, 20)=1.663, p=0.215, η^2_p=0.143 nor condition: F(2, 20)=1.403, p=0.269, η^2_p=0.123.

For %kcal from FAT, there was no significant main effect of condition for the remainder of the testing day: F(2, 22)=1.209, p=0.318, η^2_p=0.099. For the three days following the testing day, there was no significant condition x day interaction effect: F(4, 44)=0.989, p=0.424, η^2_p=0.082) and no significant main effect of condition: F(2, 22)=0.325, p=0.726, η^2_p=0.029. There was a significant main effect of day: F(2, 22)=4.134, p=0.03, η^2_p=0.273, whereby, across all conditions, %kcal from FAT was significantly greater on day 3 (38.5±6.3%) compared with day 1 (33.5±6.1%; p=0.015, d=0.81, 95% CI 0.980 – 9.000).

For %kcal from PRO on the remainder of the testing day, there was no significant main effect of condition: F(2, 22)=1.327, p=0.286, η^2_p=0.108. For the three days following the testing day, two extreme outlier were removed from the dataset (one for 4x30 day 3 and one for 2x30 day 2), resulting in n=10 for this measure. There was no condition x day interaction effect: F(4, 36)=2.014, p=0.113, η^2_p=0.183 and no significant main effect of day: F(1.192, 10.73)=1.829, p=0.189, η^2_p=0.169. There was a significant main effect of condition: F(2, 20)=4.491, p=0.026, η^2_p=0.333, whereby across all time points %kcal from PRO was significantly greater in 4x30 (17.1±3.5%) compared with 2x30 (14.1±3.5%; p=0.013, d=0.86, 95% CI 0.64 – 5.194).

Energy expenditure

There was a significant main effect of condition for TEE in each testing condition (values to nearest integer): F(2, 22)=266.46, p<0.001, η^2_p=0.960. TEE was significantly greater in 4x30 (51±8kcal) compared with both 2x30 (23±5kcal; p<0.001, d=3.92, 95% CI 22.348 – 32.819) and NoEx (7±1kcal; p<0.001, d=7.27, 95% CI 37.245 – 50.589). TEE was also significantly greater in 2x30 compared with NoEx (p<0.001, d=4.36, 95% CI 12.295 – 20.372). There was also a significant main effect of condition for AEE in each testing condition: F(2, 22)=232.493, p<0.001, η^2_p=0.955. AEE was significantly greater in 4x30 (37±7kcal) compared with 2x30 (17±4kcal; p<0.001, d=3.51, 95% CI 15.697 – 24.984) and NoEx (0±0kcal; p<0.001, 95% CI 31.177 – 43.323). AEE was also significantly greater in 2x30 compared with NoEx (p<0.001, 95% CI 13.311 – 20.507).

Wear time adherence beyond the laboratory was not met on a total of three days (one for 4x30 day 1 in one participant and one for both 2x30 day 2 and day 3 in another participant). In these cases, values were replaces using multiple imputations. For days where activity monitor adherence was met, average wear time adherence was 95.1±3.5% of the 24-hour period for each day.

For TEE responses, see table 5.3. For TEE on days following the testing day, see table 5.3. For the remainder of the testing day, there was no significant main effect of condition: F(2, 22)=0.694, p=0.510, η^2_p=0.059. For the three days following the testing day, one extreme outlier was removed from the dataset (for 2x30 day 1), resulting in n=11 for this measure. There was a trend for condition x day interaction effect: F(4, 40)=2.336, p=0.076, η^2_p=0.189. There was no significant main effect of condition: F(2, 20)=0.594, p=0.562, η^2_p=0.056 but there was a significant main effect of day: F(2, 20)=6.149, p=0.08, η^2_p=0.381. Across all conditions, TEE was reduced on day 1 following the testing day (2438±302kcal·day^{-1}) compared with day 2 (2575±222 kcal·day^{-1}; p=0.026, d=0.52) and there was a trend for it also to be reduced compared with day 3 (2571±278kcal·day^{-1}; p=0.053).

For AEE responses, see table 5.3 There was no significant main effect of condition for AEE for the remainder of the testing day: F(2, 22)=1.013, p=0.379, η^2_p=0.084. For the three days following the testing day, there was a significant condition x day interaction effect: F(4, 44)=2.771, p=0.039, η^2_p=0.201. However, there were no significant *post-hoc* tests. There were

also no significant main effects of condition: F(2, 22)=0.488, p=0.476, η^2_p=0.042 nor day: F(2, 22)=0.288, p=0.752, η^2_p=0.042.

Metabolic equivalent of task responses

For MET responses, see table 5.3. There was no significant main effect of condition for the percentage of time spent sedentary (%SED) on the remainder of the testing day: F(2, 22)=1.585, p=0.227, η^2_p=0.126. For the three days following the testing day, there was a significant condition x day interaction effect: F(4, 44)=3.512, p=0.014, η^2_p=0.242. In 4x30, there was a trend for %SED to be greater on day 1 following the testing day (79.0±7.5%) compared with 2x30 (69.5±8.3%; p=0.059). Within 4x30, there was also a trend for %SED to be greater on day 1 following the testing day (79.0±7.5%) compared with day 2 following the testing day (71.7±7.3%; p=0.077). %SED on day 3 following the testing day in 4x30 (65.2±13.5%) was also significantly lower than on day 1 following the testing day for 4x30 (79.0±7.5%, p=0.04, d=1.26, 95% CI 0.612 – 27.029). There was also a trend for a significant main effect of day: F(2, 22)=3.026, p=0.069, η^2_p=0.216, but no significant main effect of condition: F(2, 22)=0.270, p=0.766, η^2_p=0.024.

For the percentage of time spent in light-intensity PA (%LI), there was no significant main effect of condition for the remainder of the testing day: F(2, 22)=1.688, p=0.208, η^2_p=0.133. For the three days following the testing day, there was a significant condition x day interaction effect: F(4, 44)=3.073, p=0.026, η^2_p=0.218. Within 4x30, %LI was significantly lower on day 1 following the testing day (15.6±4.7%) compared with both day 2 (22.2±6.1%; p=0.019, d=1.21, 95% CI 1.074 – 12.286) and day 3 (27.1±12.7%; p=0.04, d=1.2, 95% CI 0.482 – 22.730). There was also a significant main effect of day: F(2, 22)=5.243, p=0.014, η^2_p=0.323 whereby, across all conditions, there was a trend for %LI to be greater on day 3 (23.1±6.7%) compared with day 1 (17.9±4.6%; p=0.077). There was no significant main effect of condition: F(2, 22)=0.559, p=0.58, η^2_p=0.048.

For percentage time spent in moderate-intensity physical activity (%MOD) for the remainder of the testing day, one extreme outlier was removed (for 2x30), resulting in n=11 for this measure. There was no significant main effect of condition for %MOD for the remainder of

the testing day: F(1.284, 12.839)=0.022, p=0.929, η^2_p=0.002. For the three days following the testing day, there was a significant condition x day interaction effect for %MOD: F(4, 44)=3.018, p=0.028, η^2_p=0.215. Within 4x30, %MOD was significantly greater on day 3 following the testing day (7.7±1.5%) compared with day 2 (5.8±1.9%; p=0.046, d=1.11, 95% CI 0.031 – 3.884) and there was a trend for %MOD to be greater on day 3 following the testing day compared with day 1 (4.8±3.5%; p=0.078). There was no significant main effect of day: F(2, 22)=0.693, p=0.510, η^2_p=0.059 nor condition: F(2, 22)=0.460, p=0.637, η^2_p=0.04.

For percentage time spent in vigorous-intensity physical activity (%VIG) on the remainder of the testing day, 4 extreme outliers were removed (one for 4x30, one for 2x30 and two for NoEx), resulting in n=10 for this measure. However, there was no significant main effect of condition for %VIG for the remainder of the testing day: F(1.193, 10.735)=1.456, p=0.261, η^2_p=0.139. For the three days following the testing day, three extreme outliers were removed (one for 2x30 day 1, one for NoEx day 2 and one for NoEx day 3), resulting in n=10 for this measure. There was no condition x day interaction effect: F(2.085, 18.766)=2.473, p=0.110, η^2_p=0.197. There was also no significant main effect of condition: F(2, 18)=0.269, p=0.767, η^2_p=0.029 nor day: F(2, 18)=0.269, p=0.767, η^2_p=0.029.

For percentage time spent in very vigorous physical activity (%V-VIG), 2 extreme outliers were removed (one for 4x30 and one for 2x30), resulting in n=10 for this measure. There was no significant main effect of condition for %V-VIG for the remainder of the testing day: F(1.03, 9.273)=2.689, p=0.134, η^2_p=0.230. For the three days following the testing day, 13 extreme outliers were removed from the dataset (one from 4x30 day 1, two from 4x30 day 2, two from 2x30 day 1, one from 2x30 day 2, two from NoEx day 1, two from NoEx day 2 and two from NoEx day 3), resulting in n=8 for this measure. However, there was no significant condition x day interaction effect: F(4, 28)=2.515, p=0.110, η^2_p=0.264 and no significant main effect of condition: F(1.109, 7.762)=3.306, p=0.106, η^2_p=0.321 nor day: F(2, 14)=1.379, p=0.284, η^2_p=0.165.

Table 5.3 Energy intake, total and activity energy expenditure (all values to nearest integer) and metabolic equivalent of task responses during the remainder of the testing day and day 1, day 2 and day 3 following the testing day across conditions

	4x30				2x30				NoEx			
	Remainder testing day	**Day 1**	**Day 2**	**Day 3**	**Remainder testing day**	**Day 1**	**Day 2**	**Day 3**	**Remainder testing day**	**Day 1**	**Day 2**	**Day 3**
EI (kcal·day^{-1})	994 ±422	1665 ±534	1826 ±874	1750 ±504	1018 ±539	2038 ±572	1887 ±568	2106 ±719	937 ±263	2032 ±755	1904 ±426	2051 ±990
TEE (kcal·day^{-1})	1294±212	2355 ±292	2520 ±311†	2665 ±483	1294±204	2405 ±355	2707 ±162†	2616 ±217	1245±222	2552 ±473	2500 ±475†	2433 ±320
AEE (kcal·day^{-1})	381±186	606 ±305	705 ±180	864 ±290	405±167	717 ±351	839 ±267	783 ±231	343±158	856 ±466	706 ±333	697 ±277
SED (%time·day^{-1})	70.8±11.2	79.0 ±7.4	71.7 ±7.3	65.2 ±13.5$^{\#}$	68.9±8.5	74.5 ±9.0	69.5 ±8.3	71.2 ±7.7	74.7±9.0	71.7 ±11.8	74.4 ±10.1	73.4 ±9.1
LI (%time·day^{-1})	22.3±8.9	15.6 ±4.7	22.2 ±6.1$^{\#}$	27.2 ±12.7$^{\#}$	23.6±6.5	18.6 ±6.4	22.1 ±5.3	21.6 ±5.3	18.8±7.0	19.7 ±7.9	19.0 ±7.8	20.6 ±7.3
MOD (%time·day^{-1})	5.6±2.9	4.8±3.5	5.8±1.9	7.7±1.5*	5.5±1.7	5.6±2.8	7.2±3.6	7.2±3.4	5.4±2.8	7.4±4.1	5.8±3.7	5.2±3.6
VIG (%time·day^{-1})	0.3±0.4	0.3 ±0.4	0.4 ±0.5	0.4 ±0.5	0.4 ±0.7	0.2 ±0.3	0.7 ±0.9	0.4 ±0.4	0.1 ±0.1	0.5 ±0.5	0.3 ±0.4	0.2 ±0.4
V-VIG (%time·day^{-1})	0.0±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.2±0.6	0.1 ±0.2	0.1 ±0.2	0.1 ±0.2	0.0±0.0	0.0 ±0.1	0.0 ±0.0	0.0 ±0.0

$^{\#}$ denotes significant difference compared with day 1 in that condition (p<0.05); * denotes significant difference compared with day 2 in that condition (p<0.05); '†' denotes significant difference between days across all conditions (p<0.05), 'EI' denotes 'daily energy intake', 'TEE' denotes 'daily total energy expenditure', 'AEE' denotes 'daily activity energy expenditure', 'SED' denotes '% time per day spent sedentary', 'LI' denotes '% time per day spent in light-intensity activity', 'MOD' denotes '% time per day spent in moderate-intensity activity', 'VIG' denotes '% time spent in vigorous-intensity activity', 'V-VIG' denotes '% time spent in very vigorous-intensity activity'

5.3.2 Physiological responses

HR_{peak}

For HR_{peak}, two cases were missing due to HR monitor malfunctions (monitors did not continually detect and display heart rate, resulting in n=10 for this measure). HR_{peak} was significantly greater in 4x30 (161±13 beats·min^{-1}; 89.3±6.5% HR_{max}) compared with 2x30 (152±9 beats·min^{-1}; 84.0±4.4% HR_{max}; t(9)=4.943, p<0.001, 95% CI 4.813 – 12.937; values to the nearest integer).

Blood lactate concentration

For BLa responses, see table 5.2. There were no differences at rest between conditions. A significant condition x time interaction was found for BLa: F(1.935, 21.285)=23.017, p<0.001, η^2_p=0.677. Immediately post-exercise, uncorrected BLa was significantly greater following 4x30 (6.4±1.6mmol·L^{-1}) compared with 2x30 (4.0±1.1mmol·L^{-1}; p<0.001, d=1.73, 95% CI 1.554 – 3.219) and NoEx (1.1±0.4mmol·L^{-1}; p<0.001, d=4.55, 95% CI 3.963 – 6.624). BLa was also significantly greater immediately post-condition following 2x30 compared with NoEx (p<0.001; d=3.44, 95% CI 1.968 – 3.845). Upon request, uncorrected BLa was significantly greater in 4x30 (4.1±2.8mmol·L^{-1}) compared with in 2x30 (2.4±1.4mmol·L^{-1}; p=0.049, d=0.73, 95% CI 0.009 – 3.264) and NoEx (1.2±0.4mmol·L^{-1}; p=0.019, d=1.42, 95% CI 0.456 – 5.266). Upon request, uncorrected BLa was also significantly greater in 2x30 compared with NoEx (p=0.043, d=1.17, 95% CI 0.037 – 2.411). Uncorrected BLa was also significantly increased immediately post-condition in 4x30 compared with at rest (1.3±0.4mmol·L^{-1}; p<0.001, d=4.46, 95% CI 3.708 – 6.624) and at request (p=0.014; d=1.03, 95% CI 0.482 – 4.225). Uncorrected BLa was also significantly greater upon request in 4x30 compared with rest (p=0.023, d=1.40, 95% CI 0.376 – 5.249). Uncorrected BLa was also significantly increased immediately post-condition in 2x30 compared with at rest (1.4±0.5mmol·L^{-1}; d=3.04, 95% CI 1.563 – 3.807) as well as with request (p=0.005, d=1.0, 95% CI 0.497 – 2.710).

There was also a significant main effect of condition: F(1.163, 12.796)=42.393, p<0.001, η^2_p=0.794 whereby, across all time points, uncorrected BLa was significantly greater in 4x30

(3.92±1.26mmol·L^{-1}) compared with 2x30 (2.6±0.7mmol·L^{-1}; p=0.001, d=1.28, 95% CI 0.639 – 1.980) and NoEx (1.2±0.4mmol·L^{-1}; p<0.001, d=2.96, 95% CI 1.613 – 3.915). Uncorrected BLa was also significantly greater in 2x30 compared with NoEx (p<0.001, d=2.53, 95% CI 0.839 – 2.069). There was also a significant main effect of time: F(2, 22)=27.786, p<0.001, η^2_p=0.716. Across all conditions, uncorrected BLa was significantly greater immediately post-condition (3.9±0.9mmol·L^{-1}) compared with at rest (1.3±0.4mmol·L^{-1}; p<0.001, d=3.82, 95% CI 1.752 – 3.489) as well as at request (2.58±1.31mmol·L^{-1}; p=0.003, d=1.16, 95% CI 0.475 – 2.110). Uncorrected BLa was also significantly greater at request compared with at rest (p=0.034, d=1.37, 95% CI 0.093 – 2.563).

When corrected for ΔPV, the significant condition x time interaction effect as well as the significant condition and time main effects for uncorrected BLa remained. Subsequent *post-hoc* differences also remained (all p<0.001), aside from BLa immediately post-exercise compared with request in 4x30 (p=0.062).

Blood glucose concentration

For blood glucose concentration responses, see table 5.2. There were no differences at rest between conditions. A significant condition x time interaction was found for uncorrected blood glucose concentration: F(4, 44)=3.823, p=0.009, η^2_p=0.258. Blood glucose concentration was significantly greater immediately post-condition in 4x30 (4.29±0.35mmol·L^{-1}) compared with NoEx (3.86±0.33mmol·L^{-1}; p=0.009, d=1.26, 95% CI 0.108 – 0.747). Within 4x30, uncorrected blood glucose concentration was significantly greater immediately post-condition compared with at rest (4.0±0.3mmol·L^{-1}; p=0.005, d=1.04, 95% CI 0.109 – 0.567). For blood glucose concentration, there was also a trend for a significant main effect of condition: F(2, 22)=2.866, p=0.078, η^2_p=0.207 and a significant main effect of time: F(2, 22)=4.816, p=0.018, η^2_p=0.305. Across all conditions, glucose concentration was significantly greater immediately post-condition (4.1±0.2mmol·L^{-1}) compared with rest (4.0±0.2mmol·L^{-1}; p=0.027, d=0.47, 95% CI 0.012 – 0.204).

However, when corrected for ΔPV, there was no significant condition x time interaction effect: F(4, 44)=2.044, p=0.105, η^2_p=0.157 and no significant main effect of time: F(2, 22)=1.072,

p=0.360, η^2_p=0.089. There was a significant main effect of condition: F(2, 22)=5.339, p=0.013, η^2_p=0.327 whereby, across all time points, corrected blood glucose concentration was significantly greater in NoEx (4.0±0.3mmol·L^{-1}) compared with 2x30 (3.8±0.3mmol·L^{-1}; p=0.025, d=0.69, 95% CI 0.023 – 0.364).

Plasma volume

For ΔPV responses, see table 5.2. A significant condition x time interaction effect was seen: F(2, 22)=9.626, p=0.001, η^2_p=0.467. Immediately post-condition, ΔPV was significantly greater following 4x30 (-9.4±6.6%) compared with NoEx (-0.4±6.5%; p=0.007, d=1.37, 95% CI 2.595 – 15.382) as well as following 2x30 (-10.7±8.9%) compared with NoEx (p=0.017, d=1.32, 95% CI 1.798 – 18.791). In 4x30, ΔPV immediately post-exercise (-9.4±6.6%) was significantly greater than upon request (1.1±6.4%; p=0.001, d=1.62, 95% CI 5.700 – 15.301) as well as immediately post-exercise (-10.7±9.0%) compared with upon request in 2x30 (-1.0±7.5%; p<0.001, d=1.18, 95% CI 6.250 – 13.306; statistical data not shown in table 5.2). There was no significant main effect of condition for ΔPV: F(2, 22)=2.446, p=0.110, η^2_p=0.182 but there was a significant main effect of time: F(1, 11)=49.371, p<0.001, η^2_p=0.818. Across all conditions, ΔPV was significantly greater immediately post-condition (-6.8±4.8%) compared with upon request (-0.2±3.4%; p<0.001, d=1.59, 95% CI 4.587 – 8.771).

ΔPV was significantly greater than rest immediately following 4x30: t(11)=-4.934, p<0.001, 95% CI -13.641 - -5.2247) and 2x30: t(11)=-4.146, p=0.002, 95% CI -16.4407 - -5.0382), but there was no significant difference in ΔPV from rest immediately following NoEx: t(11)=-0.236, p=0.818, 95% CI -4.586 – 3.6966). There were also no significant differences in ΔPV from rest upon request in 4x30: t(11)=0.580, p=0.573, 95% CI -2.9808 – 5.1162, 2x30: t(11)=-0.446, p=0.665, 95% CI -5.7133 – 3.7898 nor NoEx: t(11)=-0.438, p=0.670, 95% CI -4.133 – 2.7609.

Rating of perceived exertion

For RPE, two extreme outliers were removed (two for NoEx post), resulting in n=10 for this

measure. There were no differences in RPE at rest between conditions. A significant condition x time interaction was found: F(1.295, 11.658)=204.199, p<0.001, η^2_p=0.958. Immediately following each condition, RPE was significantly greater in 4x30 (16.5±1.5) compared with NoEx (6.1±0.3; p<0.001, d=9.61, 95% CI 9.003 – 11.797) as well as immediately following 2x30 (14.3±0.8) compared with NoEx (p<0.001, d=13.6, 95% CI 7.468 – 8.932). RPE was also significantly greater immediately following 4x30 compared with 2x30 (p=0.001, d=1.83, 95% CI 1.147 – 3.253). Within 4x30, RPE was significantly greater immediately following compared with rest (7.0±1.7; p<0.001, d=5.9, 95% CI 7.945 – 11.055), as well as being significantly greater immediately following 2x30 compared with rest (6.5±0.8; p<0.001, d=5.49, 95% CI 7.061 – 8.539). There was also a significant main effect of condition: F(2, 18)=224.431, p<0.001, η^2_p=0.961. Across both time points, RPE was significantly greater in 4x30 (11.8±1.2) compared with 2x30 (10.4±0.7; p=0.005, d=1.43, 95% CI 0.447 – 2.253) and NoEx (6.2±0.3; p<0.001, d=6.4, 95% CI 4.704 – 6.496). RPE was also significantly greater during 2x30 compared with NoEx (p<0.001, d=7.8, 95% CI 3.661 – 4.839). There was also a significant main effect of time: F(1, 9)=297.161, p<0.001, η^2_p=0.971. Across all conditions, RPE was significantly greater immediately post-condition (12.3±0.7) compared with rest (6.6±0.9; p<0.001, d=7.07, 95% CI 4.981 – 6.486).

5.3.3 Affective and enjoyment responses

Affective valence

For affective valence, three extreme outliers were removed from the dataset (one for 4x30 request and two for 2x30 request), resulting in n=9 for this measure. There were no differences in affective valence at rest between conditions. There was no significant condition x time interaction effect: F(4, 32)=0.887, p=0.483, η^2_p=0.100. There were also no significant main effects of condition: F(2, 16)=1.356, p=0.286, η^2_p=0.145 nor time: F(2, 16)=1.053, p=0.372, η^2_p=0.116.

Positive affect

There were no differences in positive affect at rest between conditions. A significant condition x time interaction effect was found for positive affect: F(4, 44)=4.75, p=0.003, η^2_p=0.302. Immediately post-condition, positive affect was significantly greater following 4x30 (33.3±6.9) compared with NoEx (20.8±7.2; p=0.001, d=1.71, 95% CI 5.756 – 19.244) as well as following 2x30 (31.4±4.4) compared with NoEx (p=0.004, d=1.75, 95% CI 3.739 – 17.595). Upon buffet request, positive affect was also significantly greater in 4x30 (27.2±7.7) compared with NoEx (19.3±6.5; p=0.002, d=1.06, 95% CI 3.001 – 12.666) as well as in 2x30 (26.1±8.5) compared with NoEx (p=0.011, d=0.87, 95% CI 1.542 – 11.958). Within 4x30, positive affect was significantly greater immediately post-condition compared with rest (27.0±7.5; p=0.016, d=0.80, 95% CI 1.171 – 11.329) as well as immediately following 2x30 compared with rest (25.2±7.6; p=0.02, d=0.95, 95% CI 0.961 – 11.539). Within NoEx, positive affect was significantly lower immediately post-condition compared with rest (23.0±8.3; p=0.006, d=0.27, 95% CI 0.693 – 3.807) as well as being significantly lower upon buffet request (19.3±6.5) compared with rest for NoEx (p=0.014, d=0.80, 95% CI 0.756 – 6.578). There was a significant main effect of condition: F(2, 22)=11.486, p<0.001, η^2_p=0.511. Across all time points, positive affect was significantly greater in 4x30 (29.1±6.1) compared with NoEx (21.1±7.2; p=0.003, d=1.23, 95% CI 2.923 – 13.299) and was also significantly greater in 2x30 (27.6±5.7) compared with NoEx (p=0.015, d=1.07, 95% CI 1.268 – 11.788). There was a significant main effect of time: F(2, 22)=7.341, p=0.004, η^2_p=0.400. Across all conditions, positive affect was significantly greater immediately post-condition (28.5±4.4) compared with at rest (25.1±6.1; p=0.028, d=0.59, 95% CI 0.357 – 6.476) and upon buffet request (24.2±6.6, p=0.016, d=0.70, 95% CI 0.793 – 7.762).

Negative affect

Three extreme outliers were removed from the dataset for negative affect (for each time point during the NoEx condition), resulting in n=11 for this measure. There were no differences in negative affect at rest between conditions. There was no significant condition x time interaction effect: F(4, 40)=1.663, p=0.177, η^2_p=0.143 and no significant main effect of

condition: F(2, 20)=1.257, p=0.306, η^2_p=0.112. There was a trend for a significant main effect of time for negative affect: F(1.221, 12.207)=3.997, p=0.062, η^2_p=0.286.

Enjoyment responses

There was no significant main effect of condition seen for enjoyment between 2x30 and 4x30: t(11)=-0.22, p=0.983, 95% CI -8.54 – 8.37.

5.4 Discussion

The aim of this study was to elucidate responses of appetite and eating behaviour to an apparatus-free HIIE protocol of "all-out" star jumps of varying intervals (4 x 30 seconds vs. 2 x 30 seconds vs. rest) in inactive, overweight females. No differences in feeding latency, subjective appetite nor absolute EI at the *ad libitum* lunch buffet following either 4 x 30 seconds or 2 x 30 seconds of "all-out" star jumps, or a resting control condition, were found. This suggests that an acute bout of apparatus-free HIIE does not influence either time to request feeding, subjective appetite or EI upon feeding in this population, when participants are free to eat at a participant-determined time point post-exercise.

However, it should be noted that there was also an absence of any compensatory increased in EI with exercise and, in fact, a trend (with a medium effect size) for a reduction in relative EI (of a modest 121kcal) was seen following 4 x 30 seconds of "all-out" star jumps, compared with the resting control condition. In turn, this energy deficit did not appear to be compensated for during the remainder of the testing day. Accumulations of positive energy balance as small as 24kcal per day can lead to 1kg weight gain over three years (Hall *et al.*, 2011) while reducing EI by 10kcal per day is predicted as being sufficient to prevent rates of obesity rising (Veerman *et al.*, 2007). Therefore, it is plausible to suggest that if a daily deficit in relative EI to this extent can be repeatedly induced in this way, it would prevent even small magnitudes of positive energy balance or even induce negative energy balance. Moreover, with the short duration and low volume nature of the 4 x 30 seconds exercise protocol in the present study, it would be of interest to explore the effects of repeating such a bout on a

second occurrence in the same day on relative EI, energy deficit and energy balance. If a meaningful negative energy balance can be achieved across a day in this way, theoretically this offers an effective strategy to not only increase PA levels in an inactive population, but also possibly lead to effective energy balance regulation for weight management and to facilitate body mass loss in an overweight or obese population. No study has yet explored the effects on EI when an acute HIIE protocol is conducted within close proximity to a meal either in a free-living environment or on more than one acute occasion and this would be of interest to investigate.

In turn, it is important to note the absence of compensatory increase in EI or decreases in EE across the remainder of the testing day as well as the three days following the testing day. Previous work corroborates that acute exercise is able to induce short-term energy deficits without compensatory appetite or eating behaviour responses (Hubert, King and Blundell, 1998; King *et al.*, 2011).

Furthermore, Rocha *et al.* (2015) found that, in inactive females only, EI was reduced by ~472kcal on the day following 1 hour of moderate-intensity cycling compared with active females. In the present study, a similar mean reduction of 367kcal with a medium effect size was seen on the day following 4 x 30 seconds of "all-out" star jumps compared with resting control, although this was not found to be statistically significant. It can be speculated that this may be a caused by a delayed eating behaviour response to exercise. Of note, similar findings were not seen when studied in inactive and active males (Rocha *et al.*, 2013), suggesting possible sex differences in such potential delayed energy balance responses. Again, this further supports that there is value in future research exploring beyond the acute setting and investigating the promotion of low volume, apparatus-free HIIE for initiating meaningful energy deficit over a longer period of time in an inactive and overweight female population.

At this point, however, it should also be noted that large individual variability in eating behaviour responses are seen following acute exercise in overweight and obese females (Hopkins, Blundell and King, 2013) as well as moderately active individuals (Schubert *et al.*, 2017). Furthermore, interindividual variability in subjective appetite responses as well as hormone responses to acute exercise have also been noted (Goltz *et al.*, 2018), suggesting

that findings such as those in the present study should be interpreted with caution, especially when extrapolating findings into longer term responses.

Despite a trend for a reduction in relative EI following 4 x 30 seconds of "all out" star jumping in the present study, reasons for an absence of change in subjective appetite at any time point, nor change in feeding latency compared with the resting control condition are unknown. It can be speculated that such observations may, indeed, be less related to hunger responses following exercise and more due to an earlier induction of fullness upon eating following exercise, compared with no exercise. The present study did not measure appetite and satiety hormone concentrations, nor subjective appetite and satiety responses following the a*d libitum* buffet meal. However, 4 x 30 seconds of acute HIIE has been demonstrated to increase total PYY in the transient period post-exercise relative to following no exercise (Beaulieu *et al.*, 2015). Therefore, this may, in part, be responsible for an induction of fullness or satiety sooner than with no exercise upon feeding during the transient period post-exercise and future research should explore this potential mechanism further.

Moreover, few other studies have explored feeding latency as a measure of eating behaviour and have instead prescribed *ad libitum* buffets at a pre-determined time point post-exercise. King, Burley and Blundell (1994), however, found a significant (albeit short) delay of ~5 minutes to feeding with an acute bout of high-intensity exercise compared with a resting control condition. However, authors did not see any difference in EI at the *ad libitum* buffet offered upon this request. King, Wasse and Stensel (2013) also found a significant delay of ~35 minutes with 1 hour of moderate-high-intensity continuous running compared with resting control. However, again authors did not see any differences in EI at the *ad libitum* buffet. Interestingly, King, Wasse and Stensel (2013) also saw suppressions in subjective hunger and prospective food consumption immediately following exercise compared with resting control, which is likely partly responsible for the delay in feeding seen in their study. However, there were then no differences in any measures of subjective appetite following this, even at the point that the buffet was requested, which likely demonstrates a transient 'exercise-induced anorexia', but one that did not last long enough to influence food intake when participants were free to choose when to eat. Similarly, King, Burley and Blundell (1994) also saw a brief suppression of subjective hunger during and immediately following both ~26 minutes or ~52 minutes of high-intensity continuous cycling, which is also likely partly

responsible for the short delay in feeding seen following exercise. However, again, this difference diminished by the time of request of the meal and this is likely responsible for the lack of differences in EI seen at the *ad libitum* buffet.

Such findings corroborate that parallel changes in subjective appetite and actual eating behaviour are not always seen. Certainly, corresponding responses in subjective appetite and EI at a test meal are often not demonstrated (Reger, Allison and Kuruca, 1984; Thompson, Wolfe and Eikelboom, 1988; King, Burley and Blundell, 1994; Deighton *et al.*, 2013a; Deighton *et al.*, 2013b; Beaulieu *et al.*, 2015; Holliday and Blannin, 2017a). Given this lack of correspondence between subjective appetite and actual EI shown in the present study among others, the mechanistic controls of appetite and eating behaviour can also be questioned. Indeed, differences of at least 15-25mm on a 100mm VAS appear to be required in order to ensue significant changes in subsequent EI (Sadoul *et al.*, 2014). When crudely adjusting this threshold for the utilisation of scales 150mm in length, in the present study suppressions subjective appetite immediately following both exercise conditions do not quite reach this threshold for difference in subjective appetite compared with the no exercise condition. However, it is noted that the resting VAS measures of the present study would suggest that a change in VAS measure of at least ≥25mm would be required (on a 100mm scale) in order to ensue significant changes in EI (Sadoul *et al.*, 2014). This may explain the lack of change in feeding latency as well as the insignificant reduction in absolute EI and only a trend for a reduction in relative EI with 4 x 30 seconds “all-out” star jumping. Nonetheless, it has recently been demonstrated that VAS subjective appetite ratings do not reliably predict EI (Holt *et al.*, 2017), which could also be responsible for the lack of statistically significant corresponding changes seen in subjective appetite, absolute EI and relative EI in the present study.

It is also unclear to what extent such discrepancies between subjective appetite and EI responses are due to study design, such as administering a test meal at a pre-determined time point, as discussed. In turn, differences in exercise protocols are important to consider. The present study employed 2 x 30 or 4 x 30 seconds “all-out” star jumping. It should be noted that King, Burley and Blundell (1994) employed either ~26 minutes or ~52 minutes of high-intensity continuous cycling and King, Wasse and Stensel (2013) employed 1 hour of moderate-high-intensity continuous running, which are both substantially different exercise protocols compared with the present study. It is possible that, despite subjective appetite

responses seen following exercise being unlikely to inform EI at the test meal in King, Burley and Blundell (1994) and King, Wasse and Stensel (2013), the presence of differences in subjective appetite responses in these studies compared with the present study could be due to such differences in exercise volume, mode and/or duration. However, it should be noted that findings in study 1 (Chapter 4) of this thesis demonstrate that 4 x 30 seconds of "all-out" star jumping can achieve physiological responses indicative of a high-intensity workload and similar to those of 4 x 30 seconds "all-out" cycling. In turn, physiological responses to the 4 x 30 seconds "all-out" star jumping in the present study are similar to the findings of study 1 (Chapter 4), of which these responses were similar to those seen with 4 x 30 seconds "all out" cycling. Furthermore, a suppression in composite appetite score was seen following a bout of 4 x 30 seconds of "all-out" cycling in an overweight/obese population up to 30 minutes following exercise compared with a resting control condition (Holliday and Blannin, 2017a). Similarly, suppressions of hunger and motivation to eat were demonstrated following 4 x 30 seconds "all-out" running and remained so up to 1 hour following exercise, compared with a resting control condition (Beaulieu *et al.*, 2015). Hence, it is possible for such a small volume of "all-out" exercise to induce suppressions in measures of subjective appetite, at least in apparatus-based HIIE. Why any similar responses in subjective appetite were not seen in the present study is unknown, although it is important to emphasise that a modest 121kcal deficit in relative EI was seen. Again, given that it is actual eating behaviour that will influence energy balance, this highlights the novelty and the contributions of this study.

Other studies employing HIIE protocols, however, have not always seen post-exercise suppressions in subjective appetite in overweight and obese populations. For example, there were no differences in subjective hunger, fullness, satiation, prospective food consumption nor desire to eat with two differing HIIE protocols of ~30 minutes duration in overweight/obese males (Sim *et al.*, 2014). Alkahtani *et al.* (2014) also demonstrated no differences in subjective hunger, fullness or desire to eat following a HIIE protocol of ~20 minutes duration in overweight/obese males. Alkahtani *et al.* (2014) did not measure EI, yet Sim *et al.* (2014) did and found significant reductions in EI with both HIIE protocols compared with the resting control condition, despite seeing no changes in ratings of subjective appetite. Hence, this again corroborates that parallel changes in all aspects of appetite and eating behaviour are not always present.

Although no statistically significant differences were seen in composite subjective appetite scores in the present study, overall, composite appetite AUC score was 34mm and 20mm greater with 2 x 30 seconds and 4 x 30 seconds "all out" star jumping, respectively, compared with resting control. This translates to a meaningful difference in VAS AUC in both exercise conditions of the present study compared with resting control (Blundell *et al.*, 2010; Deighton *et al.*, 2017). In turn, the tendency for a reduction in relative EI again did induce a modest energy deficit of 121kcal which is a similar deficit to that provided by the reduction in absolute EI seen with the HIIE protocol in the work of Miguet *et al.* (2018). Nonetheless, the discrepancy between the exercise duration and volume in the present study and in Miguet *et al.* (2018) as well as others who have seen reductions in absolute EI upon an *ad libitum* buffet is noted as this could be partly responsible for the effects seen on absolute and relative EI. Indeed, neither Beaulieu *et al.* (2015) nor Holliday and Blannin (2017a) saw differences in absolute or relative EI with 4 x 30 seconds of "all-out" running or cycling, respectively. Moreover, despite a transient suppression in hunger immediately post-exercise, Matos *et al.* (2019) did not see any differences in absolute EI with an acute HIIE protocol of 10 x 1 minutes of high-intensity running in overweight, inactive males. However, as previously discussed, these findings could partly due to the design of the studies meaning that the *ad libitum* test meal likely missed the transient window of appetite suppression. Again, this highlights the novelty in the design of the present study, where the *ad libitum* test meal was at a participant-determined time point. Although only a trend for a reduction in relative EI was seen at the *ad libitum* buffet in the present study, given that a reduction in relative EI across a day of 121kcal is likely to be sufficient to prevent rates of obesity rising (Veerman *et al.*, 2007) and may prevent even small accumulations of positive energy leading to weight gain (Hall *et al.*, 2011), it should be noted that such a trend with a medium effect size is still of interest. This is especially true given the growing support for HIIE to be time-efficient, accessible and practical for an inactive population (Hardcastle *et al.*, 2014; Gray *et al.,* 2016).

Although not a focus of the present study, it is important to note the greater positive affect responses seen following both exercise conditions compared with NoEx which remained significantly more positive up until request of the *ad libitum* buffet, although it is noted that similar responses were not seen in affective valence. Again, this corroborates the findings seen in study 1 (Chapter 4) of this thesis and only further supports the rationale for such an

apparatus-free HIIE protocol in an overweight/obese, inactive female population given the commonly-reported barriers to regular PA (Cerin *et al.*, 2010). Interestingly, exercise enjoyment was remarkably similar between both exercise conditions in the present study. Although again beyond the initial scope of this study, reductions in post-exercise EI have previously been noted to be correlated with increased exercise enjoyment and post-exercise affective responses (da Silva Gomes *et al.*, 2018; Unick, Michael and Jakicic, 2012). In turn, a greater rating of perceived exertion has also been shown to be associated with a greater EI post-exercise (Fearnbach *et al.*, 2017). Considering the importance of positive affective and enjoyment responses to exercise for adherence and participation in inactive individuals (Kwan and Bryan, 2010a; Hardcastle *et al.*, 2014; Biddle and Batterham, 2015) alongside their possible role in post-exercise EI responses, future work should seek acute HIIE protocols that induce positive affective and enjoyment responses. This would not only optimise adherence in an inactive population, but also post-exercise EI reduction in an overweight/obese population. Although interpreted with caution, given these associations it is possible that the greater affective responses seen following exercise protocols of the present study are somewhat responsible for the reductions, albeit non-significant, in EI upon *ad libitum* buffet request compared with the resting control condition.

Despite the novel design of the present study, it is noted that the sample size is small and is likely partly responsible for the lack of statistical significance in some measures. Although the study was initially deemed to be powered to detect differences of a medium effect size in feeding latency based on previous work, it is noted that the actual observed power of the effect of condition for both feeding latency and absolute EI in the present study were indeed underpowered.

Although sex differences in acute appetite and eating behaviour responses are not evident (Alajmi *et al.*, 2016; Thackray *et al.*, 2016), it would also be of interest to also explore the aims of the present study in a male population. Nonetheless, the present study addresses the lack of investigation in the field in a female population (Costello, Bieuzen and Bleakley, 2014) and in overweight/obese females in particular due to the applications of the present study's findings being most relevant to a population seeking effective weight management strategies. Furthermore, strengths of the present study include control for stage of the menstrual cycle in a within-subject manner as EI, amongst other appetite and eating behaviour measures, can

differ across stages of the menstrual cycle (Buffenstein *et al.*, 1995; Dye and Blundell, 1997; Brennan *et al.*, 2009). Importantly, EI over a four day period has been demonstrated to be increased in inactive females taking oral contraceptives compared with inactive females not taking oral contraceptives; this was a difference not observed in active female counterparts (Rocha *et al.*, 2018). The present study did not control for contraceptive method in females. This was due to the likely restrictions this would have induced upon recruitment. Although its potential limitations are considered, the within-subject design in the present study discounts for this somewhat. Nonetheless, future research should further elucidate explanations for the finding that EI may be influence by the taking of oral contraceptive in females.

The present study employed a single *ad libitum* meal, compared with other similar studies including Holliday and Blannin (2017a). A simple *ad libitum* buffet format was selected to avoid possible boredom or limited food choice with single item *ad libitum* buffets (Blundell *et al.*, 2010) without promoting overconsumption, whilst allowing for food selection and preference. EI and EE were then both assessed beyond the laboratory environment which are both strengths of the present study, although the inherent limitations with self-report food diaries are noted and future research to further the findings of the present study should look to use measures with greater objectivity.

5.5 Conclusion

There were no differences seen in subjective appetite, feeding latency nor absolute EI following either 2 x 30 or 4 x 30 seconds “all-out” apparatus-free HIIE in inactive, overweight females compared with no exercise. However, albeit not significant, meaningful reductions in relative EI were observed with 4 x 30 seconds “all-out” star jumps, which could induce a meaningful daily energy deficit particularly if exercise is repeated in the same day or across multiple days. Future research should explore the role of such apparatus-free HIIE timed in close proximity to meals in a free-living setting to elucidate daily energy balance responses in this population, given the commonly-reported barriers to regular PA.

Chapter 6

Study 3(a) – Appetite and energy balance responses to an 8 week apparatus-free, low volume HIIT intervention undertaken prior to meal times in overweight, inactive females

Abstract

Given current rates of physical inactivity and overweight/obesity, it is of paramount importance to develop effective PA strategies that can manipulate energy balance in a manner to promote loss in body mass. When a low volume bout of apparatus-free high-intensity intermittent star jumping is undertaken ~30 minutes prior to a meal time, relative EI is reduced by 121kcal in inactive, overweight/obese females. It is plausible that exercise in this way could induce modulations in eating behaviour to promote a negative energy balance when undertaken in an intervention format. Therefore, the purpose of this study was to determine the effects of eight weeks of apparatus-free high-intensity intermittent star jumping undertaken prior to meal times on daily EI, body mass, energy balance and appetite. Fifteen inactive, overweight/obese females (36.1±5.5 years, 28.1±2.4 $kg \cdot m^{-2}$, leisure time MVPA 29±36 $minutes \cdot week^{-1}$) undertook the intervention, undertaking 4 x 30 seconds "all out" star jumps twice a day for three days per week, either within 30 minutes prior to a meal ('pre-meal') or at any time of day outside of an hour prior to a meal ('anytime'). Mean 5-day energy and macronutrient intake were assessed at baseline and during weeks 4 and 8, while subjective appetite responses and concentrations of PYY, GLP-1 and acylated ghrelin were assessed when fasted and up to 120 minutes following a standardised breakfast at baseline and following the intervention. No differences in either mean daily EI or macronutrient intake were seen across the intervention during week 4 nor week 8 (all $p>0.05$). However, daily EI was significantly lower on exercise days compared with non-exercise days during week 8 of the intervention in both groups (by a mean of 426kcal; $p=0.033$). There were no changes in body mass in either 'anytime' (75.4±9.3kg vs. 75.7±9.4kg) or 'pre-meal' (72.2±5.1kg vs. 71.9±4.7kg; all $p>0.05$). Following the intervention, subjective appetite AUC was significantly greater in the 'pre-meal' group ($p=0.003$), whilst GLP-1 concentration AUC was significantly greater in the 'anytime' group ($p=0.037$). Low volume, apparatus-free HIIT undertaken in this way seems to induce a lower EI on exercise days, regardless of exercise timing. Reasons for

this remain to be completely elucidated but will assist in informing effective PA strategies for reducing EI in inactive, overweight populations.

6.1 Introduction

Effective strategies promoting increased PA levels as well as healthier body mass must be sought. Firstly, it is paramount to comprehend the mechanics of body mass gain, which ultimately stem from prolonged and repeated periods of positive energy balance. Such an imbalance can derive from physical inactivity (leading to reducing AEE and TEE) and/or overconsumption of EI such that EI exceeds EE over time. Hence, it is important to consider possible manipulation of both EE and EI in the shorter and longer term for pursuing effective weight management strategies.

Despite efficacy of exercise and PA alone for body mass loss (Andersen *et al.*, 1999; Shaw *et al.*, 2006) and modest reductions in body mass and/or body fat (up to ~2kg) with some higher volume HIIT interventions of eight to twelve weeks duration (Boutcher, 2011), the effectiveness of low volume HIIT for body mass loss and body composition changes remains equivocal (Boutcher, 2011; Whyte, Gill and Cathcart, 2010; Metcalfe *et al.*, 2012; De Feo, 2013; Gillen *et al.*, 2014; Allison *et al.*, 2017). Of note, the participants in studies such as these are not always overweight or obese at baseline, thereby limiting the potential for body mass loss of a meaningful magnitude. However, such a low volume of exercise likely also elicits a lower EE than typical, more prolonged and energetic continuous moderate-intensity exercise interventions (Babraj *et al.*, 2009; Deighton *et al.*, 2013a), which would similarly limit the potential for perturbation in energy balance for a meaningful reduction in body mass. Nonetheless, Martins *et al.* (2016) demonstrated a modest ~1.8kg loss in body mass over twelve weeks with a low volume HIIT intervention. Of note, the HIIT intervention elicited 125kcal EE per exercise session, compared with 250kcal EE per session in the moderate-intensity continuous protocols which only induced a loss in body mass of ~0.8kg. Therefore, the greater magnitude of body mass lost with HIIT compared with moderate-intensity exercise training in this study implies greater perturbation in energy balance in the HIIT intervention group, despite a lessened EE of the exercise session. This somewhat questions the posed equivocality of low volume HIIT interventions being effective for body mass loss

(Boutcher, 2011; De Feo, 2013), however it also implies that additional perturbation in energy balance has occurred beyond that of the exercise bouts. Given that low volume HIIE has the potential to suppress appetite in the transient period post-exercise (Beaulieu *et al.*, 2015; Holliday and Blannin, 2017a), it is possible that accumulating HIIT in this way could therefore manipulate appetite and eating behaviour over time to adjunctively influence energy balance.

Indeed, longer-term exercise programmes employing moderate-intensity, continuous exercise have demonstrated that exercise training can promote improved sensitivity of eating behaviour and subjective appetite control (Martins, Truby and Morgan, 2007; King *et al.*, 2009; Guelfi, Donges and Duffield., 2013) as well as modulation of the appetite and satiety hormone profile towards improved appetite control (Martins *et al.*, 2010; Rosenkilde *et al.*, 2013). However, the majority of this consensus stems from moderate-intensity exercise interventions. Martins *et al.* (2017) were the first to explore this relationship with HIIT specifically, although authors did not find improved appetite control nor eating behaviour with either the HIIT or the moderate-intensity intervention. Nonetheless, further research is warranted to explore the effects of low volume HIIT on the regulation of eating behaviour and EI in the longer term in overweight and obese individuals.

Furthermore, findings from study 2 (Chapter 5) of this thesis demonstrate a tendency for a reduced relative EI at a meal ~30 minutes following an acute bout of apparatus-free low volume HIIE, which seems to be maintained across the remainder of the day. Whether a meaningful perturbation in daily EI can be generated through repeating an acute bout of apparatus-free low volume HIIE in this way later in the same day (thereby increasing AEE), timed prior to a meal, remains of interest. Despite such marked suppressive and transient effects of acute HIIE on appetite, eating behaviour and EI, no study has yet incorporated HIIE into an intervention where exercise bouts are timed in close proximity to meal times, such that the meal time theoretically falls within this transient period of appetite suppression. This is of additional interest given the implications on energy balance, weight management and weight loss.

In turn, all studies exploring the longer term effects of exercise interventions on appetite and eating behaviour as well as appetite sensitivity have thus far been laboratory-based, apparatus-based and supervised. Given the commonly-reported barriers to regular PA (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017) as

well as the findings of study 1 (Chapter 4) and study 2 (Chapter 5) in this thesis, exploring the effectiveness of such appetite, eating behaviour, energy balance and body mass responses outside of a supervised, laboratory context is of current relevance to public health. Therefore, the aim of this study was to determine the effects of an 8-week apparatus-free HIIT intervention undertaken prior to meal times on daily EI, daily EE and body mass. A secondary aim of this study was to investigate the effects of an 8-week apparatus-free HIIT intervention undertaken prior to meal times on fasting and postprandial subjective appetite and plasma concentrations of appetite and satiety-related hormones following a standardised meal, as well as non-homeostatic responses including measures of habitual eating behaviour and food cravings.

6.2 Methods

6.2.1 Study design

A between-subject design was used, with participants randomly assigned to either the 'pre meal' or 'anytime' intervention group. Both intervention groups undertook an 8-week apparatus-free HIIT intervention of 4 x 30 seconds of a high-intensity intermittent star jump protocol twice a day for three days per week across the intervention. The 'pre meal' intervention group were instructed to undertake each HIIE bout such that the bout was completed at a time within 30 minutes prior to a commencing eating a meal. The 'anytime' intervention group were instructed to undertake each HIIE bout at any time of day such that the bout was completed at a time outside of one hour prior to a commencing eating a meal. Timings of exercise bouts were self-reported in an activity diary and confirmed upon collection of the HR data recordings with the time of recording provided in each data recording.

6.2.2 Participants

Overweight/obese, inactive females were recruited from the local area of Worcester.

Inclusion criteria, exclusion criteria and ethical approval were as described in sections 3.2 and 3.3. Twenty nine inactive, overweight/obese females were recruited and underwent familiarisation, while twenty one began the intervention and fifteen completed the intervention (28.1±2.4 kg·m^{-2}, 36.1±5.5 years, leisure time MVPA 29±36 minutes·week^{-1}). BMI was not statistically significant between groups (p=0.422) and neither was leisure time MVPA (p=0.233), absolute nor relative VO_{2peak} (p=0.686 and p=0.141, respectively) nor age (p=0.598).

Four participants who withdrew from the intervention cited the reason as a lack of availability to commit to the study measures and experimental trials, while one participant experienced unrelated health issues and one participant sustained a muscle strain injury which may have been due to the intervention. Table 6.1 shows participant characteristics of both experimental groups at baseline in both 'anytime' and 'pre-meal'.

Of the n=15 total participants, 12 were of white Caucasian ethnicity and 3 were of Asian ethnicity (n=2 in 'anytime' and n=1 in 'pre-meal').

Table 6.1 Baseline participant characteristics

	Anytime (n=8)	**Pre-meal (n=7)**
Body mass (kg)	75.4±9.3	72.2±5.1
BMI (kg·m^{-2})	28.5±2.5	27.7±2.1
Age (years)	35±5	37±7
Self-reported leisure time MVPA (min·week^{-1})	40±44	16±25
VO_{2peak} (mL·min^{-1}·kg^{-1})	21.6±2.5	23.8±2.8
Eating restraint	2.9±0.6	2.5±0.3

BMI' denotes 'body mass index', VO_{2peak} denotes 'cardiorespiratory fitness'

6.2.3 Preliminary trial and familiarisation

Written (appendix 5) and verbal information regarding participation in the study was given to participants, as well as the opportunity to ask any questions, before written informed consent (appendix 8) was then obtained. Based on pre-screening guidelines by the ACSM, the Physical Activity Readiness Questionnaire for Everyone (PAR-Q+; Warburto*n et al*., 2011; appendix 11) was administered to all participants, with assistance and supervision from the researcher. The

IPAQ (Craig *et al.*, 2003; appendix 1) was used to assess individuals' PA levels, again with assistance and supervision from the researcher. Following a short period of rest (>15min), a resting blood pressure measurement was taken in triplicate using an automated blood pressure monitor (Omron, Milton Keynes, UK). Participants' height was measured using a stadiometer and weight was measured using scales (Seca, Birmingham, UK) for a BMI calculation. The DEBQ (van Strien *et al.*, 1986a; appendix 2) was then administered, to assess dietary restraint. Participants were included based on the criterion of having a dietary restraint score of ≤3.5 [mean score 2.9±0.6].

Participants were randomly assigned to an intervention group, which was concealed through an opaque envelope which revealed the participant's group to them following screening procedures. Following the assignment, the researcher explained the condition in full and answered any questions regarding this. Participants were then demonstrated and undertook 4 x 30 seconds of the "all-out" star jump protocol to ensure familiarisation and feasibility. Participants were then familiarised with all measures to be taken during the remainder of the study period.

Participants then undertook an incremental exercise test to exhaustion. This was used for familiarisation purposes prior to baseline testing only (Edgett *et al.*, 2018). Participants pedalled at 50W for 2 minutes before workload was increased by 1W every 2 seconds thereafter. Participants were instructed to cycle at a cadence of 60-70RPM until volitional exhaustion was reached or until the pedal cadence consistently fell below 50RPM. This test protocol has previously been used in an overweight/obese, inactive population to assess VO_{2peak} (Gillen *et al.*, 2014).

Following a warm down recovery period, participants were provided with food diary instructions (appendix 13) and a 24-hour pre-testing day weighed food diary (appendix 14). Participants were instructed by the researcher to record their food and drink intake for the twenty four hours prior to their first experimental trial (baseline), with an explanation of how to record the weighed food diary also provided. The researcher then instructed participants to also refrain from alcohol, caffeine and any strenuous exercise in the twenty four hours prior to an experimental trial and also informed participants that they would be expected to replicate their recorded 24-hour food and drink intake from their first (baseline) experimental trial prior to the second (post-intervention) experimental trial.

6.2.4 Experimental trials

Figure 6.1 depicts the design of each experimental trial and the measures obtained at baseline, during the intervention and at post-intervention. After a minimum of seven days after the familiarisation trial, participants returned to the laboratory between 8:00-9:30am for their baseline experimental trial and at the same time of day for their post-intervention experimental trial. For both trials (baseline and post-intervention), participants were instructed to arrive fasted (overnight fast of at least 10 hours), having consumed 250mL water and minimised strenuous PA in the twenty four hours preceding the trial including arriving at the laboratory by car where possible. Upon arrival, veracity and adherence to these criteria, as well as the following of the 24-hour pre-testing day food diary (appendix 14), were checked by the researcher. Eumenorrheic participants undertook each experimental trial during the follicular phase of the menstrual cycle (days 1-14; mean 10±2 days and 7±4 days, respectively for 'anytime' and 'pre-meal' baseline trials, and mean 9±6 days and 6±5 days for 'anytime' and 'pre-meal' post-intervention trials, respectively). Participants taking contraceptive medication preventing menstruation undertook each experimental trial 28±2 days apart. However, due to unavoidable and practical reasons, a total of 4 visits fell outside of these windows.

Upon arrival, a measure of BMR was taken upon both experimental trials (see section 3.6.9 for more detail). The baseline BMR measure was inputted into the individual calibration process of the Actiheart (CamNtech, Cambridge, UK) activity monitors for the measure of EE (see section 3.6.10) at baseline and week 4, while the post-intervention measure of BMR was used for the individual calibration process at week 8. Following the BMR measurement, a fasting venous blood sample was obtained through the insertion of an intravenous (IV) cannula into the antecubital vein. Participants were then asked to rate their appetite. Following this, participants were given a breakfast meal consisting of porridge made with semi-skimmed milk, brown sugar and a glass of orange juice. This meal provided approximately 20% of predicted daily energy requirements for a sedentary day for each individual (392±30kcal; for macronutrient contributions see section 3.6.14) and was calculated as described in section 3.6.14.

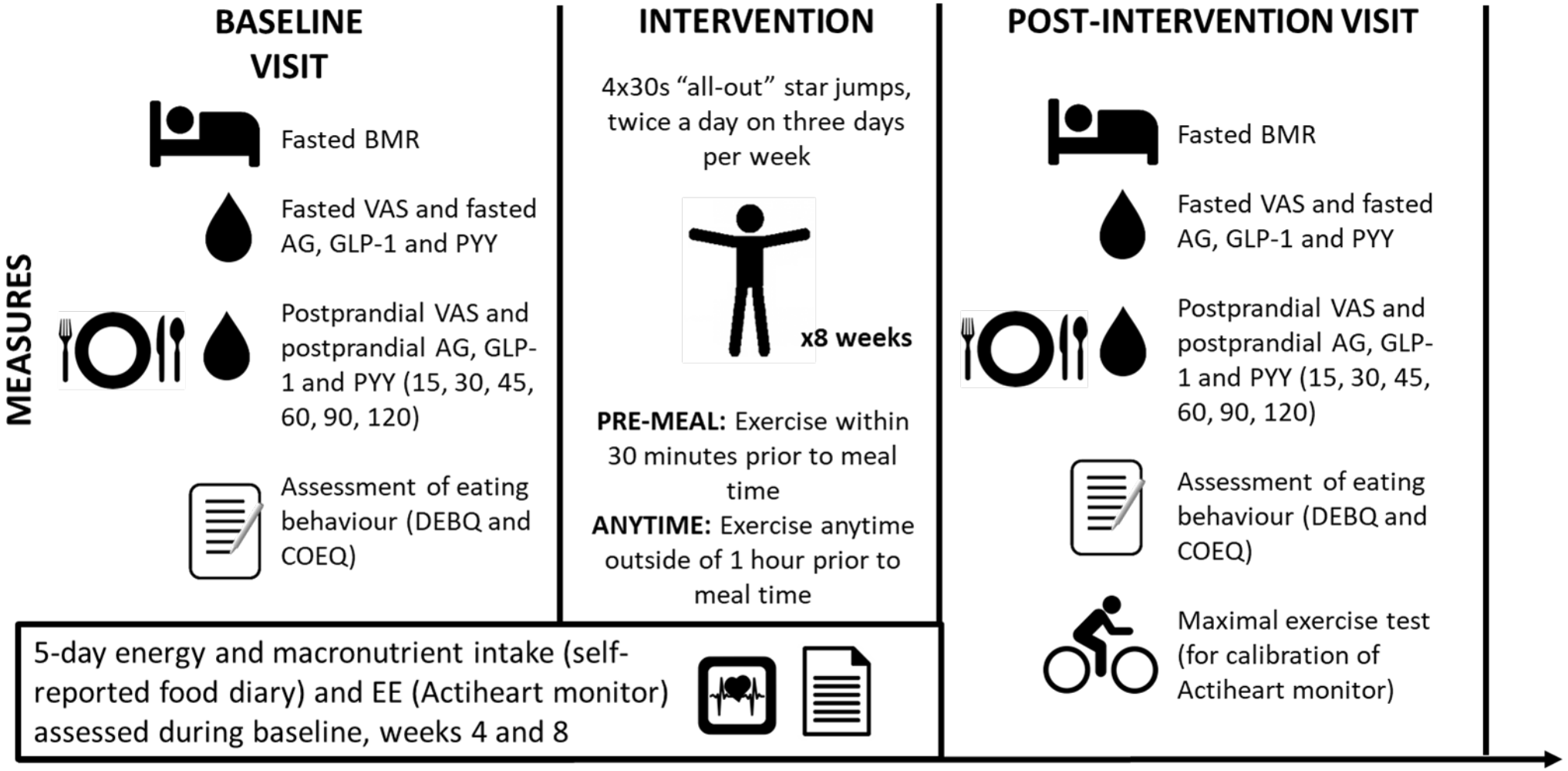

Figure 6.1 Summary of study design; 'BMR' denotes 'basal metabolic rate', 'VAS' denotes 'visual analogue scale', 'AG' denotes 'acylated ghrelin', 'DEBQ' denotes 'Dutch Eating Behaviour Questionnaire', 'COEQ' denotes 'Control of Eating Behaviour Questionnaire', '15, '30', '45', '60', '90' and '120' denote 15, 30, 45, 60, 90 and 120 'minutes post-breakfast consumption', respectively

At the time points of 15, 30, 45, 60, 90 and 120 minutes following breakfast consumption, further venous blood samples were obtained through the IV cannula and participants were again asked to rate their subjective appetite. It is noted that there can be greater anxiety in participants upon baseline trials compared with post-intervention trials, which can influence satiety hormone concentration and assessment of subjective appetite (Chandarana, 2009). To mitigate the effects of this on any hormone measurements as much as possible, ~10minutes was waited following the IV cannula insertion before blood was drawn. However, this should also be considered when interpreting subjective appetite responses.

During time periods in between each appetite rating, participants were encouraged to remain seated in the laboratory at a desk, working, watching television or reading quietly. Following the final appetite rating, participants undertook a collection of questionnaires relating to the intervention. Participants then underwent an incremental exercise test to exhaustion (identical to that of the familiarisation trial). Measures of HR, RER and rate of oxygen utilisation (VO_2) per breath were also used to calculate the predicted EE by multiplying the VO_2 with the RER-specific caloric equivalent of oxygen (appendix 19; Frayn, 1983) and was additionally used to calibrate the activity monitors (see section 3.6.10). Following the completion of the exercise test and a warm down period, participants were then free to leave the laboratory.

During the baseline experimental trial, the drinking of water was offered *ad libitum* and then this identical volume of water was offered again in the post-intervention trial. Participants' adherence to minimising strenuous physical activity on the pre-testing day was checked verbally by the researcher upon participant arrival to each experimental trial. Adherence to the pre-testing day food diary was also checked upon arrival to each experimental trial (energy and macronutrient intakes all $p>0.05$ between conditions). Room temperature was maintained by an air conditioning system at an ambient temperature of ~21˚C and humidity ranged from 29 to 56% when assessed using a hygrometer.

6.2.5 Exercise interventions

All participants were instructed to undertake two separate bouts of 4 x 30 seconds of the

star jump protocol twice a day (timing of exercise bouts aligning with their intervention group), three days per week for eight weeks. The protocol consisted of a 1-minute walking warm up, 4 x 30 seconds "all-out" star jumping interspersed with a 90 seconds walking recovery period and concluded with 1-2 minutes walking warm down. Participants were instructed to achieve as many jumps as possible in each 30-second interval, i.e. "maximal effort" jumping, as described in chapters 3 and 4. As a guide, participants were given a target HR of ≥80% HR_{max} and a HR monitor attached to a chest strap (TickrX, Wahoo Fitness, Atlanta, USA) to wear throughout each bout and instructed to record their HR continuously during each bout using a mobile phone app (Wahoo Fitness, Atlanta, USA).

6.2.6 Measures

Anthropometrics

An ISAK-qualified researcher assessed and recorded participants' height and body mass (for the calculation of BMI) and waist and hip circumferences (see section 3.6.1).

Basal metabolic rate

For the measure of BMR, participants lay supine, in a darkened quiet room in as close proximity to waking as possible for 25 minutes, with continuous measurement of pulmonary gas exchange. For the final 10 minutes of the measurement period, VO_2 measurements were averaged and multiplied with the caloric equivalent of RER and these resulting values were then averaged to estimate BMR (Frayn, 1983), as described in further detail in section 3.6.9.

Energy expenditure

Participants were instructed to wear the activity monitor (attached to the chest strap or individual electrodes, depending on preference, at chest height with instructions provided; appendix 21). TEE, AEE and METs were derived for each of the five days and a mean of the five days for each testing period. MET values were calculated according to the cut-off points described in table 3.1.

Energy intake

During the baseline testing period and during weeks 4 and 8 of the intervention for both groups, participants were asked to self-report dietary intake including timings of all meals in a weighed food diary (appendix 14) across a 5-day period. Also during weeks 4 and 8 of the intervention, participants were requested to send the researcher a message using their personal smartphone via 'WhatsApp' (WhatsApp, Dublin, Ireland) with a photograph of their meal at the time of eating following each exercise bout at the time of eating. This was followed using 'Snap-n-Send' methods previously described by Costello *et al.* (2017), however the researcher used the time that the photo was sent as confirmation of the meal time alongside the participant's HR data and self-report diary data.

Total EI and macronutrient intake were calculated for each of the five days through the use of a self-report weighed food diary (appendix 14) for a total of 5 days during baseline, week 4 and week 8. Total EI and macronutrient intake were averaged over the five days as well as averaged over the total number of exercise and non-exercise days during week 8 using the nutritional analysis software Nutritics (Nutritics Ltd, Ireland).

Subjective appetite

At the time points of fasted, 15, 30, 45, 60, 90 and 120 minutes following the breakfast meal, participants completed the VAS (Hill and Blundell, 1982; appendix 22) which assessed subjective feelings of hunger, fullness, desire to eat and prospective food consumption using 150mm VAS. A composite appetite score was then generated as the mean of these four values after fullness was reverse-scored (see section 3.6.12).

Other measures of appetite and dietary restraint

Following all postprandial measures during experimental trials, participants also completed the Control of Eating Questionnaire (COEQ; Hill, Weaver and Blundell, 1991; appendix 23) as well as the DEBQ (van Strien *et al.*, 1986a; appendix 2).

Adherence to exercise timing

Participants kept an activity diary, in which they were asked to record the timing of each exercise bout, along with the timing of the meal that followed the exercise bout.

Adherence to exercise intensity

The researcher also utilised the timing of the HR data that was sent after each exercise bout, alongside the photos of meals sent at meal times during week 4 and week 8 and the self-reported recorded timing of exercise and meals in the self-reported diary to verify that participants were adhering to their intervention group instructions and timings appropriately.

Blood sampling and analysis

A 30µL solution containing potassium phosphate buffer, p-hydroxymercuribenzoic acid and sodium hydroxide was added to 2.7mL potassium/EDTA-treated monovettes (Sarstedt, Leicester, UK) prior to collection of venous blood to prevent degradation of AG. At all blood sampling time points (fasted, 15, 30, 45, 60, 90 and 120), venous blood was collected into 6mL potassium/EDTA-treated tubes for analysis of GLP-1 and PYY as well as the pre-prepared and chilled 2.7mL potassium/EDTA-treated monovettes for analysis of AG. All collection tubes were centrifuged at 3000RPM for 10 minutes at 4˚C (Sorvall ST 8R, Thermo Fisher Scientific, Massachusetts, United States). The plasma supernatant was then aliquoted into 2mL Eppendorf tubes which were stored at -80˚C for later analysis. 50µL 1M hydrochloric acid was added to Eppendorf tubes containing 500µL plasma for the analysis of AG, to protect AG from degradation.

Biochemical analysis

PYY, GLP-1 and AG were assessed using the enzyme-linked immunosorbent assay (ELISA) technique (Merck Milipore, Massachusetts, USA). All samples from each participant were analysed in singlicate in the same assay, while CVs were obtained for triplicates and quadruplicates of particular samples in the same assay. From triplicates and quadruplicates

obtained, intra-assay coefficients of variation were 3.75%, 1.66% and 4.94% for PYY, GLP-1 and AG, respectively. Values for each of the quality controls for each analyte fell within the acceptable range. The sensitivity for the PYY, GLP-1 and AG ELISA kits were 6.5pg·mL^{-1}, 1.5pM and 15 pg·mL^{-1}, respectively.

6.2.7 Statistical analyses

An independent t-test was used to assess for any differences in measures between groups at baseline for all measures other than VAS, AG, PYY and GLP-1 where a one-way repeated measures ANOVA was used to assess for any differences in these measures across all assessed time points between groups at baseline. A 2x3 mixed design ANOVA (group x week) with repeated measures was used to assess differences between groups at baseline, week 4 and week 8 for mean daily TEE, mean daily AEE, mean daily EI, mean daily macronutrient intakes and mean daily %SED, %LI, %MOD, %VIG and %V-VIG. A 2x3 mixed design ANOVA (group x day) with repeated measures was also used to assess for differences between groups in mean daily EI during week 8 on exercise and non-exercise days compared with baseline. There was insufficient data on energy and macronutrient intakes during exercise and non-exercise days during week 4 as well as on TEE, AEE and MET responses during week 8 to undertake similar assessments. A 2x2 mixed design ANOVA (group x trial) with repeated measures was also used for assessing differences between groups at baseline and post-intervention for body mass, BMI, BMR and DEBQ and COEQ responses.

A 2x2x7 mixed design ANOVA (group x trial x time) with repeated measures was used to assess differences between groups at fasted, 15, 30, 45, 60, 90 and 120 minutes following breakfast at baseline and post-intervention in VAS (composite) responses as well as PYY, GLP-1 and AG responses. A 2x2 mixed design ANOVA (group x trial) with repeated measures was used to assess for differences between groups in AUC for VAS, PYY, GLP-1 and AG at the baseline and post-intervention trials.

Missing data analysis using the multiple imputations technique was used to replace missing data for mean AEE (11 cases), mean TEE (11 cases) mean %SED (11 cases), %LI (11 cases), %MOD (11 cases), %VIG (11 cases) and %V-VIG (11 cases) due to activity monitor malfunctions

during wear time and insufficient wear time from participants. Due to one participant not returning a weighed food diary, missing data analysis using the multiple imputations technique was used to replace missing data for mean CHO, PRO and FAT intake (1 case) and mean EI (1 case). Due to participants not undertaking at least 2 exercise days during the 5 day food diary record during week 8, missing data analysis using the multiple imputations technique was used to replace missing data for mean EI, CHO, PRO and FAT intake on exercise and non-exercise days (6 cases). Due to time restrictions and issues with cannula draws, as well as one participant feeling unwell resulting in the final 2 cannula draws of an experimental trial being aborted, missing data analysis using the multiple imputations technique was used for missing data points for PYY (12 cases), GLP-1 (12 cases), AG (12 cases) and VAS (2 cases). Cases that were extreme outliers (>3x interquartile range of the dataset) were removed from the dataset before statistical analysis. All statistical analysis was undertaken using the software SPSS (SPSS version 23.0, SPSS inc., Chicago, Illinois, USA).

6.3 Results

6.3.1 Free-living energy and macronutrient intakes

Mean daily energy and macronutrient intake

There were no differences in mean daily EI nor FAT, PRO nor CHO intake between groups at baseline. For mean daily energy and macronutrient intakes, see table 6.4. For mean daily EI, there was no significant group x week interaction effect: $F(2, 26)=1.510$, $p=0.240$, $\eta^2_p=0.104$ and no significant main effect of week: $F(2, 26)=1.229$, $p=0.309$, $\eta^2_p=0.086$. There was also no significant main effect of group: $F(1, 13)=0.286$, $p=0.602$, $\eta^2_p=0.022$.

For mean FAT intake, there was no significant group x week interaction effect: $F(2, 26)=1.379$, $p=0.270$, $\eta^2_p=0.096$, no significant main effect of week: $F(2, 26)=0.499$, $p=0.613$, $\eta^2_p=0.037$ and no significant main effect of group: $F(1, 13)=0.053$, $p=0.822$, $\eta^2_p=0.004$.

For mean PRO intake, there was no significant group x week interaction effect: $F(2, 26)=1.004$, $p=0.380$, $\eta^2_p=0.072$ and no significant main effect of week: $F(2, 26)=1.614$, $p=0.218$, $\eta^2_p=0.11$. There was also no significant main effect of group: $F(1, 13)=0.327$, $p=0.577$, $\eta^2_p=0.025$.

For mean CHO intake, there was no significant group x week interaction effect: F(2, 26)=0.677, p=0.517, η^2_p=0.049, no significant main effect of week: F(2, 26)=0.539, p=0.590, η^2_p=0.040 and no significant main effect of group: F(1, 13)=0.247, p=0.627, η^2_p=0.019.

Energy intake and % calories from macronutrients on exercise and non-exercise days

For mean EI on exercise and non-exercise days during week 8 compared with baseline, see figure 6.2. There was no significant group x day interaction effect (values to the nearest integer): F(2, 26)=0.645, p=0.533, η^2_p=0.047 and no significant main effect of group: F(1, 13)=0.339, p=0.570, η^2_p=0.025. However, there was a significant main effect of day: F(2, 26)=6.489, p=0.005, η^2_p=0.333. Across both groups, mean EI was significantly reduced on exercise days (1733±478kcal·day-1) compared with non-exercise days (2159±606kcal ·day-1; p=0.033, d=0.78, 95% CI 31.867 – 820.847). There was also a trend for mean EI on non-exercise days to be greater than baseline days (1824±367kcal·day-1; p=0.058).

For %kcal from FAT intake on exercise and non-exercise days, there was no significant group x day interaction effect: F(1.322, 17.181)=0.025, p=0.976, η^2_p=0.002 and no significant main effect of group: F(1, 13)=0.530, p=0.480, η2p=0.039. However, there was a trend for a significant main effect of day: F(1.322, 17.181)=3.896, p=0.055, η^2_p=0.231, whereby post-hoc tests demonstrated %kcal from FAT intake on exercise days (33.3±6.3%) was significantly lower than non-exercise days (37.8±8.1%; p=0.006, d=0.62, 95% CI 1.343 – 7.798).

For %kcal from CHO intake, there was no significant group x day interaction effect: F(2, 26)=0.918, p=0.412, η^2_p=0.066, no significant main effect of day: F(2, 26)=1.691, p=0.204, η^2_p=0.066 and no significant main effect of group: F(1, 13)=0.814, p=0.383, η^2_p=0.059.

For %kcal from PRO intake, there was no significant group x day interaction effect: F(2, 26)=0.157, p=0.856, η^2_p=0.012, no significant main effect of day: F(2, 26)=1.973, p=0.159, η^2_p=0.132 and no significant main effect of group: F(1, 13)=0.006, p=0.940, η^2_p=0.000.

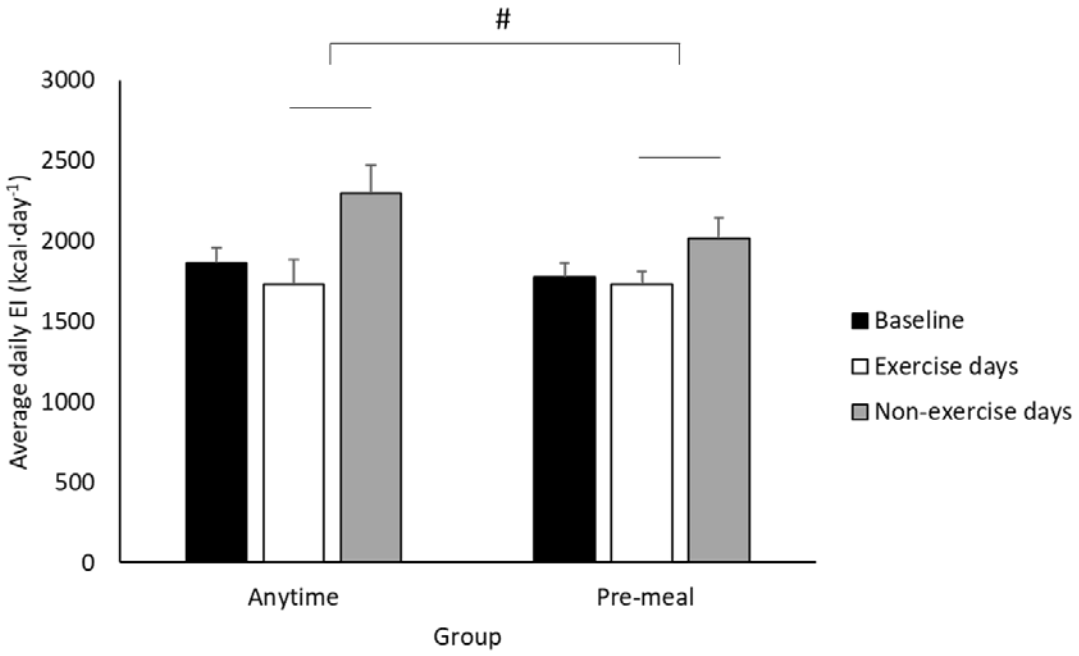

Figure 6.2 Mean daily energy intake at baseline and on exercise and non-exercise days during week 8 in both groups; # denotes difference between exercise and non-exercise days across both groups (p<0.05)

6.3.2 Body mass and BMI

There were no differences in body mass nor BMI between groups at baseline. For body mass, there was no significant group x trial interaction effect: F(1, 13)=0.527, p=0.481, η^2_p=0.039 and no significant main effect of trial: F(1, 13)=0.011, p=0.918, η^2_p=0.001 nor group: F(1, 13)=0.777, p=0.394, η^2_p=0.056. For BMI, there was no significant group x trial interaction effect: F(1, 13)=0.358, p=0.560, η^2_p=0.027, no significant main effect of trial: F(1, 13)=0.018, p=0.896, η^2_p=0.001 and no significant main effect of group: F(1, 13)=0.575, p=0.462, η^2_p=0.042.

Table 6.2 Body mass and BMI responses for baseline and post-intervention

	Baseline		Post	
	Anytime	**Pre-meal**	**Anytime**	**Pre-meal**
Body mass (kg)	75.4±9.3	72.2±5.1	75.7±9.4	71.9±4.7
BMI (kg·m^{-2})	28.5±2.5	27.7±2.1	28.6±2.5	27.6±2.1

'BMI' denotes 'body mass index'

6.3.3 Energy expenditure

There were no differences in mean daily TEE nor AEE between groups at baseline. Wear time adherence was not met on a total of fifteen days (one day during baseline for three participants, two days during baseline for one participant, three days during week 4 for one participant, one day during week 8 for two participants, two days during week 8 for one participant and four days during week 8 for another participant). In these cases, values were replaced using multiple imputations. In study 3, for days where activity monitor adherence was met, average adherence was 97.1±2.2% of the 24-hour period for each day.

For mean daily TEE and AEE, one participant was removed from the dataset (in the 'pre-meal' group due to insufficient data and a malfunction of the activity monitor), resulting in n=6 for the 'pre-meal' group. For mean daily TEE, there was no significant group x week interaction effect: $F(2, 24)=0.381$, $p=0.687$, $\eta^2_p=0.031$ and no significant main effect of week: $F(1.366, 16.398)=0.227$, $p=0.714$, $\eta^2_p=0.019$. There was also no significant main effect of group: $F(1, 12)=0.559$, $p=0.469$, $\eta^2_p=0.045$. For mean daily AEE, there was no significant group x week interaction effect: $F(2, 24)=1.324$, $p=0.285$, $\eta^2_p=0.099$ and no significant main effect of week: $F(2, 24)=1.080$, $p=0.356$, $\eta^2_p=0.0.083$. There was also no significant main effect of group: $F(1, 12)=1.168$, $p=0.301$, $\eta^2_p=0.089$. There was insufficient data in week 8 to assess differences between exercise and non-exercise days for both groups in TEE, AEE and MET responses.

Metabolic equivalent of task responses

There were no differences in %SED, %LI, %MOD, %VIG nor %V-VIG between groups at baseline. For MET responses, see table 6.3. For %SED, %LI, %MOD, %VIG and %V-VIG, again, one participant was removed from each dataset ('pre-meal' group) due to insufficient data due to insufficient wear time and activity monitor malfunctions, resulting in n=6 for the 'pre-meal' group. For %SED, there was a significant group x week interaction effect: $F(2, 24)=4.124$, $p=0.029$, $\eta^2_p=0.256$. Within 'pre-meal', %SED was significantly greater during week 4 (68.9±7.6%) compared with at baseline (60.4±7.6%; $p=0.022$, $d=1.12$, 95% CI 1.162 – 15.806). There was also a trend for %SED to be greater during week 4 in 'pre-meal' compared with during week 8 (63.4±6.2%; $p=0.085$). There was also a significant main effect of week: $F(2, 24)=4.205$, $p=0.027$, $\eta^2_p=0.259$ whereby, across both groups, there was a trend for %SED to

be greater during week 4 (68.2±9.3%) compared with baseline (63.9±11.1%; p=0.096). However, there was no significant main effect of group: F(1, 12)=0.376, p=0.551, η^2_p=0.03.

For %LI, there was no significant group x week interaction effect: F(1.324, 15.887)=1.115, p=0.344, η^2_p=0.085 but there was trend for a main effect of week: F(1.324, 15.887)=3.710, p=0.063, η^2_p=0.236.

For %MOD, there was no significant group x week interaction effect: F(2, 24)=0.702, p=0.506, η^2_p=0.055 and no significant main effect of week: F(2, 24)=0.218, p=0.806, η^2_p=0.018. There was also no significant main effect of group: F(1, 12)=0.008, p=0.932, η^2_p=0.01.

For %VIG, two extreme outliers were removed from the dataset (one at baseline and one during week 4 in the 'anytime' group), resulting in n=7 in the 'anytime' group for this measure. There was no significant group x week interaction effect: F(2, 22)=0.800, p=0.462, η^2_p=0.068 and no significant main effect of group: F(1, 11)=1.002, p=0.338, η^2_p=0.083. However, there was a significant main effect of week: F(2, 22)=5.657, p=0.01, η^2_p=0.340. Across both groups, %VIG was significantly greater at week 8 (0.5±0.3%·day^{-1}) compared with baseline (0.2±0.2%·day^{-1}; p=0.045, d=1.08, 95% CI 0.006 – 0.579).

For %V-VIG, five extreme outliers were removed from the dataset (one at baseline in the 'anytime' group, two during week 4 in the 'anytime' group and two during week 8 in the 'anytime' group), resulting in n=6 for the 'anytime' group for this measure. There was no significant group x week interaction effect: F(2, 20)=0.715, p=0.501, η^2_p=0.067 and no significant main effect of group: F(1, 10)=0.855, p=0.377, η^2_p=0.079. However, there was a significant main effect of week: F(2, 20)=11.903, p<0.001, η^2_p=0.543. Across both groups, there was a significant increase in %V-VIG during week 8 (0.1±0.1%·day^{-1}) compared with baseline (0.0±0.0%·day^{-1}; p=0.001, d=1.48, 95% CI 0.035 – 0.113). There was also a trend for %V-VIG during week 4 (0.1±0.1%·day^{-1}) to be greater than during baseline (p=0.064).

6.3.4 Basal metabolic rate

There were no differences in BMR between groups at baseline. One extreme outlier was removed from the dataset (one in the 'anytime' group at baseline and post-intervention), resulting in n=7 for this measure in the 'anytime' group. There was no significant group x trial

Table 6.3 Mean daily energy expenditure and metabolic equivalent of task responses during baseline, week 4 and week 8 in both groups

	Anytime			Pre-meal		
	Baseline	**Week 4**	**Week 8**	**Baseline**	**Week 4**	**Week 8**
TEE (kcal·day^{-1})	2594±487	2650±420	2642±494	2789±372	2722±272	2821±136
AEE (kcal·day^{-1})	792±333	834±312	864±292	1012±199	889±170	1010±148
SED (%time·day^{-1})	67.4±12.9	67.4±10.2$^{\#}$	67.0±9.8	60.4±7.6	68.9±7.6$^{\#*}$	63.4±6.2
LI (%time·day^{-1})	25.5±10.7	23.6±9.6	23.1±8.2	30.5±4.9	24.8±6.7	27.9±5.2
MOD (%time·day^{-1})	6.6±4.2	7.6±5.0	7.7±3.6	7.7±2.4	7.1±1.6	7.5±2.5
VIG (%time·day^{-1})	0.1±0.1	0.4±0.3	0.4±0.3$^{\#}$	0.3±0.2	0.4±0.1	0.6±0.3$^{\#}$
V-VIG (%time·day^{-1})	0.0±0.0	0.1±0.1	0.1±0.1$^{\#}$	0.0±0.0	0.1±0.1	0.1±0.1$^{\#}$

$^{\#}$ denotes significant difference compared with baseline across both groups (p<0.05); * denotes significant difference compared with baseline in that group (p<0.05); 'TEE' denotes 'total energy expenditure', 'AEE' denotes 'activity energy expenditure', 'SED' denotes '% time per day spent sedentary', 'LI' denotes '% time per day spent in light-intensity activity', 'MOD' denotes '% time per day spent in moderate-intensity activity', 'VIG' denotes '% time spent in vigorous-intensity activity', 'V-VIG' denotes '% time spent in very vigorous-intensity activity'

interaction effect: F(1, 12)=0.194, p=0.668, η^2_p=0.016, no significant main effect of trial: F(1, 12)=2.029, p=0.180, η^2_p=0.145 and no significant main effect of group: F(1, 12)=0.629, p=0.443, η^2_p=0.05. BMR at baseline was 1630±106kcal·day^{-1} and 1607±143kcal·day^{-1} for 'anytime' and 'pre-meal', respectively (values to the nearest integer). At post-intervention, BMR was 1585±169kcal·day^{-1} for 'anytime' and 1522±104kcal·day^{-1} for 'pre-meal'.

6.3.5 Subjective appetite responses

There were no differences in subjective appetite responses at any time point between groups at baseline. For subjective appetite (VAS composite) responses, see figure 6.3. Four extreme outliers were removed (one from 30 and one from 45 minutes following breakfast at baseline

in the ‘anytime’ group, one from 15 and one for 30 minutes following breakfast in post-intervention in the ‘pre-meal’ group), resulting in n=7 for the ‘anytime’ group and n=6 for the ‘pre-meal’ group for this measure. There was a significant group x trial x time interaction effect: F(2.963, 32.588)=4.936, p<0.001, η^2_p=0.310. Post-intervention, VAS was significantly greater at 45 minutes post-breakfast in ‘pre-meal’ (40.7±30.0mm) compared with ‘anytime’ (11.9±27.8mm; p=0.027, 95% CI 3.864 – 53.805). Post-intervention, there were also trends for VAS to be greater in ‘pre-meal’ compared with ‘anytime’ at 30 minutes (26.4±18.6mm vs. 12.6mm±7.3; p=0.097), 60 minutes (44.4±36.0mm vs. 14.9±10.0mm; p=0.061), 90 minutes (54.4±42.3mm vs. 18.6±12.7; p=0.055) and 120 minutes post-breakfast (59.4±48.8mm vs. 24.6±14.8mm; p=0.098). Within the ‘anytime’ group, there was a trend for fasted VAS to be lower at baseline (73.8±28.1mm) compared with post-intervention (93.9±20.0mm; p=0.089). Within ‘anytime’, VAS was also significantly greater at baseline (46.6±50.8mm) compared with post-intervention (24.6±47.2mm) at 120 minutes post-breakfast (p=0.026, d=0.45, 95% CI 3.243 – 40.828). Within ‘pre-meal’, VAS was significantly greater post-intervention compared with baseline at 45 minutes post-breakfast (40.7±29.9mm vs. 15.0±16.9mm; p=0.004, d=1.06, 95% CI 10.269 – 41.031) as well as at 60 minutes post-breakfast (17.2±22.7mm vs. 44.4±37.5mm; p=0.008, d=0.88, 95% CI 8.731 – 45.603), whilst there was also a trend for VAS to be greater post-intervention at 90 minutes post-breakfast (54.4±42.3mm) compared with baseline (39.2±37.7mm; p=0.059).

There was also a significant group x trial effect: F(1, 11)=5.745, p=0.035, η^2_p=0.343. Within ‘pre-meal’, across all time points, VAS was significantly greater post-intervention (46.5±28.3) compared with baseline (34.0±24.5mm; p=0.033, d=0.47, 95% CI 1.21 – 23.916). Post-intervention across all time points, there was a trend for VAS to be greater in ‘pre-meal’ (46.5±28.3mm) compared with ‘anytime’ (34.0±24.5mm; p=0.099). There was a significant main effect of time: F(1.704, 18.748)= 40.296, p<0.001, η^2_p=0.786 whereby, across both baseline and post-intervention and across both groups, fasted VAS was significantly greater than all time points post-breakfast (all p<0.05, effects not shown in figure 6.3). VAS at 120 minutes post-breakfast was also significantly greater than at 45, 60 and 90 minutes post-breakfast (all p<0.05; not shown in figure 6.3). There was no significant group x time interaction effect: F(1.704, 18.748)=1.779, p=0.198, η^2_p=0.139 and no significant time x trial

Table 6.4 Mean daily energy and macronutrient intakes for both groups during baseline, week 4 and week 8 of the intervention

	Anytime			Pre-meal		
	Baseline	**Week 4**	**Week 8**	**Baseline**	**Week 4**	**Week 8**
EI (kcal·day^{-1})	1864±367	1866±508	1849±670	1778±344	1592±381	1856±396
CHO intake (g·day^{-1})	212.5±61.1	207.3±72.4	208.8±86.5	188.2±48.6	187.4±46.5	208.3±61.8
PRO intake (g·day^{-1})	74.2±17.9	72.8±18.5	73.9±29.2	72.7±14.5	60.7±16.5	72.6±15.6
FAT intake (g·day^{-1})	73.5±20.9	75.9±20.5	73.1±30.2	75.0±22.1	64.7±18.0	75.9±16.6

'EI' denotes 'daily energy intake', 'CHO' denotes 'daily carbohydrate intake', 'PRO' denotes 'daily protein intake', 'FAT' denotes 'daily fat intake'

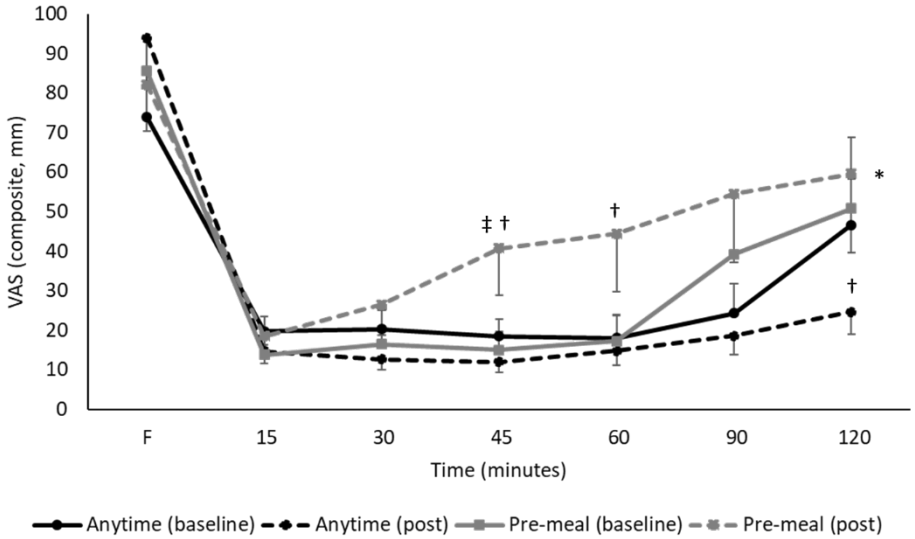

*Figure 6.3 VAS (composite) scores at baseline and post-intervention in both groups; ‡ denotes difference between groups at that time point for that trial (p<0.05), † denotes difference between visits at that time point within a group (p<0.05), * denotes difference between visits over all time points within a group (p<0.05), 'F' denotes 'fasted', '15', '30', '45', '60', '90' and '120' denote 15, 30, 45, 60, 90 and 120 'minutes post-breakfast consumption', respectively*

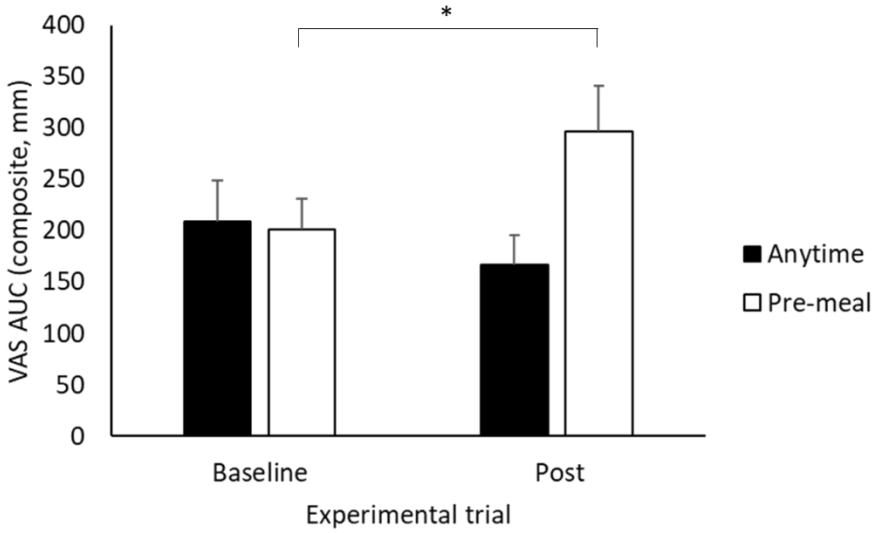

Figure 6.4 VAS (composite) AUC scores at baseline and post-intervention ('post') in both groups; # denotes difference between time points (p<0.05)

interaction effect: F(2.963, 32.588)=0.2163, p=0.112, η^2_p=0.164. There was also no significant main effect of group: F(1, 11)=1.336, p=0.272, η^2_p=0.108.

For VAS (composite) AUC, see figure 6.4. There were no differences in VAS (AUC) between groups at baseline. There was a significant group x trial interaction effect: F(1, 13)=14.347, p=0.002, η^2_p=0.525. Within the 'pre-meal' group, VAS AUC was significantly greater post-intervention (288.1±180.7mm) compared with baseline (196.4±118.5mm; p=0.003, d=0.60, 95% CI 38.014 – 151.751). There was no significant main effect of trial: F(1, 13)=2.181, p=0.163, η^2_p=04432 and no significant main effect of group: F(1, 13)=02.181, p=0.399, η^2_p=0.055.

6.3.6 Appetite and satiety hormone responses

PYY

There were no differences in PYY responses at any time point between groups at baseline.

There was no significant group x trial x time interaction effect: F(2.369, 28.426)=0.731, p=0.627, η^2_p=0.057, nor was there a significant group x trial interaction effect: F(1, 12)=0.026, p=0.876, η^2_p=0.002. There was no significant trial x time interaction effect: F(2.369, 28.426)=1.412, p=0.261, η^2_p=0.105 and no significant group x time interaction effect: F(1.845, 22.141)=0.881, p=0.513, η^2_p=0.068. There was a significant main effect of time: F(1.845, 22.141) =9.165, p<0.001, η^2_p=0.433. Across both groups and experimental trials, fasted PYY was significantly lower (92.3±27.0) compared with 15 minutes post-breakfast (117.6±17.9; p=0.021, d=1.10, 95% CI 2.835 –47.607) and with 120 minutes post-breakfast (126.8±27.9; p=0.01, d=1.26, 95% CI 6.530 – 62.349). There was no significant main effect of trial: F(1, 12)=2.488, p=0.141, η^2_p=0.172 and no significant main effect of group: F(1, 12)=0.23, p=0.598, η^2_p=0.024.

For change in PYY concentration from fasted (ΔPYY), see figure 6.5. The significant main effect of time and subsequent *post-hoc* effects remained (all p<0.05, effects not shown in figure 6.5): F(1.845, 22.141)=9.164, p=0.002, η^2_p=0.433. There were no differences in PYY AUC between groups at baseline. For PYY AUC, there was no significant group x trial interaction

effect: F(1, 12)=0.044, p=0.837, η^2_p=0.004 and no significant main effect of trial: F(1, 12)=2.755, p=0.123, η^2_p=0.187 or group: F(1, 12)=0.166, p=0.691, η^2_p=0.014.

GLP-1

GLP-1 concentration was found to be significantly greater in the 'pre-meal' group compared with 'anytime' at 120 minutes following breakfast at baseline, therefore change in GLP-1 concentration from fasted (ΔGLP-1) responses should also be considered (see below). One extreme outlier was removed from the dataset (one in the 'pre-meal' group at 15 minutes following breakfast at baseline), resulting in n=5 for the 'pre-meal' group for this measure. There was no significant group x trial x time interaction effect: F(6, 66)=1.125, p=0.358, η^2_p=0.093 and no significant group x time interaction effect: F(6, 66)=0.414, p=0.867, η^2_p=0.036. There was a significant group x trial interaction effect: F(1, 11)=9.512, p=0.01, η^2_p=0.464, whereby within the 'anytime' group only, GLP-1 concentration was significantly greater post-intervention (28.6±5.2pM) compared with baseline (25.3±5.0pM; p=0.002, d=0.65, 95% CI 1.492 – 5.223). There was also a significant trial x time interaction effect: F(6, 66)=2.586, p=0.026, η^2_p=0.19. GLP-1 concentration was significantly greater post-intervention compared with baseline, across both groups, at 15 minutes (33.9±6.1pM vs. 29.0±8.0pM) and 30 minutes (31.0±7.0pM vs. 27.4±7.0pM) post-breakfast. There was also a significant main effect of time: F(6, 66)=22.322, p<0.001, η^2_p=0.670 whereby GLP-1 concentration was significantly greater at every time point following breakfast compared with fasted (all p<0.05, effects not shown). There was no significant main effect of group: F(1, 11)=0.438, p=0.522, η^2_p=0.038 but there was a trend for a main effect of trial: F(1, 11)=3.346, p=0.095, η^2_p=0.233.

For ΔGLP-1, see figure 6.6. The trend for a main effect of trial no longer remained: F(1, 12)=0.795, p=0.39, η^2_p=0.062 and there was no longer a significant trial x time interaction: F(6, 66)=1.712, p=0.131, η^2_p=0.125. The significant main effect of time and subsequent *post-hoc* effects remained (all p<0.05, effects not shown in figure 6.6): F(6, 72)=25.454, p<0.001, η^2_p=0.680. The significant group x trial interaction effect also remained: F(1, 12)=4.785, p=0.049, η^2_p=0.285. In the 'anytime' group, across all time points, GLP-1 concentration was significantly greater at post-intervention (12.1±4.8pM) compared with baseline (8.0±5.5pM, p=0.037, d=0.79, 95% CI 0.302 – 7.911). There were no differences in GLP-1 AUC between

groups at baseline. For GLP-1 AUC, see figure 6.7. There was a significant group x trial interaction effect: F(1, 12)=5.301, p=0.04, η^2_p=0.306, whereby within the 'anytime' group only, GLP-1 AUC concentration was greater post-intervention (170.7±43.4pM) compared with baseline (141.0±42.7pM; p=0.037, d=0.97, 95% CI 2.045 – 57.364). There was also a trend for GLP AUC concentration to be greater in 'pre-meal (187±57pM) compared with 'anytime' at baseline (p=0.071). There was no significant main effect of trial: F(1, 12)=0.579, p=0.461, η^2_p=0.046 or group: F(1, 12)=1.223, p=0.291, η^2_p=0.092.

Acylated ghrelin

Fasted AG concentration was found to be significantly greater in the 'pre-meal' group compared with 'anytime' as well as at 120 minutes following breakfast at baseline, therefore change in AG concentration from fasted (ΔAG) responses should also be considered (see below). There was no significant group x trial x time interaction effect: F(6, 72)=1.382, p=0.234, η^2_p=0.103 and no significant group x trial interaction effect: F(1, 12)=0.025, p=0.878, η^2_p=0.002. There was no trial x time interaction effect: F(2.875, 34.494)=1.352, p=0.274, η^2_p=0.101 but there was a significant group x time interaction effect: F(6, 72)=3.298, p=0.006, η^2_p=0.216. AG concentration was significantly greater in the 'pre-meal' group compared with 'anytime' at fasted (1533±514 vs. 921±515pg·mL^{-1}, p=0.048, d=1.19, 95% CI 7.158 – 1216.899) and at 60 (807±225 vs. 511±226pg·mL$^{-1,}$ p=0.032, d=1.31, 95% CI 30.343 – 562.257), 90 (1078±380 vs. 626±379pg·mL$^{-1,}$ p=0.048, d=1.19, 95% CI 5.191 – 898.493) and 120 minutes following breakfast (1293±473 vs. 734±475pg·mL^{-1}, p=0.049, d=1.18, 95% CI 1.631 – 1116.618). Within the 'anytime' group, AG concentration was significantly greater at fasted (921±512pg·mL^{-1}) compared with 15 (690±421pg·mL^{-1}, p=0.037, d=0.83, 95% CI 9.632 – 452.401), 30 (560±339pg·mL^{-1}, p=0.014, d=1.19, 95% CI 56.434 – 664.757) and 120 minutes following breakfast (734±475pg·mL^{-1}, p=0.025, d=0.7, 95% CI 16.921 – 356.477). Within the 'pre-meal' group, fasted AG concentration (1533±514pg·mL^{-1}) was significantly greater than at 15 (1059±421pg·mL^{-1}, p<0.001, d=1.0, 95% CI 218.665 – 729.930), 30 (885±340pg·mL^{-1}, p<0.001, d=1.49, 95% CI 296.455 – 998.886), 45 (694±250 pg·mL^{-1}, p=0.001, d=2.08, 95% CI 342.190 – 1334.909), 60 (807±225pg·mL^{-1}, p=0.009, d=1.83, 95% CI 147.602 – 1303.492), 90 (1078±380pg·mL^{-1}, p=0.027, d=1.0, 95% CI 37.723 – 872.695) and 120 minutes following

breakfast (1293±473pg·mL^{-1}, p=0.011, d=0.49, 95% CI 43.560 – 435.645). AG concentration was also significantly lower at 45 (694±250pg·mL^{-1}, p=0.04, d=1.05, 95% CI 11.651 – 716.852) compared with 15 minutes following breakfast (1059±421pg·mL^{-1}). AG concentration was also significantly greater at 120 minutes following breakfast (1293±473pg·mL^{-1}) compared with at 15 (1059±421pg·mL^{-1}, p=0.04, d=0.52, 95% CI 7.243 – 462.148), 30 (885±340 pg·mL^{-1}, p=0.004, d=0.99, 95% CI 111.616 – 704.520) and 45 minutes following breakfast (694±250pg·mL^{-1}, p=0.007, d=1.58, 95% CI134.385 – 1063.509) as well as at 90 (1078±380pg·mL^{-1}, p=0.034, d=0.5, 95% CI 19.677 – 747.005) compared with 45 minutes following breakfast.

There was no significant main effect of trial: F(1, 12)=0.359, p=0.560, η^2_p=0.029, but there was a trend for a significant main effect of group: F(1, 12)=4.254, p=0.061, η^2_p=0.262. There was a significant main effect of time: F(1.864, 22.372)=24.648, p<0.02, η^2_p=0.673 whereby AG concentration was significantly greater when fasted compared with all post-breakfast time points (all p<0.05, effects not shown). AG concentration was also significantly greater at 15 minutes post-breakfast compared with 30 and 45 minutes post-breakfast as well as significantly greater at 120 minutes post-breakfast compared with 30, 45 and 60 minutes post-breakfast (all p<0.01).

For ΔAG, see figure 6.8. Six extreme outliers were removed (one at 15 minutes following breakfast at post-intervention in the 'pre-meal' group and one at 45, 60 and 120 minutes following breakfast at post-intervention in the 'pre-meal' group and two at 90 minutes following breakfast in the 'pre-meal' group at post-intervention), resulting in n=3 for the 'pre-meal' group for this measure. For ΔAG, there became a trend for a group x trial interaction: F(1, 9)=4.709, p=0.058, η^2_p=0.344 and the group x time interaction became a trend: F(6, 54)=2.088, p=0.07, η^2_p=0.188. The significant main effect of time and subsequent *post-hoc* effects remained (all p<0.05, effects not shown in figure 6.8): F(2.4, 21.603)=37.989, p<0.001, η^2_p=0.808. There were no differences in AG AUC between groups at baseline. For AG AUC, there was no significant group x trial interaction effect: F(1, 12)=0.019, p=0.893, η^2_p=0.002 and no significant main effect of trial: F(1, 12)=0.703, p=0.418, η^2_p=0.055. There was a trend for a significant main effect of group: F(1, 12)=4.057, p=0.067, η^2_p=0.253.

6.3.7 Eating behaviour questionnaires

DEBQ

There were no differences in DEBQ responses between groups at baseline. For DEBQ responses, see table 6.5. One extreme outlier was removed from the dataset for 'eating restraint' (at baseline in the 'anytime' group), resulting in n=7 for the 'anytime' group for this measure. There was no significant group x trial interaction (see table 6.5): F(1, 12)=0.630, p=0.443, η^2_p=0.05, no significant main effect of trial: F(1, 12)=1.416, p=0.257, η^2_p=0.106 and no significant main effect of group: F(1, 12)=1.184, p=0.298, η^2_p=0.09. Similarly, there was no significant group x trial interaction effect for 'external eating': F(1, 13)=0.920, p=0.355, η^2_p=0.066. There was also no significant main effect of trial: F(1, 13)=0.920, p=0.355, η^2_p=0.066 and no significant main effect of group: F(1, 13)=0.088, p=0.771, η^2_p=0.007. For 'emotional eating', there was also no significant group x trial interaction: F(1, 13)=2.055, p=0.175, η^2_p=0.137. There was also no significant main effect of trial: F(1, 13)=0.216, p=0.650, η^2_p=0.016 and no significant main effect of group: F(1, 13)=0.284, p=0.603, η^2_p=0.021.

COEQ

There were no differences in COEQ responses between groups at baseline. For COEQ responses, see table 6.5. For 'hunger', there was no significant group x trial interaction: F(1, 13)=0.241, p=0.631, η^2_p=0.018 and no significant main effect of trial: F(1, 13)=0.000, p=0.987, η^2_p=0.000. There was also no significant main effect of group: F(1, 13)=0.013, p=0.921, η^2_p=0.001. For 'fullness', there was no significant group x trial interaction: F(1, 13)=0.174, p=0.683, η^2_p=0.013, nor was there a significant main effect of trial: F(1, 13)=0.246, p=0.629, η^2_p=0.019. There was also no significant main effect of group: F(1, 13)=0.275, p=0.609, η^2_p=0.021. For 'positive mood', one extreme outlier was removed for the dataset (at baseline in the 'anytime' group), resulting in n=7 for the 'anytime' group for this measure. There was no significant group x trial interaction: F(1, 12)=0.620, p=0.446, η^2_p=0.049 and no significant main effect of trial: F(1, 12)=0.306, p=0.591, η^2_p=0.025. There was also no significant main effect of group: F(1, 12)=0.215, p=0.651, η^2_p=0.018. For 'savoury craving', there was no

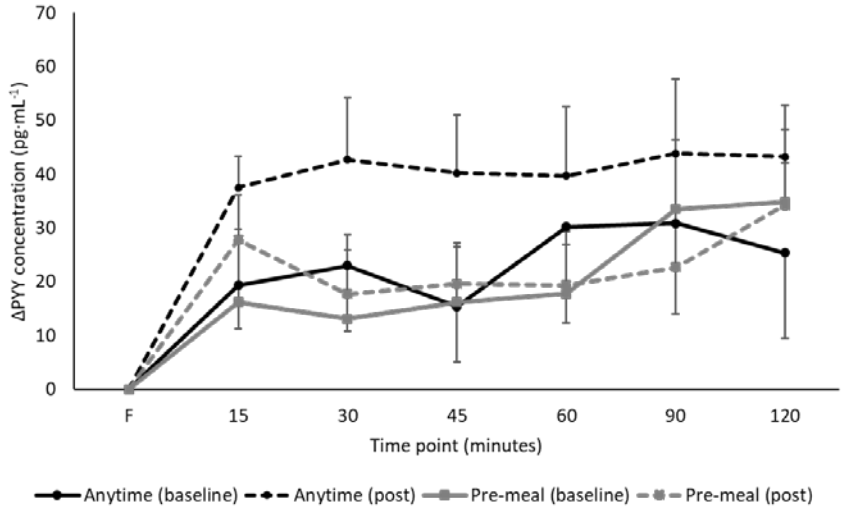

Figure 6.5 ΔPYY concentrations at baseline and post-intervention in both groups; for figures 6.5 to 6.7, 'F' denotes 'fasted', '15', '30', '45', '60', '90' and '120' denote 15, 30, 45, 60, 90 and 120 'minutes post-breakfast consumption', respectively

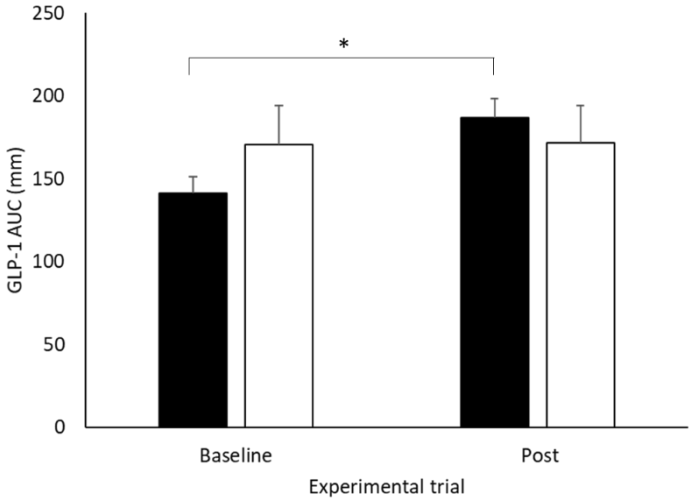

Figure 6.7 GLP AUC scores at baseline and post-intervention in both groups; * denotes difference between visits within group (p<0.05)

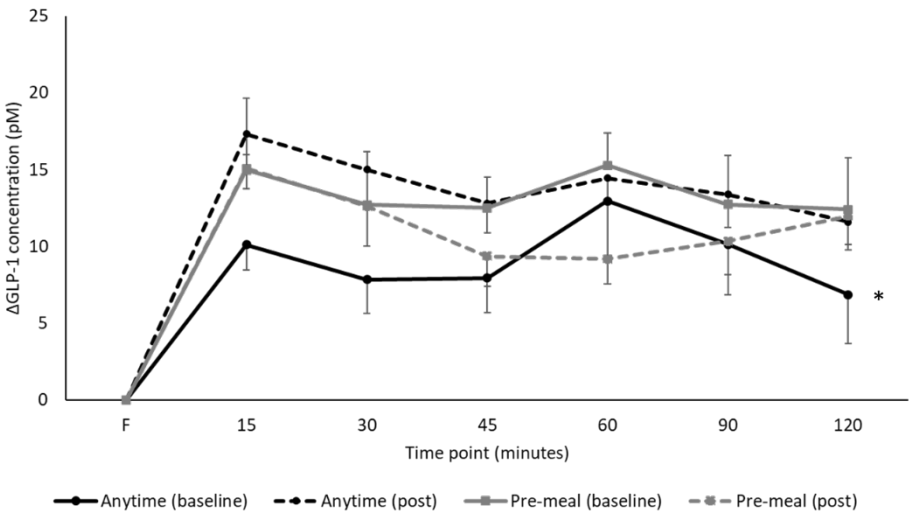

Figure 6.6 ΔGLP-1 concentrations at baseline and post-intervention in both groups; * denotes difference between visits over all time points within a group (p<0.05)

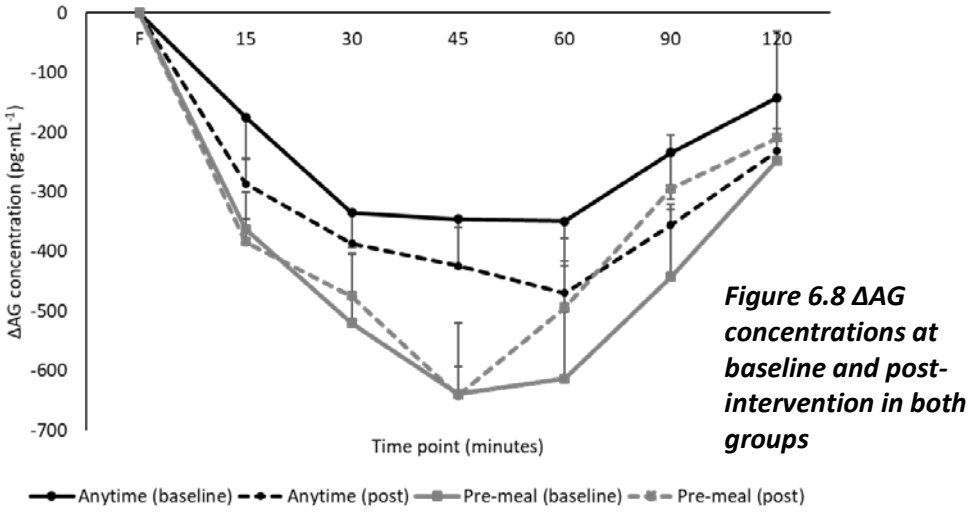

Figure 6.8 ΔAG concentrations at baseline and post-intervention in both groups

significant group x trial interaction effect: F(1, 13)=0.299, p=0.594, η^2_p=0.022. There was also no significant main effect of trial: F(1, 13)=0.642, p=0.437, η^2_p=0.047 and no significant main effect of group: F(1, 13)=0.226, p=0.642, η^2_p=0.017. For 'craving control', there was nosignificant group x trial interaction effect: F(1, 13)=0.741, p=0.405, η^2_p=0.054 and no significant main effect of trial: F(1, 13)=1.822, p=0.200, η^2_p=0.123. There was also no significant main effect of group: F(1, 13)=0.131, p=0.723, η^2_p=0.01. For 'sweet craving', there was no significant group x trial interaction effect: F(1, 13)=0.099, p=0.758, η^2_p=0.008 and no significant main effect of trial: F(1, 13)=1.714, p=0.213, η^2_p=0.117. There was also no significant main effect of group: F(1, 13)=1.299, p=0.275, η^2_p=0.091. For 'difficulty in resisting one food', there was also no significant group x trial interaction effect: F(1, 13)=3.081, p=0.103, η^2_p=0.192 and no significant main effect of trial: F(1, 13)=0.003, p=0.961, η^2_p<0.001. There was also no significant main effect of group: F(1, 13)=0.968, p=0.343, η^2_p=0.069.

Table 6.5 Responses from the DEBQ and COEQ for baseline and post-intervention for both groups

	Anytime		**Pre-meal**	
	Baseline	**Post**	**Baseline**	**Post**
Eating restraint	2.7±0.5	2.8±0.4	2.5±0.3	2.6±0.2
External eating	3.2±0.4	3.2±0.5	3.3±0.4	3.1±0.3
Emotional eating	2.7±0.8	2.8±0.8	2.6±0.9	2.5±1.1
Hunger	57.8±23.6	53.9±22.0	52.7±26.3	56.9±22.5
Fullness	64.1±21.5	63.6±25.6	71.9±19.2	66.0±22.3
Positive mood	68.0±9.6	67.0±12.4	61.7±20.4	67.4±13.4
Savoury craving	44.4±8.1	43.3±11.9	49.8±20.9	44.0±16.6
Craving control	55.1±19.6	42.1±17.9	46.6±20.6	43.7±28.2
Sweet craving	54.1±12.9	50.0±11.7	46.6±17.4	39.9±24.4
Difficulty resisting one food	66.8±16.9	51.6±23.6	59.1±28.3	73.4±14.0

6.4 Discussion

The aim of this study was to determine the effects of an 8-week apparatus-free HIIT intervention, undertaken prior to meal times, on daily EI, body mass, energy balance and

measures of appetite. There were no differences between mean daily EI during either week 4 or week 8 in either group compared with baseline. However, mean daily EI on exercise days during week 8 was lower than non-exercise days during this period, across both the 'pre-meal' and 'anytime' groups. Furthermore, on exercise days during week 8, there was a trend for a reduced proportion of EI from fat and compared with non-exercise days during this time across both groups. However, no changes in body mass occurred in either group across the 8-week period. There were also no changes in fasted nor postprandial AG, nor PYY, following the intervention. In the 'anytime' group, there was a significant increase in GLP-1 AUC following the intervention compared with baseline, but this observation did not occur in the 'pre-meal' group. In fact, postprandial subjective appetite was increased following the intervention in this group only.

The rationale to explore daily energy and macronutrient intakes on both exercise and non-exercise days was based of the findings of chapter 5 (study 2) of this thesis, where a meaningful (although, non-significant) reduction in relative EI (121kcal) was observed at an *ad libitum* buffet ~30 minutes following 4 x 30 seconds of "all-out" star jumping in overweight, inactive females. It was therefore of interest to explore the effects of two bouts of 4 x 30 seconds "all-out" star jumping per day for an 8-week free-living intervention period on daily EI, in the interest of energy balance. In the present study, EI on exercise days was reduced by a mean of 426kcal compared with non-exercise days when assessed during the final week (week 8) of the intervention. However, that this reduction was seen across both groups suggests that, in fact, exercising in this manner within 30 minutes prior to meal times did not induce this reduction *per se* and that timing of exercise in this way is not be required for inducing changes in eating behaviour.

Mean daily EI in the present study, however, was not influenced in either group which is likely partly responsible for the lack of changes seen in body mass. Although improved appetite sensitivity and coupling of EE and EI is seen as levels of PA increase above sedentary (Mayer, Roy and Mitra, 1956; Beaulieu *et al.*, 2017; Beaulieu *et al.*, 2018), a lack of change in daily EI is in agreement with many previous studies that have explored appetite and eating behaviour responses to chronic aerobic exercise interventions, even when reductions in body mass are also seen (Martins, Morgan and Truby, 2007; Martins *et al.* 2010). However, Martins, Morgan and Truby (2007) and Martins *et al.* (2010) did instruct participants to avoid changing their

food intake throughout the intervention, whereas in the present study participants were not explicitly instructed to do so. Moreover, the present study exhibits differences in exercise parameters and is one of the first to explore appetite and eating behaviour responses to a HIIT intervention. Nonetheless, it is important that no compensatory increases in EI were seen in the present study, which has previously occurred in overweight/obese females undertaking twelve weeks of aerobic exercise (Myers *et al.*, 2019).

Conversely, a reduction in EI (of ~472kcal) occurred on the day following exercise in inactive females when compared with a resting control condition (Rocha *et al.*, 2015), whereas mean EI on non-exercise days in the present study was greater than that of the exercise days. It is possible that some degree of compensation in EI occurred on non-exercise days, although no significant differences were seen on non-exercise days compared with baseline days whilst mean EI on exercise days was also not statistically significantly different to baseline days either. Of note, the findings of Rocha *et al.* (2015) were following an acute bout of exercise, so the discrepancy with the present study could indeed arise from the repeated exercise bouts or the differences in exercise mode and protocol. However, such potential compensation is also in contrast to the findings of chapter 5 (study 2), as a mean reduction in EI of ~367kcal was seen on the day following one bout of 4 x 30 seconds "all-out" star jumps compared with resting control. However, these findings in study 2 were again an acute occurrence and were also not statistically significant.

It is well known that increasing EE through exercise is often partially compensated for through greater EI in females but not males (Stubbs *et al.*, 2002a; Stubbs *et al.*, 2002b). It has also been demonstrated that, overall, compensatory increases in EI are responsible for many of the attenuated losses in body mass typically seen in a large sample of exercise intervention studies (Westerterp, 2018). Therefore, it is not unlikely that the potentially compensatory increases in EI on non-exercise days were, indeed, compensatory in response to the EE and EI responses on exercise days and therefore somewhat responsible for the resultant absence of modulation in body mass in the present study. At this point, it is important to acknowledge that marked variability in adaptive metabolic and behavioural responses to exercise training exist and likely influence the individual differences seen regulation in energy balance and resultant outcomes in body mass (King *et al.*, 2007; Boutcher and Dunn, 2009; King *et al.*, 2012; Hopkins *et al.*, 2014; MacLean *et al.*, 2017). Individual responses in change in body mass

in the present study should be considered when interpreting these findings, especially given its small sample size.

Certainly, both caloric restriction and exercise studies have been demonstrated to attenuate predicted loss in body mass by up to 12-44% and 55-64%, respectively, which are also likely conservative estimates as these do not include assumptions of behavioural compensation (Dhurandhar *et al.*, 2015). Although evidence has previously suggested that generally, chronic exercise does not seem to influence energy or macronutrient intake (Donnelly *et al.*, 2014), it is also acknowledged that this is again limited by a lack of adequately powered trials to assess this as well as inadequate methods to assess EE and EI appropriately and that this conclusion was based on studies of a maximum duration of 2 weeks only, thus warranting further study. Indeed, caloric restriction alone can induce greater compensatory increases in appetite and EI compared with exercise alone (Cameron *et al.*, 2016), thereby supporting the promotion of exercise over caloric restriction for effective weight management. Nonetheless, it would certainly be of interest to explore the effects of caloric restriction on non-exercise days only in an intervention similar to the present study and explore any greater effects on body and/or fat mass as well as any resulting effects on appetite or eating behaviour.

At this point, however, it should also be noted that some degree of measurement error is likely observed with free-living, behavioural the measures of EI, EE and macronutrient intake in the present study. Assessments of EI and EE suggest that, on average, participants were in energy deficit at all three time points (baseline, week 4 and week 8). Baseline EI values are of a similar magnitude to those seen in the larger study of overweight and obese females at baseline (Holliday *et al.*, 2018; Batrakoulis *et al.*, 2018). However, that all participants in the present study were in negative energy balance is reasonable to suggest that this was not the case that participants, given that they were overweight or obese at baseline and did not see any changes in body mass across the duration of the intervention. Of note, similar mean measures of TEE were observed when the Actiheart device was used in an overweight/obese and inactive population, albeit male (Rocha *et al.*, 2016). Given the self-report nature of energy and macronutrient intake assessments, reporting can be subject to bias and inaccuracies, especially in overweight/obese, female populations (Prentice *et al.*, 1986; Bingham, 1987; Borrelli, 1990; Schoeller, Bandini and Dietz, 1990; Hebert *et al.*, 1997; Macdiarmid and Blundell, 1998; Wehling and Lusher, 2017), which may be responsible for this

large discrepancy seen in EE and EI. Nonetheless, there were no differences between groups nor time points in AEE nor TEE at any time points in the present study. This suggests that, overall, the low volume HIIT protocol did not lead to significant increases in TEE or AEE across the 5- day periods. Measures of TEE and AEE specifically on exercise and non-exercise days, however, were not feasible to analyse in the present study due to insufficient data for multiple imputations to be used. Nonetheless, a greater %VIG and %V-VIG during week 8 of the intervention in both exercise groups demonstrates that the HIIT intervention significantly increased time spent in vigorous and very vigorous activity across this 5-day measurement period, which was therefore likely to be increased on exercise days too.

Indeed, in the present study, there was a trend for a reduction in %kcal from FAT on exercise days compared with non-exercise days which is likely, at least in part, to be driving the EI deficit between exercise and non-exercise days. Alkahtani *et al.* (2014) previously demonstrated that a HIIT intervention of four weeks duration reduced fat intake at an *ad libitum* test meal by 16%, but the novelty of the present study means that few comparable studies have assessed daily macronutrient intake across an intervention period. Moreover, a reduced daily mean fat intake is a common finding in regular exercisers among cross-sectional studies (Donnelly *et al.*, 2014), while cravings of high-fat foods and fast food fats were reduced following twelve weeks of moderate-intensity exercise (Rocha *et al.*, 2016).

Although only a trend was observed for a main effect of 'day', this suggests that, acutely, exercise in this way may improve fat intake which is considered beneficial to an overweight/obese population, although it is noted that this was observed on exercising days only. Despite this, no changes in non-homeostatic appetite control were found when assessed using either the DEBQ or the COEQ in the present study. Similarly, Rocha *et al.* (2016) found no changes in aspects of the eating restraint using the TFEQ (Stunkard and Messick, 1985) following an exercise intervention of twelve weeks duration. It is possible that improvements in dietary restraint usually accompany body mass loss (Rocha *et al.*, 2016) which may explain the null findings in assessments of eating control and restraint in the present study.

Although the novelty of the present study means that there are few comparable studies that have explored HIIT interventions where exercise is timed around meal times, body mass is a common assessment in many other HIIT interventions. Many studies of similar duration to the present study did not see modulations in body mass in overweight/obese individuals

(Kong *et al.*, 2016; Phillips *et al.,* 2017). Thus, these observations corroborate with the null findings for change in body mass observed in both groups of the present study, despite attempts to specifically manipulate EI at meal times through the strategic timing of exercise, which should still be considered a novel aspect to the present study. Reasons for null findings in change in body mass likely include an insufficient energy deficit being induced. The protocol in the present study, as well as that of Phillips *et al.* (2017), accumulated just 2 and 5 minutes of high-intensity activity, respectively. Martins *et al.* (2016) employed a larger volume of HIIT whereby ~10 minutes total duration of repeated 8 seconds of high-intensity cycling and 12 seconds of recovery saw improvements in body mass (a mean loss of 1.8kg) over 12 weeks. Reductions in fat mass of ~1.0kg were also seen with 4 x 20 seconds of high-intensity cycling on three days per week, although only a trend for accompanying reductions in body mass were seen and it is noted that the duration of this study was 12 weeks (Bagley *et al.*, 2016). Hence, this suggests that a slightly greater volume of HIIT and/or a duration of twelve weeks or more is required in order to result in modulations in body and/or fat mass in an overweight/obese population undertaking low volume HIIT.

It should, however, still be noted that the protocols of Bagley *et al.* (2016) and Martins *et al.* (2016) were laboratory-based, undertaken on a cycle ergometer and supervised; thus, the practical application and effectiveness of these findings is limited due to commonly-reported barriers to regular PA (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017), as previously mentioned. Moreover, a high-intensity, circuit-based exercise intervention of forty weeks duration lead to reductions in body mass and body fat of ~6% and ~5.5%, respectively, along with a 21% improvement in VO_{2peak} (Batrakoulis *et al.*, 2018). Of note, an increase in BMR was also seen in Batrakoulis *et al.* (2018) and not in the present study, again suggesting that perhaps a longer duration than eight weeks is required to observe meaningful changes in this measure. However, the exercise bouts in this study were still supervised and were of 23-41 minutes duration, which somewhat questions the time efficiency and effectiveness of the intervention, but does again pose the idea that perhaps a greater volume of HIIT than the present study is required to have an influence on body mass.

It is also important to consider how timing of exercise in the present study influenced eating behaviour in any other way in both groups. It is plausible that participants in both groups and

in particular the 'pre-meal' group, may have planned or began to prepare a meal in advance of undertaking exercise. However, Barutcu, Witcomb and James (2019) suggest that when a meal is planned in advance of undertaking exercise, post-exercise EI is increased in anticipation. Although mean daily EI was reduced on exercise days in both groups, it is possible that anticipation of exercise attenuated the reduction in EI that was hypothesised with the 'pre-meal' group. However, this is less likely to explain the reduction in mean daily EI seen in the 'anytime' group, which had a greater amount of time allowed between cessation of exercise and meal time although it is noted that this could be due to, at least in part, non-exercise day compensation.

Not dissimilar to the present study, no changes in body mass were seen over six weeks when 10 x 1 minutes of high-intensity cycling was undertaken either in the fed or fasted state in overweight/obese females (Gillen *et al.*, 2013). Nonetheless, reductions in total, abdominal and gynoid fat mass were observed, suggesting that such favourable modulations can occur in the absence of body mass loss, although these were not able to be measured in the present study. The findings of Gillen *et al.* (2013) are of note given that exercising before breakfast, in the fasted state, induced a more negative energy balance (~400kcal energy deficit) compared with exercising after breakfast, in the fed state (Edinburgh *et al.*, 2019). This was seen following an acute bout of exercise in active, healthy weight males, however and again does not fully explain the similar changes seen in EE and EI in the present study. Nonetheless, exercising before breakfast also improves adaptations to the skeletal muscle metabolic profile compared with fed exercise, although findings are again currently limited to young, healthy males (Wallis and Gonzales, 2018) and thus this warrants future research. The present study was not designed or able to assess such metabolic adaptations, so future research should do so to better determine whether there is a preferable time to undertake exercise for these benefits.

Of note, when a greater choice in exercise mode is available and with a self-selected intensity, EI was reduced by ~190kcal compared with when no choice in exercise mode nor intensity was available (Beer *et al.*, 2017). It could be considered that the 'anytime' group in the present study certainly had less restriction with regard to exercise timing, given that the 'pre-meal' group were required to complete two HIIE bouts within a 30-minute window prior to meal times in the same day. However, again no differences in EI were seen between the 'anytime'

and the 'pre-meal' group as to explain any attenuated loss in body mass in the 'pre-meal' group. Nonetheless, implications in restricting exercise timing should be considered when designing and interpreting findings of future studies that manipulate exercise timing where there are, inherently, greater choice and/or flexibility in exercise timing for particular conditions.

Other mechanistic reasons that may underpin attenuated losses in body mass include an increased orexigenic drive and an altered profile of appetite and satiety hormones accordingly (Casanova *et al.*, 2019). Indeed, after four consecutive days of acute moderate-intensity, continuous exercise, compensatory increases in AG were seen in response to a meal in females and not males, regardless of energy status (Hagobian *et al.*, 2009). Again, this could have been responsible for possible compensation in EI on non-exercise days in both groups in the present study, however, no changes in either fasted or postprandial AG were seen during experimental trials in the present study. Twelve weeks of moderate-intensity continuous exercise has also previously induced compensatory increases in fasted (Martins *et al.*, 2010) and postprandial subjective appetite (King *et al.*, 2009) in inactive, overweight/obese individuals. Similarly, twelve weeks of HIIT in inactive, overweight/obese individuals also demonstrated increased fasted and postprandial hunger, although this was not accompanied by similar increases in fasted and postprandial AG (Martins *et al.*, 2017). Likewise, increased subjective appetite (VAS AUC) was seen in the 'pre-meal' group in the present study, following the intervention, which was a finding not seen in the 'anytime' group.

In fact, an increase in overall GLP-1 was seen in the 'anytime' group along with suppressed subjective appetite at 120 minutes following breakfast, following the intervention, which again were findings not observed in the 'pre-meal' group. Improvements in postprandial subjective fullness and postprandial satiety hormone concentration in response to a meal with exercise training have indeed also been demonstrated, at least with moderate-intensity, continuous exercise (King *et al.*, 2009; Martins *et al.*, 2010; Guelfi, Donges and Duffield, 2013; Rosenkilde *et al.*, 2013), although this is the first study to demonstrate this occurring with HIIT and that this was demonstrated in absence of changes in body mass suggests that reductions in body mass are not required to see reductions in subjective and objective markers of appetite. However, it is noted that the present study did not assess body composition although reductions in waist circumference did occur (see section 7.3.2), suggesting that

reductions in abdominal fat likely occurred (Clasey *et al.*, 1999), thus the role of body mass and body composition change in appetite control still requires additional exploration.

In the present study, there was as an increased subjective appetite response that was not accompanied by any underpinning appetite nor satiety hormones. Due to a lack of other mechanistic data in the present study, reasons for this would, at this stage, be only speculative. It is also acknowledged that the small sample size of the present study does limit these implications, such that the effect of individual variability within groups is likely somewhat responsible for these differing responses. Indeed, reasonable variability is noted within body mass responses in the present study. Notably, large individual variability in eating behaviour responses are seen following acute exercise in overweight and obese females (Hopkins, Blundell and King, 2013). Furthermore, variability in between individuals in subjective appetite responses as well as hormone responses to acute exercise have also been noted (Goltz *et al.*, 2018). It is therefore plausible that large interindividual variability may have occurred in these measures in the present study and hence findings should be interpreted with caution. Certainly, large interindividual variability can be seen with the appetite and satiety hormones in the present study, which is why changes in these hormones from fasted are also presented.

In light of the findings of this study, it is acknowledged that the self-report nature of energy and macronutrient intakes is a limitation and should be considered when interpreting findings of this study. Both complete 5-day adherence and poor functionality of some of the activity monitors during weeks 4 and 8 also meant that, with a small sample size, there was an insufficient volume of data to run multiple imputations to replace missing data. Again, the small sample size of the present study likely increases the chance of type II error, while some measures were likely underpowered to detect differences between groups. It is also acknowledged that the presence of a non-exercise control group would have strengthened the findings and implications of the present study, although this was not practically nor logistically feasible during the study period. Furthermore, although it was deemed that the choice of gut peptide hormones assessed were appropriate for the objective of the study given the surrounding literature, it is acknowledged that the assessment of the tonic hormone leptin may have added further insight into the role of leptin in appetite and energy balance. This was due to limited resources available to assess any further appetite associated

hormones, but given its role in fuel homeostasis (see section 2.5.4), it would be of interest for similar studies with greater resource to assess any changes in circulating leptin concentrations in the future.

6.5 Conclusion

Undertaking 4 x 30 seconds of "all out" star jumping twice per day either within 30 minutes prior to a meal or outside of 1 hour prior to a meal reduces daily EI by a mean of 426kcal compared with non-exercise days. Despite this, undertaking 4 x 30 seconds of "all out" star jumping within 30 minutes prior to a meal seems to increase subjective appetite, with no accompanying effects from appetite nor satiety hormones. Undertaking this exercise any time outside of 1 hour prior to meal times increases postprandial GLP-1 concentration and suppresses postprandial subjective appetite. Reasons for such responses remain to be fully elucidated and warrant further research in a free-living setting. They do, however, suggest that performing two bouts of low volume HIIE per day on three days per week in this way, at any time that does not fall within 1 hour prior to a meal time leads to beneficial changes in eating behaviour and appetite control on these days. How manipulation of eating behaviour on non-exercising days can further influence appetite and energy balance in this population remains of interest and will assist in informing effective PA strategies for reducing EI in inactive, overweight/obese populations. Manners in which energy and macronutrient intakes can be manipulated with low volume HIIT should be further explored as these are of interest to an inactive, overweight/obese population with regard to energy balance and weight management across a longer duration, in a larger study to consolidate the findings of the present study.

Chapter 7

Study 3(b) – Cardiorespiratory fitness, metabolic health and enjoyment responses to an 8 week apparatus-free, low volume HIIT intervention in overweight, inactive females

Abstract

Low volume HIIT has been demonstrated to improve cardiorespiratory fitness and metabolic health, which are important risk markers of the 'metabolic syndrome' and all-cause mortality. Nonetheless, much of this evidence arises from studies that are typically laboratory-based, supervised and/or require access to specialised apparatus, such as a cycle ergometer. Cycle ergometer-based HIIE is posed to induce aversive affective responses which would likely limit adherence in the longer term, especially in an inactive, overweight population. Thus, the effectiveness of such exercise programmes is limited. Physiological responses of acute apparatus-free HIIE are not dissimilar to those of apparatus-based HIIE, therefore the purpose of this study was to determine the effects of an 8-week apparatus-free HIIT intervention on VO_{2peak}, markers of metabolic health as well as affective valence and enjoyment responses. Fifteen inactive, overweight/obese females (36.1±5.5 years, 28.1±2.4 $kg \cdot m^{-2}$, leisure time MVPA 29±36 $minutes \cdot week^{-1}$) undertook 4 x 30 seconds "all out" star jumps twice a day for three days per week, either within 30 minutes prior to a meal ('pre-meal') or at any time outside of an hour prior to a meal ('anytime'). VO_{2peak} significantly improved by a mean of 8% in both groups (p=0.007) and both groups demonstrated a significant mean reduction of 1.3cm in waist circumference (p=0.011). Changes in fasted TAG, glucose or insulin, as well as changes in postprandial glucose or insulin were not seen (all p>0.05). There were also no changes in HOMA-IR, LDL-C, HDL-C nor TC (all p>0.05). However, both groups demonstrated significant improvements in affective valence post-exercise compared with pre-exercise, across the eight weeks (p=0.005). Enjoyment of the intervention in the 'pre-meal' group was significantly reduced in weeks 6, 7 and 8 compared with 'anytime' (p=0.033, p=0.002 and p=0.018, respectively) while in the 'anytime' group it remained similar across the eight weeks (all p>0.05). Ratings of acceptability of the intervention were similar between groups (p>0.05). This suggests that apparatus-free, low volume HIIT in a free-living setting can be effective in improving cardiorespiratory fitness and markers of metabolic health in inactive,

overweight/obese females while still inducing positive affective, enjoyment and acceptability responses.

7.1 Introduction

Strong associations exist between low cardiorespiratory fitness and both increased risk of all-cause mortality as well as increased cardiovascular disease-related mortality (Kodama *et al.*, 2009; Mandsager *et al.*, 2018). Hence, the improvements in VO_{2max} often seen with low volume HIIT interventions are of note for public health benefit (Whyte, Hill and Cathcart, 2010; Trilk *et al.*, 2011; Adamson *et al.*, 2014; Gillen *et al.*, 2014; Foster *et al.*, 2015; Kong *et al.*, 2016; Allison *et al.*, 2017). Moreover, each 1-MET improvement in cardiorespiratory fitness is associated with a 15% and a 19% reduction in all-cause and cardiovascular disease-related mortality, respectively (Lee *et al.*, 2011).

Furthermore, improvements in insulin sensitivity with a magnitude of ~11% are also seen with low volume HIIT interventions in inactive and overweight/obese adults of four to six weeks duration (Cocks *et al.*, 2016; Allison *et al.*, 2017). Such improvements are also important as insulin resistance is implicated in a clustering of metabolic disorders (DeFronzo and Ferrannini, 1991), typically referred to as the 'metabolic syndrome', which also encompasses additional risk factors including central obesity, hyperglycaemia, hypertension and low concentrations of HDL-C (Reaven, 1998; Eckel, Grundy and Zimmet, 2005). Moreover, low volume HIIT interventions of six to eight weeks duration can improve 24-hour mean blood glucose concentration and postprandial glucose by ~9% and 6%, respectively (Gillen *et al.*, 2014; Adamson *et al.*, 2014). Furthermore, a 2.4cm reduction in waist circumference, ~8% reduction in TC and a ~10% reduction in LDL-C have also been seen with low volume HIIT of eight weeks duration in inactive adults (Stavrinou *et al.*, 2018), demonstrating the efficacy for low volume HIIT in reducing the incidence of 'metabolic syndrome' (Reaven, 1998; Eckel, Grundy and Zimmet, 2005).

Although some important improvements in cardiorespiratory fitness have been seen with HIIT interventions that are not laboratory nor apparatus-based (Blackwell *et al.*, 2017), many studies that demonstrate such improvements in metabolic health are typically laboratory-

based and/or supervised. Therefore, the effectiveness of unsupervised, free-living HIIT interventions on such metabolic health benefits remain to be elucidated. Furthermore, adherence to a free-living exercise intervention is a likely important factor in determining the effectiveness of HIIT interventions on health benefits in inactive individuals. Hence, barriers and correlates to regular PA and exercise should be considered to promote most effective improvements in health. Many low volume cycle-ergometer based HIIT interventions still require at least ~24 minutes (Whyte, Hill and Cathcart, 2010; Trilk *et al.*, 2011; Freese *et al.*, 2015; Ho *et al.*, 2018) per exercise bout, alongside access to specialised apparatus and facilities, which all remain barriers to regular PA in inactive populations (Trost *et al.*, 2002; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017).

In turn, findings from study 1 (Chapter 4) of this thesis indicate that 4 x 30 seconds of "all-out" star jumping achieved similar HR responses to 4 x 30 seconds of "all-out" cycling. Additionally, 4 x 30 seconds of "all-out" star jumping demonstrated BLa responses akin to high-intensity exercise and previous HIIE protocols. Given the commonly-reported barriers to regular PA and exercise, such acute physiological responses to apparatus-free HIIE protocols warrant their incorporation and investigation within longer term interventions. Additionally, the acute physiological responses observed in study 1 (Chapter 4) were seen alongside a reduced RPE as well as greater affective responses, with a tendency for greater enjoyment responses compared with 4 x 30 seconds of "all-out" cycling, which is important given that HIIE may not be tolerable nor appealing for inactive individuals (Gibala *et al.*, 2012). In addition, aversive affective responses likely lead to a reduction in adherence (Biddle and Batterham, 2015) whilst positive affective responses are associated with increased adherence up to 12 months later (Williams *et al.*, 2008). Moreover, how affective responses to HIIT change over time in an intervention format remains to be determined (Stork *et al.*, 2017).

Lack of enjoyment can also be a prominent barrier to regular PA (Trost *et al.*, 2002; Salmon *et al.*, 2003; Burgess, Hassmén and Pumpa, 2017). However, enjoyment levels of an eight week unsupervised, free-living HIIT intervention remained high throughout the intervention and indifferent to that of a moderate-intensity, continuous exercise intervention (Vella, Taylor and Drummer, 2017). Moreover, VO_{2peak} improved by 8% in the HIIT intervention, with no improvement seen in the moderate-intensity intervention while LDL-C concentration improved by 20% in the HIIT intervention only (Vella, Taylor and Drummer, 2017), suggesting

that free-living HIIT can be effective for metabolic health and be enjoyable in inactive, overweight/obese individuals, although this intervention was apparatus-based. Improvements in VO_{2peak} of ~13% were seen over six weeks with both a HIIT and a moderate-intensity, continuous exercise intervention, however enjoyment of the HIIT intervention increased across the intervention while enjoyment of the moderate-intensity intervention remained constant and lower (Heisz *et al.*, 2016). Nonetheless, both Vella, Taylor and Drummer (2017) and Heisz *et al.* (2016) were supervised interventions and so again raises the question regarding both affective and enjoyment responses during unsupervised exercise outside of the laboratory setting. Importantly, positive social cognitions (such as intentions towards and to continue with HIIT) have been shown following HIIT interventions (Stork *et al.*, 2017).

Given the findings from study 1 (Chapter 4) of this thesis, along with evident metabolic health benefits with supervised and/or apparatus-based HIIT interventions, there is rationale to explore an unsupervised apparatus-free HIIT intervention in a free-living setting and its effects on important metabolic health markers and responses that are likely to influence longer-term adherence. Therefore, the aim of this study was to determine the effects of an eight week apparatus-free HIIT intervention on VO_{2peak} and markers of metabolic health (including waist circumference, insulin sensitivity and fasted lipid concentrations), as well as markers of affective valence, enjoyment and social cognitions of the HIIT intervention in inactive, overweight/obese females.

7.2 Methods

7.2.1 Study design

This study was part of the larger study that also formed the study of chapter 6, thus the study design of this study was a between-subjects design was used and participants were randomly assigned to either the 'pre meal' or 'anytime' intervention group. Instructions given to both intervention groups are described in more detail in study 3a (Chapter 6).

7.2.2 Participants

Inclusion and exclusion criteria, as well as ethical approval, were as described in section 3.3 and the same as those required for study 3a (Chapter 6). Ethical approval was as described in section 3.2. Participants and participant characteristics were as described in section 6.2.2.

7.2.3 Preliminary trial and familiarisation

The design of the familiarisation trial was as detailed in section 6.2.3.

7.2.4 Experimental trials

After a minimum of seven days after the familiarisation trial, participants returned to the laboratory between 8:00-9:30am. As described in section 6.2.4, participants were instructed to arrive fasted (overnight fast of at least 10 hours), having consumed 250mL water and minimised strenuous PA in the twenty four hours prior to the trial including arriving at the laboratory by car where possible. Upon arrival, veracity and adherence to these criteria, as well as the following of the 24-hour pre-testing day food diary (appendix 14), were checked by the researcher.

Prior to the breakfast meal, measures of body mass, waist and hip circumference were taken. A resting blood pressure measurement was taken using an automatic blood pressure monitor (Omron, Milton Keynes, UK) and a fasting venous blood sample was then obtained through the insertion of an IV cannula into the antecubital vein. A sterile lancet was inserted into a fingertip and a capillary blood sample was obtained. Participants were then given a breakfast meal (as described in section 6.2.4). At the time points of fasted, 15, 30, 45, 60, 90 and 120 minutes following breakfast consumption, further venous blood samples were obtained through the IV cannula. Following the 2-hour rest period (for other measures taken during this period see section 6.2.4), participants undertook a collection of questionnaires relating to the intervention, described in more detail below. Participants then underwent an incremental exercise test to exhaustion, as described in sections 3.2.3 and 6.2.3. Measures of

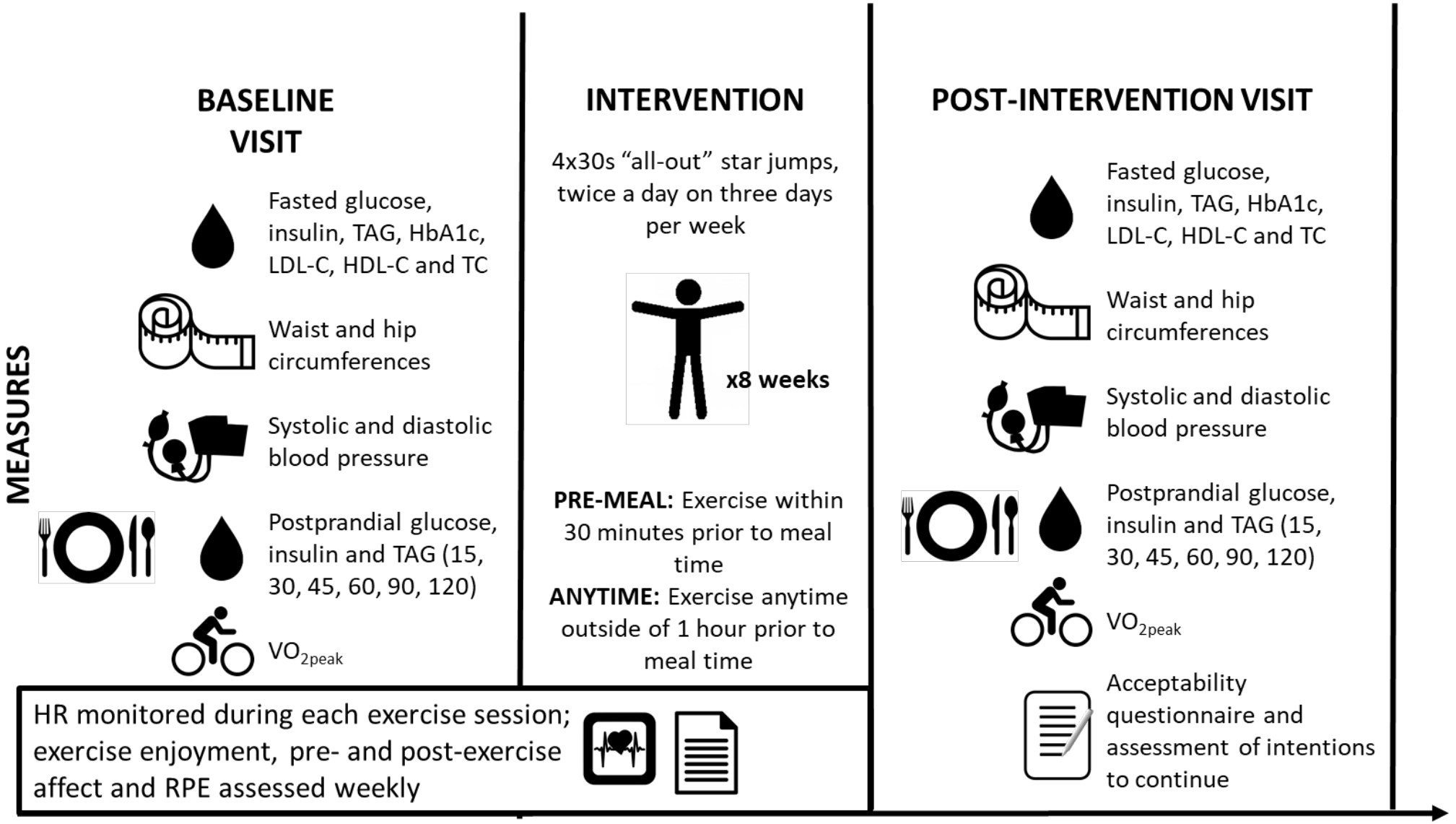

Figure 7.1 Summary of study design; '15, '30', '45', '60', '90' and '120' denote 15, 30, 45, 60, 90 and 120 'minutes post-breakfast consumption', respectively, 'TAG' denotes 'triglycerides', 'HbA1c' denotes 'glycated haemoglobin', 'LDL-C' denotes 'low-density lipoprotein cholesterol', 'HDL-C' denotes 'high-density lipoprotein cholesterol', 'TC' denotes 'total cholesterol', 'HR' denotes 'HR', 'RPE' denotes 'rating of perceived exertion', 'VO_{2peak}' denotes 'peak maximal oxygen uptake'

VO_{2peak}, peak power output (PPO) and time to exhaustion (TTE) were also assessed. Following a warm down period, participants were then free to leave the laboratory.

7.2.5 Exercise intervention

All participants were instructed to undertake two separate bouts of the 4 x 30 seconds of the high-intensity intermittent star jump protocol twice a day (aligning with their intervention group), three days per week for eight weeks. Instructions regarding timings of exercise according to group allocation were given to both intervention groups are described in more detail in section 6.2.5.

7.2.6 Measures

Cardiorespiratory fitness

As well as being used to individually calibrate the activity monitors for the measure of EE, VO_{2peak} was measured during the exercise test to volitional exhaustion (described in more detail in section 3.6.8). HR was recorded (Polar H7, Polar, Kempele, Finland) as well as oxygen production, using an online gas calorimetry system (MetaLyzer 3B, Cortex Medical, Peipzig, Germany). VO_{2peak} was calculated as the highest thirty seconds of VO_2 value recording during the test. TTE and PPO were also assessed as the total time from start until cessation of the test at voluntary exhaustion (in seconds) and the highest power output (in Watts) achieved during the test, respectively.

Anthropometrics

An ISAK-qualified researcher assessed and recorded participants' waist and hip circumferences.

Blood pressure

Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed according to methods described in section 3.6.2. Estimated mean arterial pressure (MAP) was calculated as 2/3 DBP + 1/3 SBP (Phillips *et al.*, 2017).

Blood Sampling and Analysis

Fasted venous blood only was collected into 4mL serum clot activator tubes for the analysis of triglycerides (TAG), non-esterified fatty acids (NEFA), TC, LDL-C and HDL-C. Further fasted and postprandial blood samples were collected at 15, 30, 45, 60, 90 and 120 minutes following the breakfast meal into 4mL serum clot activator tubes for analysis of insulin as well as 4mL sodium fluoride/potassium oxalate tubes for analysis of glucose. A fasted capillary blood draw was collected in a 4µL cuvette and analysed for HbA1c (HemoCue® HbA1c System, Ängelholm, Sweden). All collection tubes were centrifuged at 3000RPM for 10 minutes at 4˚C (Sorvall ST 8R, Thermo Fisher Scientific, Massachusetts, United States). The plasma supernatant was then aliquoted into 2mL Eppendorf tubes which were stored at -80˚C for later analysis.

Biochemical Analysis

Fasted and postprandial insulin concentrations were assessed using the ELISA technique (IBL International, Hamburg, Germany). Values for each of the quality controls fell within the acceptable range, while the sensitivity for the insulin ELISA kits was 1.76-100 µlU·mL^{-1}. All samples from each participant were analysed in singlicate in the same assay, while CVs were obtained for duplicates, triplicates and quadruplicates of particular samples in the same assay. From duplicates, triplicates and quadruplicates obtained, intra-assay coefficients of variation were 9.58%. Fasted and postprandial glucose concentrations were assessed using the desktop analyser YSI 2900 (YSI Incorporated, Yellow Springs, Ohio, USA). CVs were again obtained for duplicates, triplicates and quadruplicates of particular samples in the same assay, such that the intra-assay coefficients of variation were 4.31%. Insulin sensitivity was determined for each participant at baseline and post-intervention using the Homeostatic

Model Assessment of Insulin Resistance (HOMA-IR) formula, calculated according to Wallace, Levy and Matthews (2004) as:

$$\frac{[\text{Fasted plasma insulin concentration} \times \text{fasted plasma glucose concentration}]}{22.5}$$

Fasted and postprandial TAG concentrations were assessed using direct spectrophotometry where samples were mixed with an appropriate reagent (Instrumentation Laboratory Ltd, Warrington, UK). All samples from each participant were analysed in singlicate in the same assay, while CVs were obtained for duplicates, triplicates and quadruplicates of particular samples in the same assay. From duplicates, triplicates and quadruplicates obtained, intra-assay coefficients of variation were 4.64%. The concentration of LDL-C, HDL-C and total cholesterol (TC) were assessed using a fully automated clinical chemistry analyser (Daytona Plus, Randox Laboratories Ltd, County Antrim, UK). This method uses direct photometry to measure a coloured endpoint and automatically determined concentrations using computer software. All samples from each participant were analysed in duplicate, with the mean value taken. Calculated CVs for duplicate measurements were 3.64%, 1.61% and 1.03% for LDL-C, HDL-C and TC concentrations, respectively.

Weekly assessment of enjoyment, RPE, affective valence and number of star jumps achieved

On the final exercising day of each week, participants were instructed to record their self-reported levels of enjoyment of the intervention for the past week using the PACES (Kendzierski and DeCarlo, 1991; appendix 18), with the amended wording as described in section 3.6.6. Participants were also instructed to record their pre- and post-exercise affective valence using the FS (Hardy and Rejeski, 1989; appendix 16) and rating of perceived exertion (RPE) using the RPE scale (Borg, 1982; appendix 15) for the final exercise bout on the final exercising day of each week. Participants were also asked to record the number of star jumps achieved in each 30-second interval on that occasion in a separate recording sheet, all of which were mailed back to the main researcher.

Social cognitions

On the post-intervention experimental trial only, participants' intentions to continue with the exercise intervention were then assessed using methods previously described by Focht (2013) and Krinski *et al.* (2017). Participants were asked to rate their intentions to continue undertaking 4 x 30 seconds of "all out" star jumps twice a day on a 100m VAS for a) tomorrow; and for three days per week for b) the next week; c) the next 2 weeks and d) the next month (appendix 24). The final score was calculated by mean the responses to each of the four time intervals. Acceptability of the intervention was assessed following methods previously used by Boereboom *et al.* (2016). Participants were asked to rate their magnitude of agreement on a 1-5 likert scale of the following statements: 'HIIT was well explained'; 'I enjoyed HIIT'; 'I would recommend HIIT to others'; 'HIIT was more demanding than expected'; 'I would do HIIT again'; 'the physical strain interfered with my life'; 'I believe my fitness has improved' and 'I would have liked to exercise in a group' where 1 denoted 'strongly disagree' and 5 denoted 'strongly agree' (appendix 25).

Adherence to exercise timing and intensity

Participants were asked to record confirmation of completion of their respective exercise intervention in their activity diary. They were also given a HR monitor attached to a chest strap (TickrX, Wahoo Fitness, Atlanta, USA) to wear during each bout and instructed to record their HR continuously during each bout using a mobile phone app (Wahoo Fitness, Atlanta, USA) where the data was saved. Throughout the 8-week intervention, following each exercise session, participants were required to export and send the HR data to the researcher via 'WhatsApp' (WhatsApp, Dublin, Ireland). HR_{peak} for each of the four 30-second intervals was averaged across weeks 1-8 for each participant and presented as both absolute values and as a percentage of HR_{max} calculated using the Karvonen formula (Karvonen, Kentala and Mustala, 1957).

7.2.7 Statistical analyses

An independent t-test was used to assess for any differences in measures between groups at baseline for all measures other than glucose and insulin where a one-way repeated measures ANOVA was used to assess for any differences in these measures across all assessed time points between groups at baseline. A 2x2 mixed design ANOVA (group x trial) with repeated measures was used to assess differences between groups at baseline and post-intervention for waist and hip circumference, waist to hip ratio, systolic and diastolic blood pressure, mean arterial pressure, HbA1c, VO_{2peak}, PPO, TTE and HR at 50W, 75W, 100W and 125W. A 2x2x7 mixed design ANOVA (group x trial x time) with repeated measures was used to assess differences between groups at between groups at fasted, 15, 30, 45, 60, 90 and 120 minutes following breakfast at baseline and post-intervention in insulin and glucose responses. A 2x2 mixed design ANOVA (group x trial) with repeated measures was used to assess for differences in AUC for insulin and glucose at baseline and post-intervention. A 2x2 mixed design ANOVA (group x trial) with repeated measures was also used to assess for differences between groups in fasted TAG, LDL-C and HDL-C concentration as well as total cholesterol concentration and HOMA-IR at baseline and post-intervention. A 2x8 mixed design ANOVA (group x week) with repeated measures was used to assess differences between groups in ratings of enjoyment and mean number of star jumps per exercise interval (each 30 seconds) as well as mean HR_{peak} during each week of the intervention. A 2x8x2 mixed design ANOVA (group x week x time) with repeated measures was used to assess differences between groups in affective valence and ratings of perceived exertion pre and post-exercise during each week of the intervention.

Independent samples t-tests were used to assess differences between groups in intentions to continue the exercise intervention as well as adherence to the exercise intervention and adherence to exercise timing according to group allocation. Mann-Whitney tests were used to assess differences in ratings of components of acceptability of the intervention between groups due to non-normal distribution.

Missing data analysis using the multiple imputations technique was used for missing data points for LDL-C (2 cases) and TC (4 cases) due to insufficient sample volume available for analysis. Due to time restrictions and issues with cannula draws, as well as one participant

feeling unwell resulting in the final 2 cannula draws of an experimental trial being aborted, missing data analysis using the multiple imputations technique was used for missing data points for insulin (14 cases) and glucose (18 cases). Due to participants not adhering to completing the weekly recordings and to three participants not fully completing all exercise sessions across all weeks of the intervention, missing data analysis using the multiple imputations technique was used for missing data points for weekly assessment of enjoyment (10 cases), RPE (25 cases), affective valence (27 cases), number of star jumps (17 cases). Due to one participant not adhering to wearing the heart rate monitor during the intervention and due to two participants not completing the exercise sessions across one and two weeks of the intervention, respectively, missing data analysis using the multiple imputations technique was used for missing data points for mean HR across each week (7 cases). Extreme outliers (>3x interquartile range of the dataset) were removed from the dataset before statistical analysis. All statistical analysis was undertaken using the software SPSS (SPSS version 23.0, SPSS inc., Chicago, Illinois, USA).

7.3 Results

7.3.1 Cardiorespiratory fitness responses

VO_{2peak}

There were no differences in VO_{2peak} between groups at baseline. For VO_{2peak} responses, see figure 7.2 and table 7.2. There were no significant group x trial interaction effects for VO_{2peak} either expressed relative to body mass ($mL \cdot min^{-1} \cdot kg^{-1}$): $F(1, 13)=0.000$, $p=0.984$, $\eta^2_p<0.001$ or in absolute values ($L \cdot min^{-1}$): $F(1, 13)=0.003$, $p=0.961$, $\eta^2_p<0.001$. However, there was a significant main effect of trial for VO_{2peak} when expressed relative to body mass: $F(1, 13)= 10.399$, $p=0.007$, $\eta^2_p=0.444$, whereby across both groups VO_{2peak} significantly increased from baseline ($22.7\pm2.7 mL \cdot min \cdot kg^{-1}$) to post-intervention ($24.4\pm2.3 mL \cdot min^{-1} \cdot kg^{-1}$; $p=0.007$, $d=0.69$, 95% CI 0.574 – 2.904). There was a trend for a main effect of group: $F(1, 13)=3.435$, $p=0.087$, $\eta^2_p=0.209$. There was also a significant main effect of trial for VO_{2peak} when expressed in absolute values: $F(1, 13)=10.004$, $p=0.007$, $\eta^2_p=035$. Across both groups, VO_{2peak} significantly

increased from baseline (1.7±0.3L·min^{-1}) to post-intervention (1.8±0.3L·min^{-1}; p=0.007, d=0.40, 95% CI 0.037 – 0.199). There was no significant main effect of group: F(1, 13)=0.146, p=0.708, η^2_p=0.011.

Peak power output

There were no differences in PPO between groups at baseline. For PPO responses, see table 7.3. There was no significant group x trial interaction: F(1, 13)=0.009, p=0.924, η^2_p=0.001. However, there was a significant main effect of trial: F(1, 13)=39.095, p<0.001, η^2_p=0.75 such that, across both groups, PPO was significantly increased from baseline (165.3±27.9W) compared with post-intervention (179.1±27.1W, p<0.001, d=0.50, 95% CI 9.023 – 18.549). There was no significant main effect of group: F(1, 13)=0.203, p=0.660, η^2_p=0.015.

Time to exhaustion

There were no differences in TTE between groups at baseline. For TTE responses, see table 7.3. There was no significant group x trial interaction for TTE: F(1, 13)=0.332, p=0.575, η^2_p=0.025. However, a significant main effect of trial was present: F(1, 13)=59.026, p<0.001, η^2_p=0.82 whereby TTE significantly increased from baseline (348.5±55.4 seconds) to post-intervention (377.7±55.4 seconds, p<0.001, d=0.53, 95% CI 20.98 – 37.395). There was no significant main effect of group: F(1, 13)=0.244, p=0.630, η^2_p=0.018.

HR at submaximal intensities

There were no differences in HR responses at submaximal intensities between groups at baseline. For HR responses at submaximal intensities, see table 7.1. There was no significant group x trial interaction effect for HR at 50W: F(1, 13)=1.748, p=0.209, η^2_p=0.119, but there was a significant main effect of trial: F(1, 13)=5.803, p=0.032, η^2_p=0.309. HR was significantly reduced at 50W across both groups from baseline (114.9±13.6) to post-intervention (110.8±10.1; p=0.032, d=0.34, 95% CI 0.425 – 7.807). There was also a trend for a significant main effect of group: F(1, 13)=4.189, p=0.061, η^2_p=0.244.

Table 7.1 Submaximal heart rate responses at baseline and post-intervention ('post') in both groups during the exercise test at 50, 75, 100 and 125W

	Anytime		Pre-meal	
HR (beats·min^{-1})	**Baseline**	**Post**	**Baseline**	**Post**
50W	122.1±16.8	115.8±11.9$^{\#}$	107.7±8.1	105.9±7.4$^{\#}$
75W	123.3±14.5	119.4±9.6	115.0±10.4	113.1±9.1
100W	138.9±16.6	135.6±15.3	126.0±13.4	125.7±10.3
125W	151.6±13.6	147.8±13.5	140.9±15.4	139.7±12.2

$^{\#}$ denotes difference between trials across both groups (p<0.05), 'HR' denotes heart rate

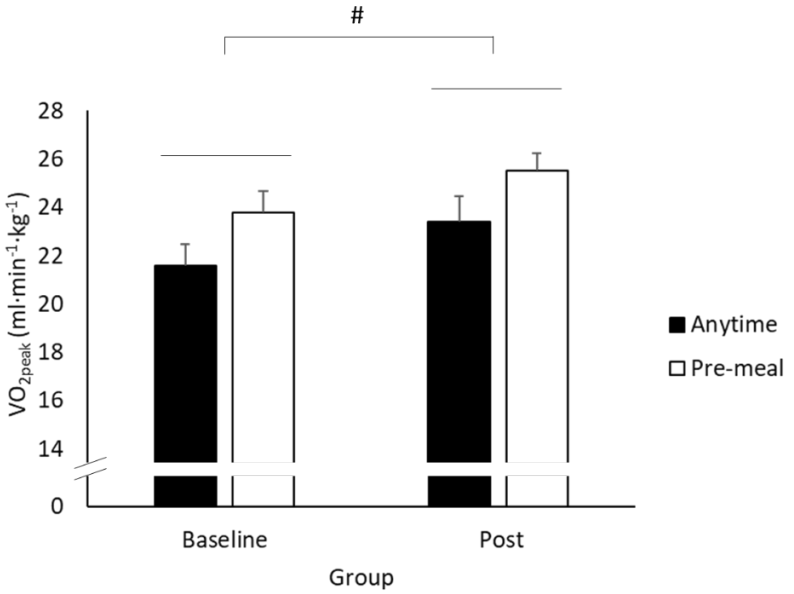

Figure 7.2 VO_{2peak} responses at baseline and post-intervention ('post') in both groups; $^{\#}$ denotes difference between trials across both groups (p<0.05), 'VO_{2peak}' denotes 'peak maximal oxygen uptake', 'post' denotes 'post-intervention'

Table 7.2 Cardiorespiratory fitness responses at baseline and post-intervention ('post') for both groups

	Anytime		Pre-meal	
	Baseline	**Post**	**Baseline**	**Post**
VO_{2peak} (mL·min^{-1}·kg^{-1})	21.6±2.5	23.4±2.5$^{\#}$	23.8±2.8	25.5±1.9$^{\#}$
VO_{2peak} (L·min^{-1})	1.7±0.4	1.8±0.4$^{\#}$	1.7±0.3	1.8±0.2$^{\#}$
Peak power output (PPO; W)	162.0±32.3	176.0±31.6$^{\#}$	168.6±21.9	182.1±21.0$^{\#}$
Time to exhaustion (TTE; seconds)	340.4±63.3	371.8±63.1$^{\#}$	356.6±44.0	383.6±44.6$^{\#}$

$^{\#}$ denotes difference between trials across both groups (p<0.05), 'VO_{2peak}' denotes 'peak maximal oxygen uptake', 'PPO' denotes 'peak power output', 'TTE' denotes 'time to exhaustion', 'post' denotes 'post-intervention', 'W' denotes 'Watts'

For HR at 75W, one extreme outlier was removed from the dataset (at baseline in the 'anytime' group), resulting in n=7 for the 'anytime' group for this measure. There was no significant group x trial interaction effect: F(1, 12)=0.539, p=0.477, η^2_p=0.043 but there was a trend for a main effect of trial: F(1, 12)=4.4, p=0.058, η^2_p=0.268. There was no significantmain effect of group: F(1, 12)=1.584, p=0.232, η^2_p=0.117.

For HR at 100W, there was no significant group x trial interaction effect: F(1, 13)=1.131, p=0.307, η^2_p=0.08, whilst there was also no significant main effect of trial: F(1, 13)=1.61, p=0.227, η^2_p=0.11). There was no significant main effect of group: F(1, 13)=2.480, p=0.139, η^2_p=0.160.

For HR at 125W, there was no significant group x trial interaction effect: F(1, 13)=1.024, p=0.330, η^2_p=0.073 and no significant main effect of trial: F(1, 13)=3.455, p=0.086, η^2_p=0.210. There was also no significant main effect of group: F(1, 13)=1.821, p=0.20, η^2_p=0.123.

7.3.2 Metabolic health responses

Table 7.3 shows waist and hip circumferences, waist to hip ratio, systolic and diastolic blood pressure, mean arterial pressure and HbA1c responses at baseline and post-intervention for both groups.

Table 7.3 Metabolic responses at baseline and post-intervention ('post') in both groups

	Anytime		Pre-meal	
	Baseline	**Post**	**Baseline**	**Post**
Waist circumference (cm)	82.4±8.0	80.7±7.8[#]	77.8±4.2	76.9±4.1[#]
Hip circumference (cm)	107.2±5.6	105.8±5.7[‡]	104.1±4.3	104.8±4.3
Waist to hip ratio (2d.p.)	0.77±0.1	0.76±0.1	0.75±0.0	0.73±0.1
Systolic blood pressure (mmHg)	110.3±8.4	109.3±10.3	114.1±8.6	113.4±9.9
Diastolic blood pressure (mmHg)	78.5±5.7	75.6±7.7	80.4±6.6	80.9±8.1
Mean arterial pressure (mmHg)	87.2±3.6	84.3±4.4	89.9±5.6	89.0±5.0
HbA1c (%)	5.7±0.2	5.6±0.2	5.7±0.3	5.7±0.3
HbA1c (mmol·mol^{-1})	39.4±2.2	38.1±1.9	39.3±2.6	39.3±3.7

[#] denotes difference between trials across both groups (p<0.05); [‡] denotes difference between trials within that group (p<0.05), 'HbA1c' denotes 'glycated haemoglobin'

Waist circumference

There were no differences in waist circumference between groups at baseline. There was no significant group x trial interaction effect for waist circumference: F(1, 13)=0.731, p=0.408, η^2_p=0.053. However, there was a significant main effect of trial: F(1, 13)=8.697, p=0.011, η^2_p=0.401, such that across both groups waist circumference was significantly reduced from baseline (80.1±6.5cm) to post-intervention (78.8±6.4cm, p=0.011, d=0.2, 95% CI 0.355 - 2.3). There was no significant main effect of group: F(1, 13)=1.589, p=0.23, η^2_p=0.109.

Hip circumference

There were no differences in hip circumference between groups at baseline. There was a significant group x trial interaction effect for hip circumference: F(1, 13)=5.015, p=0.043, η^2_p=0.278. Within 'anytime', there was a significant reduction in hip circumference from baseline (107.2±5.6cm) to post-intervention (105.8±5.7cm, p=0.04, d= 0.20, 95% CI 0.73 – 2.727). No main effect of trial was seen: F(1, 13)=0.763, p=0.398, η^2_p=0.055. There was no significant main effect of group: F(1, 13)=0.598, p=0.453, η^2_p=0.044.

Waist to hip ratio

There were no differences in waist to hip ratio between groups at baseline. There was no significant group x trial interaction effect for waist to hip ratio: F(1, 13)=0.668, p=0.429, η^2_p=0.049, but there was a trend for a main effect of trial F(1, 13)=4.432, p=0.055, η^2_p=0.254. There was no significant main effect of group: F(1, 13)=0.888, p=0.363, η^2_p=0.064.

Systolic blood pressure

There were no differences in systolic blood pressure between groups at baseline. There was no significant group x trial interaction effect for systolic blood pressure: F(1, 13)=0.017,

p=0.899, η^2_p=0.001 and no significant main effect of trial: F(1, 13)=0.606, p=0.45, η^2_p=0.045. There was no significant main effect of group: F(1, 13)=0.737, p=0.406, η^2_p=0.054.

Diastolic blood pressure

There were no differences in diastolic blood pressure between groups at baseline. There was no significant group x trial interaction effect for diastolic blood pressure: F(1, 13)=1.915, p=0.190, η^2_p=0.128. There was also no main effect of trial present: F(1, 13)=1.05, p=0.324, η^2_p=0.075. There was no significant main effect of group: F(1, 13)=1.078, p=0.318, η^2_p=0.077.

Mean arterial pressure

There were no differences in mean arterial pressure between groups at baseline. Two extreme outliers were removed from the dataset (one at post-intervention in the 'anytime' group and one at post-intervention in the 'pre-meal' group), resulting in n=7 for the 'anytime' group and n=6 for the 'pre-meal' group for this measure. However, there was no significant group x trial interaction effect: F(1, 11)=0.863, p=0.373, η^2_p=0.073 nor was there a significant main effect of trial: F(1, 11)=3.055, p=0.108, η^2_p=0.217. There was no significant main effect of group: F(1, 11)=2.390, p=0.150, η^2_p=0.178.

Insulin sensitivity (HOMA-IR)

There were no differences in HOMA-IR between groups at baseline. For HOMA-IR, one extreme outlier was removed from the 'anytime' group (for baseline), resulting in n=7 for the 'anytime' group for this measure. There was no significant group x trial interaction effect: F(1, 11)=2.094, p=0.176, η^2_p=0.160 and no significant main effect of trial: F(1, 11)=2.016, η^2_p=0.155 nor group: F(1, 11)=0.609, p=0.452, η^2_p=0.052 (see table 7.4).

HbA1c

There were no differences in HbA1c between groups at baseline when expressed with as a percentage or in mmol·mol^{-1}.There was no significant group x trial interaction for HbA1c when expressed with as a percentage: F(1, 13)=1.671, p=0.219, η^2_p=0.114, or in mmol·mol^{-1}: F(1, 13)=1.123, p=0.308, η^2_p=0.08. There were also no significant main effects of trial when expressed either as a percentage: F(1, 13)=1.056, p=0.323, η^2_p=0.075, or in mmol·mol^{-1}: F(1, 13)=1.123, p=0.308, η^2_p=0.08 . There were also no significant main effects of group for either HbA1c expressed as a percentage: F(1, 13)=0.249, p=0.626, η^2_p=0.019, or in mmol·mol^{-1}: F(1, 13)=0.188, p=0.671, η^2_p=0.014.

Fasted and postprandial insulin

There were no differences in insulin responses at any time point between groups at baseline. Four extreme outliers were removed from the dataset (two for fasted at baseline in the 'anytime' group, one for fasted at baseline in the 'pre-meal group and one for fasted post-intervention in the 'pre-meal' group), resulting in n=6 for the 'anytime' group and n=5 for the 'pre-meal' group for this measure. There was no significant group x trial x time interaction effect: F(6, 54)=0.465, p=0.831, η^2_p=0.049. There was no significant trial x time interaction effect: F(6, 54)=1.131, p=0.357, η^2_p=0.112 and no significant group x time interaction effect: F(6, 54)=0.729, p=0.628, η^2_p=0.075. There was also no significant group x trial interaction effect: F(1, 9)=0.000, p=0.988, η^2_p=0.000, no significant main effect of trial: (1, 9)=2.457, p=0.151, η^2_p=0.214 and no significant main effect of group: F(1, 9)=0.931, p=0.360, η^2_p=0.094. There was a significant main effect of time: F(2,452, 22.069)=35.877, p<0.001, η^2_p=0.799 whereby, across both groups and both trials, insulin concentration was greater at every time point following breakfast compared with fasted (all p<0.05, effects not shown). Insulin concentration was also reduced at 90 minutes following breakfast compared with 15, 30, 45 and 60 minutes following breakfast (all p<0.05, effects not shown) as well as being reduced at 120 minutes following breakfast compared with 15, 30, 45 and 60 minutes following breakfast (all p<0.05, effects not shown).

For change in insulin concentration from fasted (Δinsulin), see figure 7.3. There became a trend for a main effect of trial: F(1, 12)=4.531, p=0.055, η^2_p=0.274. Across all time points and both groups, there was a trend for Δinsulin be greater post-intervention (38.2±17.1 μlU·mL^{-1}) compared with at baseline (31.8±19.1 μlU·mL^{-1}; p=0.055). The significant main effect of time and subsequent *post-hoc* effects remained (all p<0.05, effects not shown in figure 7.3): F(2.633, 31.601)=34.438, p<0.001, η^2_p=0.742. There were no differences in insulin AUC between groups at baseline. For insulin AUC, there was no significant group x trial interaction effect: F(1, 12)=0.201, p=0.662, η^2_p=0.210 and no significant main effect of group: F(1, 12)=0.423, p=0.528, η^2_p=0.034. However, there was a trend for a main effect of trial: F(1, 12)=3.195, p=0.099, η^2_p=0.210, whereby there was a trend for insulin AUC to be greater post-intervention (297.8±130.0) compared with baseline (270.5±143.5; p=0.099).

Fasted and postprandial glucose

There were no differences in glucose responses at any time point between groups at baseline. There was no significant group x trial x time interaction effect: F(6, 72)=1.167, p=0.334, η^2_p=0.089, no significant trial x time interaction effect: F(3.183, 28.192)=0.718, p=0.637, η^2_p=0.056 and no significant group x time interaction effect: F(6, 72)=1.191, p=0.321, η^2_p=0.09. There was also no significant group x trial interaction effect: F(1, 12)=1.738, p=0.212, η^2_p=0.127 and no significant main effect of trial: F(1, 12)=0.014, p=0.909, η^2_p=0.001 nor group: F(1, 12)=0.08, p=0.782, η^2_p=0.007. There was a significant main effect of time: F(2.951, 35.414)=23.712, p<0.001, η^2_p=0.664. Across both groups and all time points, glucose concentration was significantly greater at 15 minutes following breakfast compared with fasted as well as 45, 60, 90 and 120 minutes following breakfast (all p<0.05, effects not shown). Glucose concentration was also significantly greater at 30 minutes following breakfast compared with 90 and 120 minutes following breakfast (all p<0.05, effects not shown).

For change in glucose concentration from fasted (Δglucose), see figure 7.4. There became a trend for a group x trial interaction effect: F(1, 12)=3.211, p=0.098, η^2_p=0.211. Post-intervention, across all time points, there was a trend for Δglucose to be greater in the

'anytime' group (0.25±0.72 mmol·L^{-1}) compared with the 'pre-meal' group (0.01±0.70mmol·L^{-1}; p=0.068). The significant main effect of time and subsequent *post-hoc* effects remained (all p<0.05, effects not shown in figure 7.4): F(2.952, 35.421)=23.742, p<0.001, η^2_p=0.664. There were no differences in glucose AUC between groups at baseline. For glucose AUC, there was no significant group x trial interaction effect: F(1, 12)=2.025, p=0.180, η^2_p=0.144 and no significant main effect of trial: F(1, 12)=0.006, p=0.938, η^2_p=0.001 nor group: F(1, 12)=0.034, p=0.858, η^2_p=0.003.

Fasted TAG

There were no differences in fasted TAG between groups at baseline. For fasted TAG responses, see table 7.4. There was no significant group x trial interaction effect: F(1, 12)=0.008, p=0.930, η^2_p=0.001, no significant main effect of trial: F(1, 12)=0.316, p=0.584, η^2_p=0.026 and no significant main effect of group: F(1, 12)=0.03, p=0.866, η^2_p=0.002.

Fasted LDL-C, HDL-C and TC

There were no differences in fasted LDL-C, HDL-C nor TC between groups at baseline. For fasted LDL-C, HDL-C and TC responses, see table 7.4. For fasted LDL-C concentration, there was no significant group x trial interaction effect: F(1, 12)=0.859, p=0.372, η^2_p=0.067 and no significant main effect of trial: F(1, 12)=0.325, p=0.579, η^2_p=0.026. There was also no significant main effect of group: F(1, 12)=0.376, p=0.551, η^2_p=0.03.

For fasted HDL-C concentration, one participant was removed from the 'anytime' group due to insufficient data, resulting in n=7 for the 'anytime' group for this measure. There was no significant group x trial interaction effect: F(1, 11)=1.014, p=0.336, η^2_p=0.084 and no significant main effect of trial: F(1, 11)=0.089, p=0.771, η^2_p=0.008. There was also no significant main effect of group: F(1, 11)=0.279, p=0.608, η^2_p=0.025.

For TC concentration, there was no significant group x trial interaction effect: F(1, 12)=1.244, p=0.287, η^2_p=0.094 and no significant main effect of trial: F(1, 12)=2.779, p=0.121, η^2_p=0.188

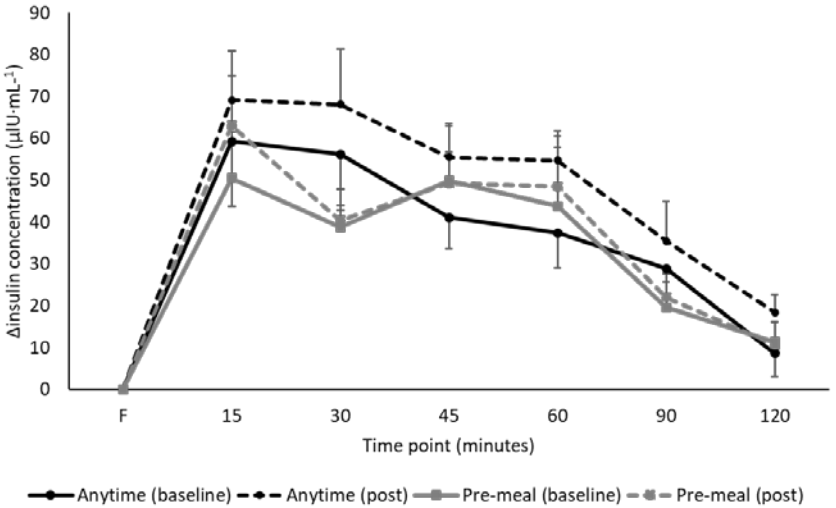

Figure 7.3 Δinsulin concentrations at baseline and post-intervention in both groups; for figures 7.3 to 7.5, 'F' denotes 'fasted', '15', '30', '45', '60', '90' and '120' denote 15, 30, 45, 60, 90 and 120 'minutes post-breakfast consumption', respectively

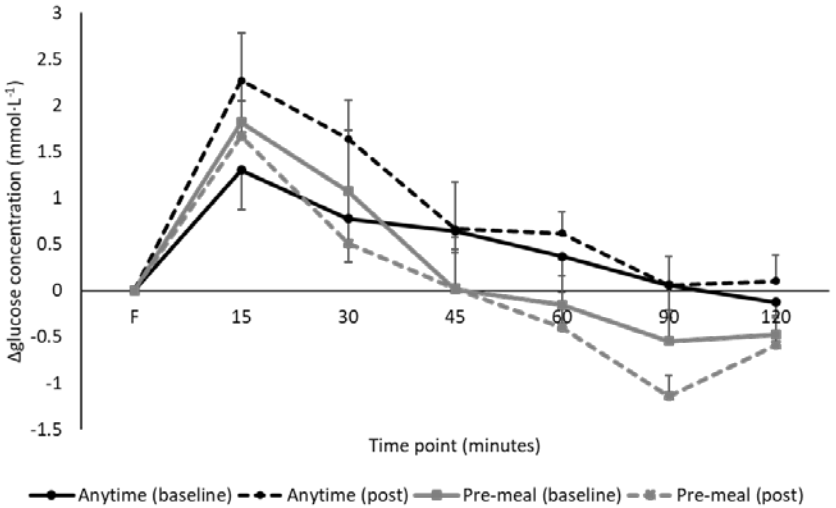

Figure 7.4 Δglucose concentrations at baseline and post-intervention in both groups

Table 7.4 Fasted LDL-C, HDL-C, TC, TAG and HOMA-IR responses at baseline and post-intervention ('post') in both groups

	Anytime		Pre-meal	
	Baseline	**Post**	**Baseline**	**Post**
LDL-C (mmol·L^{-1})	2.9±0.5	2.8±0.5	3.0±0.6	3.1±0.7
HDL-C (mmol·L^{-1})	1.4±0.2	1.4±0.3	1.5±0.4	1.5±0.3
TC (mmol·L^{-1})	4.6±0.5	4.4±0.6	4.6±0.6	4.5±0.8
TC:HDL ratio	3.5±0.4	3.2±0.4	3.2±0.7	3.2±0.6
TAG (mg·dL^{-1})	76.4±22.3	73.4±28.4	78.8±23.2	74.0±22.7
HOMA-IR	1.4±0.7	1.1±0.4	1.6±1.0	1.6±1.0

'LDL-C' denotes 'low-density lipoprotein cholesterol', 'HDL-C' denotes 'high-density lipoprotein cholesterol', 'TC' denotes 'total cholesterol', 'TAG; denotes 'triglycerides', 'HbA1c' denotes 'glycated haemoglobin'

nor group: F(1, 12)=0.04, p=0.845, η^2_p=0.003.

For TC: HDL ratio, one participant was removed from the 'anytime' group due to insufficient data, resulting in n=7 for the 'anytime' group for this measure. There was no significant group x trial interaction: F(1, 11)=1.737, p=0.214, η^2_p=0.136 and no significant main effect of trial: F(1, 11)=2.216, p=0.165, η^2_p=0.168. There was also no significant main effect of group: F(1, 11)=0.329, p=0.578, η^2_p=0.029.

7.3.3 Weekly measures

Enjoyment

Figure 7.5 shows mean enjoyment responses per week in each group. There was no significant main effect of week: F(3.172, 41.240)=2.142, p=0.106, η^2_p=0.141, but there was a significant group x week interaction effect: F(7, 91)=5.988, p<0.001, η^2_p=0.315. During week 6, enjoyment was greater in 'anytime' (88.1±8.9) compared with 'pre-meal' (72.3±16.4; p=0.033, d=1.2, 95% CI 1.446 – 30.233). Enjoyment was also greater during week 7 in 'anytime' (90.4±11.6) compared with 'pre-meal' (66.7±12.2; p=0.002, d=1.99, 95% CI 10.397 – 36.924) as well as during week 8 (92.3±13.0 vs. 73.3±14.2; p=0.018, d=1.4, 95% CI 3.788 – 34.140). There was also a trend for enjoyment to be greater during week 3 in 'anytime' (87.3±7.8) compared with 'pre-meal' (76.3±13.5; p=0.08). Within 'anytime', there was a trend for enjoyment to be lower during week 3 (76.3±13.5) compared with during week 5 (87.0±9.2; p=0.058). Within 'pre-meal', enjoyment was greater during week 3 (87.3±7.8) compared with week 7 (66.7±12.2; p=0.013, d=2.01) as well as during week 4 (82.1±11.1) compared with week 7 (p=0.036, d=1.32, 95% CI 0.640 – 30.217). Within 'pre-meal', enjoyment was also significantly greater during week 5 (89.4±8.9) compared with both week 6 (72.3±16.4; p=0.009, d=1.3, 95% CI 3.348 – 30.938) and week 7 (66.7±12.2; p=0.001, d=2.13, 95% CI 8.849 – 36.580). There was no significant main effect of group: F(1, 13)=2.320, p=0.152, η^2_p=0.151.

RPE

RPE responses per exercise bout per week in each group are shown in table 7.6. One participant was removed from the dataset due to having insufficient data to run multiple imputations to replace missing data. There was no significant group x week x time interaction: $F(37, 84)=0.662$, $p=0.703$, $\eta^2_p=0.052$, nor a significant group x time interaction: $F(1, 12)=1.295$, $p=0.277$, $\eta^2_p=0.097$. There was no significant group x week interaction: $F(7, 84)=1.7$, $p=0.12$, $\eta^2_p=0.124$, nor a significant time x week interaction: $F(3.603, 43.241)=1.344$, $p=0.24$, $\eta^2_p=0.101$. There was no significant main effect of week: $F(7, 84)=0.930$, $p=0.488$, $\eta^2_p=0.072$ but there was a significant main effect of time: $F(1, 12)=39.496$, $p<0.001$, $\eta^2_p=0.767$. Across all weeks and within both groups, RPE was significantly greater post-exercise (15.5±1.9) compared with before exercise (10.2±2.0; $p<0.001$, $d=2.72$, 95% CI 3.457 – 7.126). There was no significant main effect of group: $F(1, 12)=0.329$, $p=0.577$, $\eta^2_p=0.027$.

Affective valence

Affective valence responses per exercise bout per week in each group are shown in table 7.6. One participant was removed from the dataset due to having insufficient data to run multiple imputations to replace missing data and three extreme outliers were removed from the dataset (one from the 'pre-meal' group during week 4, one from the 'pre-meal' group during week 7 and one from the 'anytime' group during week 8), resulting in n=7 for the 'anytime' group and n=4 for the 'pre-meal' group for this measure. There was no significant group x week x time interaction: $F(2.842, 25.575)=0.648$, $p=0.714$, $\eta^2_p=0.067$ and no significant time x week interaction: $F(2.842, 25.575)=0.652$, $p=0.581$, $\eta^2_p=0.068$. There was also no group x time interaction: $F(1, 9)=1.65$, $p=0.231$, $\eta^2_p=0.155$ and no group x week interaction: $F(7, 63)=1.017$, $p=0.428$, $\eta^2_p=0.102$. There was no significant main effect of week: $F(7, 63)=1.034$, $p=0.417$, $\eta^2_p=0.103$ but there was a significant main effect of time: $F(1, 9)=13.609$, $p=0.005$, $\eta^2_p=0.602$. Across all weeks and within both groups, affective valence was significantly greater post-exercise (2.6±0.7) compared with before exercise (1.7±1.0; $p=0.005$, $d=1.04$, 95% CI 0.35 – 1.458). There was no significant main effect of group: $F(1, 9)=1.476$, $p=0.255$, $\eta^2_p=0.141$.

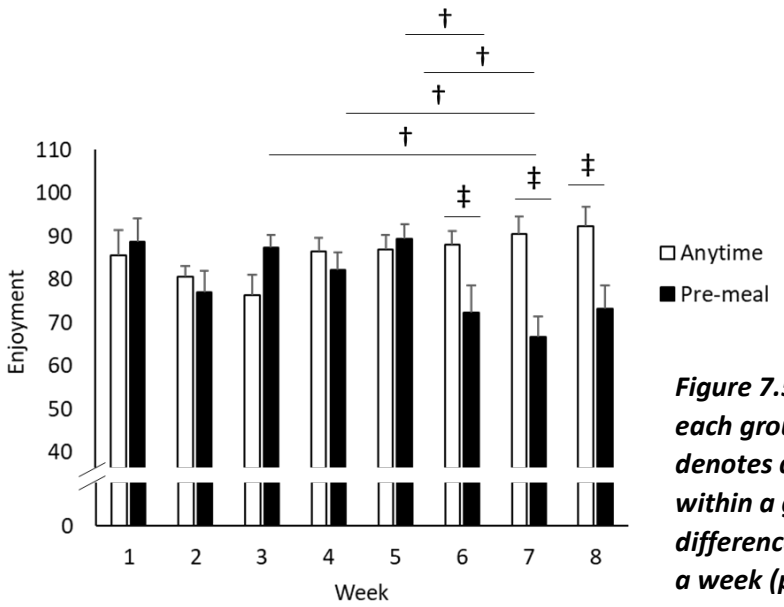

Figure 7.5 Mean enjoyment in each group during each week; † denotes difference between weeks within a group (p<0.05), ‡ denotes difference between groups within a week (p<0.05)

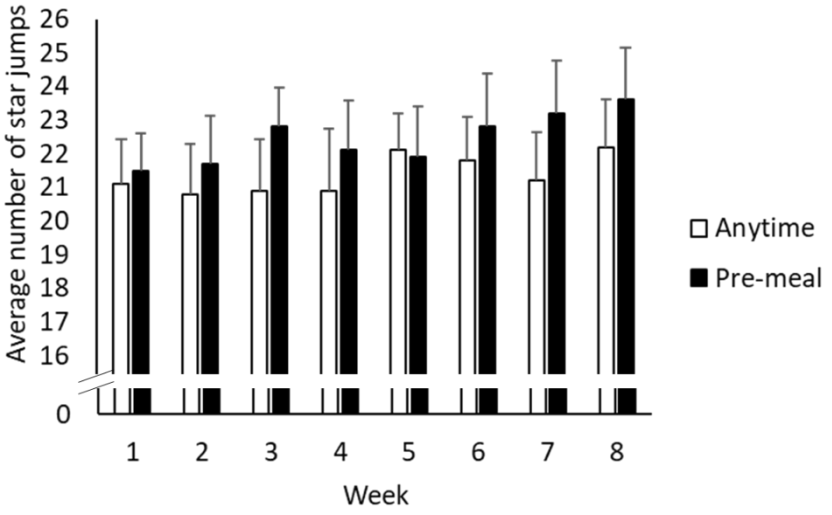

Figure 7.6 Mean number of star jumps achieved in each group across per exercise interval in each week

Table 7.5 Mean affective valence pre-exercise ('pre') and post-exercise ('post') in each group during each week

Week		Time point	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Affective valence	Anytime	Pre	2.0±1.2	1.9±3.2	1.4±1.5	2.3±1.0	3.0±1.5	2.0±1.4	2.3±1.4	2.1±1.8
		Post#	2.7±1.0	2.3±2.1	1.7±1.1	2.9±0.9	3.3±1.4	3.0±1.4	2.9±0.9	3.0±1.2
	Pre-meal	Pre	1.5±2.4	1.3±0.96	2.0±0.82	0.25±2.2	2.8±1.3	0.5±2.5	1.3±0.96	0.8±1.71
		Post#	1.8±2.2	2.8±1.7	3.3±0.96	2.5±1.0	3.5±0.6	2.0±2.2	2.5±0.58	1.75±1.9
RPE	Anytime	Pre	8.9±2.5	10.3±2.8	9.8±2.1	9.8±1.2	9.0±2.3	10.5±2.6	9.4±1.8	9.1±1.6
		Post#	16.3±2.7	16.9±2.6	15.6±2.0	15.5±2.2	15.5±2.6	16.5±2.3	15.3±1.7	15.1±2.1
	Pre-meal	Pre	10.3±3.8	9.5±4.9	12.8±3.4	10.7±3.2	10.7±2.4	11.3±3.1	11.7±1.8	10.3±2.8
		Post#	14.8±2.7	14.8±3.9	15.3±3.5	15.5±3.3	17.0±1.1	14.8±3.2	15.3±1.9	14.3±3.4

 # denotes difference between 'pre' and 'post' across all weeks for both groups (p<0.05), 'RPE' denotes 'rating of perceived exertion'

Number of star jumps

Figure 7.6 shows mean number of star jumps achieved per interval per week in each group. There was no significant group x week interaction effect for the mean number of star jumps completed per interval per week: $F(7, 91)=0.789$, $p=0.599$, $\eta^2_p=0.057$ and no significant main effect of week: $F(2.404, 31.257)=1.883$, $p=0.162$, $\eta^2_p=0.127$. There was no significant main effect of group: $F(1, 13)=0.322$, $p=0.580$, $\eta^2_p=0.024$.

7.3.4 Intentions to continue

There were no significant differences in ratings of intentions to continue with the intervention between 'anytime' (87.4±38.4) and 'pre-meal' (90.3±47.5): $t(13)=-0.127$, $p=0.901$.

7.3.5 Acceptability of the intervention

Acceptability responses are shown in table 7.7. Values were assessed using Mann Whitney tests due to all datasets failing the assumption of normal distribution (all $p<0.05$ for Shapiro-Wilk tests). However, there were no significant differences between groups for 'HIIT was well explained': $U=24.0$, $N_1=8$, $N_2=7$, $p=0.694$; 'I enjoyed HIIT': $U=22.0$, $N_1=8$, $N_2=7$, $p=0.536$; 'I would recommend HIIT to others': $U=26.0$, $N_1=8$, $N_2=7$, $p=0.867$; 'I would do HIIT again': $U=26.5$, $N_1=8$, $N_2=7$, $p=0.867$; 'the physical strain interfered with my life': $U=20.5$, $N_1=8$, $N_2=7$, $p=0.397$; 'I believe my fitness has improved': $U=27.5$, $N_1=8$, $N_2=7$, $p=0.955$ nor 'I would have liked to exercise in a group': $U=19.5$, $N_1=8$, $N_2=7$, $p=0.336$, but there was a trend for 'HIIT was more demanding than expected' to be agreed to a greater extent by the 'anytime' group compared with the 'pre-meal' group: $U=11.5$, $N_1=8$, $N_2=7$, $p=0.054$.

7.3.6 Adherence

Intervention

One extreme outlier (from the 'anytime' group) was removed from the dataset (33.4%),

resulting in n=7 in the 'anytime' group for this measure. There were no significant differences between 'anytime' (92.0±10.6%; mean 45±3 out of 48 sessions) and 'pre-meal' (93.8±7.0%; 41±11 out of 48 sessions) for adherence to the intervention: t=-0.376, df=12, p=0.713.

Table 7.6 Acceptability responses following the intervention in both groups

	Anytime		Pre-meal	
	Median	***Range***	***Median***	***Range***
HIIT was well explained	5	5	5	4-5
I enjoyed HIIT	4	3-5	4	3-5
I would recommend HIIT to others	4.5	4-5	5	4-5
HIIT was more demanding than expected	4	3-5	3	2-4
I would do HIIT again	5	4-5	5	4-5
The physical strain interfered with my life	2.5	1-5	2	1-5
I believe my fitness has improved	4	3-5	4	2-5
I would have liked to exercise in a group	2	1-4	2	1-5

Exercise timing

Two extreme outliers (both from the 'anytime' group) were removed from the dataset (18.75% and 23.8%) which occurred due to poor adherence to recording of exercise timing in relation to meals, resulting in n=6 in the 'anytime' group for this measure. There were no significant differences between 'anytime' (95.3±5.1%) and 'pre-meal' (84.5±17.3%): t=1.465, df=11, p=0.171.

Not including the two participants with poor adherence to recording of exercise timing in relation to meals, the mean window of time that exercise was undertaken prior to the following meal was calculated using self-report timings of exercise during weeks 1-3 and 5-7 and using the time of images of the meal sent during weeks 4 and 8, all alongside the timing of each exercise bout according to that recorded in the HR data. For the 'anytime' group, the mean window of time that exercise was undertaken prior to the following meal was 2 hours 24 minutes±22 minutes and 14 minutes±7 minutes for the 'pre-meal group'.

Exercise intensity

One participant was removed from the dataset due to having insufficient data to run multiple imputations to replace missing data (one from 'pre-meal' due to HR monitor malfunctions). For HR_{peak} as a $\%HR_{max}$, there was no significant group x week interaction effect: F(7, 84)=1.112, p=0.363, η^2_p=0.085, but there was a significant main effect of week: F(7, 84)=20.164, p<0.001, η^2_p=0.627. Across both groups, mean HR_{peak} as a percentage of HR_{max} was significantly greater during week 1 (88.1±3.9%) compared with during weeks 3-8: week 3 (86.0±4.2%; p=0.001, d=0.52, 95% CI 0.794 – 3.322); week 4 (85.9±4.0%; p=0.05, d=0.56, 95% CI 0.001 – 4.284); week 5 (84.2±4.4%; p=0.002, d=0.94, 95% CI 1.198 – 6.557); week 6 (83.8±3.5%; p=0.002, d=1.16, 95% CI 1.427 – 7.011); week 7 (82.9±3.8%; p<0.001, d=1.35, 95% CI 2.771 – 7.598) and week 8 (82.6±4.1%; p<0.001, d=1.37, 95% CI 2.758 – 8.069). Mean HR_{peak} was also significantly lower during week 6 (83.8±3.5%) compared with week 2 (86.7±3.9%; p=0.006, d=0.78, 95% CI 0.700 – 5.098), as well as being significantly lower during week 7 (82.9±3.8%) compared with week 2 (86.7±3.9%; p=0.001, d=0.99, 95% CI 1.389 – 6.340), week 3 (86.0±4.2%; p=0.004, d=0.77, 95% CI 0.825 – 5.428) and week 4 (85.9±4.0%; p=0.006, d=0.77, 95% CI 0.737 – 5.347). Mean HR_{peak} during week 8 (82.6±4.1%) was also significantly lower compared with during week 2 (86.7±3.9%; p=0.004, d=1.02, 95% CI 1.125 – 7.061), week 3 (86.0±4.2%; p=0.008, d=0.82, 95% CI 0.686 – 6.024) and week 4 (85.9±4.0%; p=0.005, d=0.81, 95% CI 0.802 – 5.740). There was a trend for a significant main effect of group: F(1, 12)=4.395, p=0.058, η^2_p=0.268.

7.4 Discussion

The aim of this study was to determine the effects of an eight week apparatus-free HIIT intervention on VO_{2peak} and markers of metabolic health (waist circumference, insulin sensitivity and fasted lipid concentrations) as well as affective, enjoyment and social cognition responses in inactive, overweight/obese females. Firstly, both intervention groups experienced a similar improvement in VO_{2peak} (a mean improvement of 8.3% for 'anytime' and of 7.1% for 'pre-meal'), with a medium effect size. Similarly, both groups demonstrated a significant mean reduction of 1.3cm in waist circumference (a mean reduction of 1.7cm for

'anytime' and of 0.9cm for 'pre-meal'). However, changes in neither fasted nor postprandial glucose nor insulin were seen. There were also no changes in HOMA-IR nor fasted lipids consisting of TAG, LDL-C, HDL-C nor TC. Both groups did demonstrate significant improvements in affective valence post-exercise compared with pre-exercise, across the eight weeks, yet enjoyment of the intervention in the 'pre-meal' group was significantly reduced in weeks 6, 7 and 8 compared with 'anytime'.

Such an improvement in VO_{2peak} is noteworthy as increased cardiorespiratory fitness is linked with reductions in all-cause mortality and cardiovascular disease-related mortality (Kodama *et al.*, 2009; Mandsager *et al.*, 2018). Although not assessed in the present study, it is demonstrated that improvements in cardiorespiratory fitness are due to mechanisms including cardiac and skeletal remodelling as well as increased maximal cardiac output and stroke volume, which thereby allows greater capacity for aerobic metabolism and oxygen delivery (Astorino *et al.*, 2017; Gibala, Bostad and McCarthy, 2019). However, improvements in cardiac output are not always seen alongside increases in aerobic performance, suggesting that peripheral adaptations are also partly responsible (MacPherson *et al.*, 2011). An 8% improvement in cardiorespiratory fitness is a similar magnitude of improvement to previous HIIT studies of six to eight weeks duration (Trilk *et al.*, 2011; Adamson *et al.*, 2014; Kong *et al.*, 2016; Allison *et al.*, 2017). It is not as large an improvement as some studies that have shown up to 19% improvements over 4-8 weeks (Foster *et al.*, 2015), however this is likely due to protocol and/or exercise parameter differences. Foster *et al.* (2015) employed protocols consisting of either 8 x 20 seconds or 13 x 30 seconds, which therefore would total a substantially greater training volume than the present study. Nonetheless, the protocols of Trilk *et al.* (2011), Adamson *et al.* (2014), Foster *et al.* (2015) and Kong *et al.*, (2016) were also all cycle ergometer-based (and therefore, laboratory-based). Hence, the VO_{2peak} improvement seen in the present study, achieved in the absence of a laboratory facility during the intervention period and without the requirement of a cycle ergometer, suggests that such improvements in cardiorespiratory fitness can be achieved without these requirements. This is important given that a lack of access to facilities and apparatus is a common barrier to regular PA (Trost *et al.*, 2002; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017).

Indeed, improvements in cardiorespiratory fitness without the need for specialist facilities or apparatus have previously been seen. Cardiorespiratory fitness improved by ~11% following

five weeks of whole-body aerobic resistance exercise performed maximally, which did not require specialist equipment but was laboratory-based and supervised (Myers *et al.*, 2015). Similarly, an ~8% improvement was seen with 5 x 1 minutes of whole body high-intensity exercises (star jumps, squat thrusts and static sprints), which was unsupervised and home-based, over four weeks (Blackwell *et al.*, 2017). However, interestingly, this improvement was attenuated (although non-significantly different) when compared with an identical, but laboratory-based, supervised version of 5 x 1 minutes of high-intensity cycling where the improvement in VO_{2peak} was ~17% (Blackwell *et al.*, 2017). Supervised and/or laboratory-based HIIT interventions appear more effective in increasing cardiorespiratory fitness than unsupervised/home-based interventions and this should be considered when interpreting the findings of the present study. However, given the commonly-reported barriers to regular PA, that include lack of access to facilities and apparatus (Trost *et al.*, 2002; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017) therefore somewhat limit the effectiveness and practical applicability of such interventions. Still observing improvements in VO_{2peak} without such requirements is a novel aspect of this study.

Similar to findings in both groups of the present study, Blackwell *et al.* (2017) did not see improvements in systolic nor diastolic blood pressure following either the laboratory- or home-based intervention. However, this may have been due to the reasonably small sample size (n=18) or shorter duration of the study (four weeks). A similar intervention consisting of 5 x 1 minutes high-intensity cycling over six weeks (n=189) saw improvements in mean arterial pressure of around ~3%, as well as improvements of ~10% in cardiorespiratory fitness (Phillips *et al.*, 2017), although this was a supervised, laboratory-based intervention and, thus, effectiveness is limited and this magnitude of improvement may not be seen if it were home-based and unsupervised.

The present study did see improvements in waist circumference despite not seeing accompanying improvements in body mass (see section 6.3.2). A mean reduction of 1.3cm across both groups in the present study is not dissimilar to improvements in waist circumference seen over eight weeks with 10 x 1 minutes of high-intensity cycling, undertaken either twice or three times per week for eight weeks in inactive adults (Stavrinou *et al.*, 2018). This was associated with improvements in body and trunk fat percentage, but only when undertaken on three days per week (Stavrinou *et al.*, 2018) suggesting that a particular

threshold frequency of HIIT is required for optimal adaptations, although further work is required to establish this. Thus, given these findings, although body and trunk fat percentage were not assessed in the present study, it is therefore plausible that the improvement in waist circumference seen in the present study was associated with similar improvements in trunk (and possibly, body) fat percentage, given that modest improvements in body composition can often occur in the absence of improvements in body mass with HIIT interventions (Wewege *et al.*, 2017). Furthermore, as waist circumference can be seen as an accurate predictor of total abdominal fat as well as abdominal visceral fat (Clasey *et al.*, 1999) and given the association between increased trunk fat and heightened risk of cardiovascular disease in females even with a healthy BMI (Chen *et al.*, 2019), any reduction in waist circumference in this population should be considered beneficial. It is noted, however, that use of a DEXA scanner in the present study would have bettered assessments of total and abdominal fat and future, similar studies should look to focus on this measure.

Mechanistically, it has been demonstrated that acute HIIE (consisting of repeated 8 seconds of high-intensity cycling interspersed with 12 seconds of recovery for a total duration of 20 minutes) induces fat oxidation during and following exercise in untrained females (Trapp, Chisholm and Boutcher, 2007). Therefore, the reductions in waist circumference, leg and trunk fat mass seen in Martins *et al.* (2016) that utilised this exact protocol over for three times a week over twelve weeks could be underpinned by this mechanism. It is possible that this mechanism also underpinned the findings of a reduction in waist circumference in the shorter duration protocols of the present study, although further insight into whether there is a particular threshold of exercise volume to be met for this mechanism to occur is required. It should also be noted that, alongside reductions in waist circumference and trunk and leg fat mass, trunk and leg FFM also increased in all exercise groups (Martins *et al.,* 2016). The combination of fat loss and FFM gain is likely to counteract each other when it comes to body mass assessment, such that reductions in body mass are attenuated when FFM is gained. This is a common observation with exercise interventions (Parr, Coffey and Hawley, 2013). Therefore, it is possible that (although not measured) in the present study, FFM was also gained which would again counteract any reductions in body mass as a result of fat mass loss (observed with the proxy measure of waist circumference).

In the present study, there were no changes seen in assessments of fasted nor postprandial metabolic blood markers, including no changes observed in fasted TAG, LDL-C, HDL-C nor HDL-C in either group. This is similar to other studies that have shown a lack of change in such lipid profiles with eight weeks of low volume HIIT (Ho *et al.*, 2018), although studies with larger exercise volumes than Ho *et al.* (2018) and the present study have found improvements in HDL-C (Musa *et al.*, 2009), LDL-C and TC (Sandvei *et al.*, 2012; Stavrinou *et al.*, 2018). Similarly, there were no changes in fasted nor postprandial glucose nor insulin in either group in the present study. Reductions in fasted and postprandial glucose have previously been seen with five to eight weeks of low volume HIIT (Sandvei *et al.*, 2012; Kong *et al.*, 2016), although again larger exercise volumes may have been responsible for the presence of these modulations compared with the present study. Of note, undertaking an acute bout of 6 x 1 minutes of high-intensity hill walking thirty minutes before breakfast reduced postprandial glucose for up to three hours following breakfast, compared with a resting control condition (Francois *et al.*, 2014). However, such a response following eight weeks of low volume HIIE undertaken within 30 minutes prior to a meal twice a day three days a week over eight weeks was not seen in the present study. This suggests this response is not seen as a result of accumulated HIIE across a HIIT intervention, or it may be as participants in the study of Francois *et al.* (2014) were already insulin resistant and so were perhaps more likely to see such responses in glucose and glycaemic control. Insulin resistance, assessed by HOMA-IR did also not change as a result of the intervention in either group in the present study. Previous studies have demonstrated improvements in insulin resistance as well as postprandial insulin with low volume HIIT interventions of similar exercise volume to the present study (Babraj *et al.*, 2009), therefore this warrants future research to provide greater insight into reasons for this.

Thus far no differences between the 'pre-meal' and the 'anytime' group have been seen in the measures discussed in this chapter. However, this is not surprising given that there is little or no rationale for the timing of exercise to have any differential effect at least on cardiorespiratory fitness and metabolic health markers such as blood pressure. Given that a lack of enjoyment is associated with low PA levels (Trost *et al.*, 2002; Salmon *et al.*, 2003; Burgess, Hassmén and Pumpa, 2017), any between-group differences in enjoyment were worth assessing in the present study. Generally, although acute aversive responses are

assumed, HIIT has been demonstrated more enjoyable than moderate-intensity, continuous exercise in inactive, overweight adults (Heisz *et al.*, 2016; Kong *et al.*, 2016; Vella, Taylor and Drummer, 2017). Indeed, eight weeks of high-intensity apparatus-free HIIE induced improvements in cardiorespiratory fitness of ~8% whilst intentions to engage in, and enjoyment of, low volume whole-body HIIE increased following the intervention (Foster *et al.*, 2015). However, this was a supervised intervention and was demonstrated in recreationally active females; whether such findings extend to previously inactive and/or overweight/obese females remains to be elucidated. Furthermore, Foster *et al.* (2015) found that enjoyment of all HIIT interventions declined across eight weeks in inactive individuals, although this response also occurred in the moderate-intensity continuous exercise intervention. In the present study, it appeared that enjoyment of the 'pre-meal' intervention seemed to decline in weeks 6, 7 and 8 compared with the 'anytime' group. Reasons for this are unclear, but could be related to the timing restriction that the 'pre-meal' group were required to adhere to. Conversely, enjoyment did not seem to decline in the 'anytime' group. Considering this, along with the lack of group differences in cardiorespiratory fitness and many of the metabolic health measures in the present study, the greater level of flexibility allowed in the 'anytime' group may have led to greater feelings of enjoyment. Certainly, greater choice and flexibility in exercise programmes can lead to a greater sense of autonomy in exercisers which, in turn, is associated with increased intrinsic motivation as well as increased uptake and adherence to PA (Ryan and Deci, 2000). Importantly, "all out" star jumping for 4 x 30 seconds was also shown to improve post-exercise affective valence across both groups in the present study. This corroborates recent literature that demonstrates HIIT induces positive psychological responses (Oliveira *et al.*, 2018) and challenges the assumptions that this would not be the case for inactive and/or overweight populations (Hardcastle *et al.*, 2014; Biddle and Batterham, 2015). Again, this certainly warrants further research into affective responses to HIIT programmes in these populations in the future.

Moreover, there were no differences in intentions to continue with the intervention between groups, while in fact, both groups' mean ratings of intentions were reasonably high. Additionally, there were no group differences in any aspects of the acceptability assessment and again the intervention was deemed highly acceptable by both groups. Of note, nine out of fifteen participants disagreed to some extent that they would have preferred to exercise

in a group. This is an interesting and worthy finding, especially given that a perceived lack of time and a lack of accessibility to facilities are also prominent barriers to joining activity classes for some females, along with the cost and low awareness of classes as well as the requirement to arrange childcare facilities (Withall, Jago and Fox, 2011). Hence, this provides further support for an increased focus and an increased evidence base for home-based or free-living PA programmes that approach and address many of these barriers, ultimately to increase PA levels in female populations. Furthermore, as activity levels increase, increased interest and enjoyment is associated with retention of activity levels (Withall, Jago and Fox, 2011). Hence, home-based or free-living PA programmes that are enjoyable could be a suitable initial stage for many inactive females to increase PA levels, which then may lead on to greater interest in alternative and additional PA strategies.

Interestingly, further improvements in cardiorespiratory fitness are seen when interval duration and volume as well as study duration increase (Wen *et al.*, 2019). Future studies should explore HIIT interventions that do not require laboratory access or apparatus and that progress to a longer duration and a greater volume than the present study. Importantly, mean adherence in both groups of the present study was high, although it is demonstrated that adherence to supervised interval training is greater than when unsupervised over a duration of twenty four weeks (Wen and Ang, 2019). However, it is also plausible that a lack of variety in the exercise protocol (in the case of Wen and Ang (2019), the same cycle protocol five times per week) across such a long duration reduced adherence alone. A greater choice and flexibility in exercise programmes is associated with increased intrinsic motivation, as well as increased uptake and adherence to PA (Ryan and Deci, 2000). Furthermore, in the present study, mean HR_{peak} of exercise sessions appeared to be highest during week 1 of the intervention before it began to decline across most of the remaining weeks. Although this is, in part, likely due to the improvements in fitness seen, participants were still instructed to undertake exercise at an “all out” intensity and given that maximal HR does not seem to change over the course of a HIIT intervention (Astorino *et al.*, 2017), this implies that either a greater exercise stimulus would be required across the course of a longer duration intervention (by increasing interval or total exercise volume) and/or some aspect of greater choice or supervision is required in order to maintain sufficient adherence (Ryan and Deci, 2000).

The free-living aspect and approach to commonly-reported barriers to regular PA in the present study is a strength, although its small sample size and lack of resting control group are all noted as limitations. Moreover, findings in small sample sizes are under greater influence from individual variability in measures and this should be considered alongside interpretation of the findings of this study. Certainly, large interindividual variability can be seen with the measures of glucose and insulin in the present study, in particular, which is why changes in these hormones from fasted are also presented. This may have increased the chance of type II error with some measures being underpowered to detect significant differences between groups in the intervention. Therefore, findings should be interpreted with caution. However, given the commonly-reported barriers to regular PA along with the findings of this study, it is clear that future studies should explore longer duration, HIIT interventions in a free-living setting with larger sample sizes to consolidate these findings. Effects on cardiorespiratory fitness and metabolic health as well as measures important for influencing exercise enjoyment and adherence must also be considered.

7.5 Conclusion

Important improvements in cardiorespiratory fitness and waist circumference were seen with eight weeks of apparatus-free HIIT in a free-living setting with no effect of exercise timing in proximity to meals, although no improvements in glucose, insulin, TAG nor lipid profiles were seen. However, enjoyment decreased towards the end of the intervention for the 'pre-meal' group compared with the 'anytime' group, suggesting that the greater element of choice and flexibility with the 'anytime' condition offers increased exercise enjoyment. Nonetheless, acceptability and intentions to continue with the intervention were positive and similar for both intervention groups. This suggests that apparatus-free low volume HIIT in a free-living setting can be efficacious and effective in improving cardiorespiratory fitness and waist circumference in inactive, overweight/obese females while still inducing positive affective, enjoyment and acceptability responses. Certainly, this also warrants further future research in free-living, low volume HIIT for cardiorespiratory fitness and metabolic health in inactive, overweight and obese females to further explore the effectiveness and practical application of low volume HIIT.

Chapter 8

General discussion

8.1 Rationale of thesis

Levels of overweight and obesity have risen globally since 1980 (Finucane *et al.*, 2011). A further 11 million adults are predicted to be obese by 2030 in the UK which is likely to instil a multitude of potentially fatal chronic diseases (Wang *et al.*, 2011). Meanwhile, particularly in females, proportions of UK adults meeting PA guidelines remain low (Craig and Hirani, 2009; Health and Social Care Information Centre, 2015; Guthold *et al.*, 2018). Many reported contributing reasons for this exist and include a perceived lack of time, (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017), a lack of access to apparatus or facilities as well as limited access to apparatus at home (Trost *et al.*, 2002; Cerin *et al.*, 2010). It is clear, therefore, that PA strategies that can increase PA levels and induce loss or, at least, prevent gain in body mass in females are required. In order to do so, it is imperative to consider how both sides of the energy balance equation can best be manipulated for the regulation of energy balance, both effectively and efficaciously in an inactive, overweight, female population. Females are also significantly underrepresented in sport and exercise medicine research (Costello, Bieuzen and Bleakley, 2014).

8.2 Summary of aims, objectives and findings of thesis

The primary aims and objectives of this thesis and whether these were met by the work undertaken in this thesis are detailed below.

Aim 1: To assess physiological, affective and enjoyment responses as well as appetite, eating behaviour and acute energy balance responses to apparatus-free HIIE in inactive, overweight and/or obese females.

> **Objective 1 (Study 1, chapter 4):** Identify an appropriate mode of apparatus-free HIIE that can replicate the physiological responses, as well as better the affective and

enjoyment responses, seen with traditional apparatus-based HIIE in inactive, overweight and/or obese females.

Objective 1 was met through the findings of study 1 (Chapter 4) which demonstrated the physiological responses achieved with "all out" intermittent star jumping are comparable to those attained with traditional "all out" intermittent cycling. A more positive affective response, as well as a tendency for a preferable enjoyment response, were both seen with "all out" intermittent star jumping compared with "all out" intermittent cycling. Given the commonly-reported barriers to regular PA in an overweight/obese female population, this demonstrated the potential efficacy and effectiveness of a PA programme incorporating high-intensity intermittent star jumping in this population.

Objective 2 (Study 2, chapter 5): Determine dose responses to the apparatus-free mode of HIIE of varying intervals (4x30 seconds vs. 2x30 seconds vs. rest), in order to identify the minimum number of intervals required to elicit a meaningful effect on appetite feeding latency, EI and energy balance in inactive, overweight and/or obese females.

Objective 2 was met through the findings of study 2 (Chapter 5) which found no differences in feeding latency, subjective appetite nor absolute EI at a participant-selected time point following either 2 x 30 seconds or 4 x 30 seconds of acute "all out" star jumping compared with a resting control. There was, however, a trend seen for relative EI to be reduced by 121kcal following 4 x 30 seconds of high-intensity intermittent star jumping compared with the resting control condition. It was therefore considered plausible that exercise in this way, when repeated over time, could promote a meaningful negative energy balance in this population whilst increasing PA levels. Together, the findings of studies 1 and 2 (objectives 1 and 2) answered aim 1 of this thesis.

Aim 2: To explore the effective utilisation of apparatus-free HIIE bouts within a HIIT intervention for inactive, overweight and/or obese females.

Objective 3 (Study 3, chapters 6 and 7): Incorporate findings of objectives 1 and 2 to develop an innovative approach to increasing PA levels in overweight and/or obese,

inactive females in order to promote meaningful improvements in metabolic health markers, appetite, eating behaviour and energy balance, as well as tackle specific barriers faced by this population, through the incorporation of time-efficient exercise. Objective 3 is split across chapter 6 (focusing on measures of EI, EE, resulting energy balance as well as fasting and postprandial subjective appetite and plasma levels of appetite and satiety-related hormones following a standardised meal) and chapter 7 (focusing on measures of VO_{2peak}, fasted insulin, glucose and lipid profiles as well as markers of affect, enjoyment and social cognitions of the intervention).

Objective 3 was met through the findings of both study 3a (Chapter 6) and 3b (Chapter 7). Study 3b (Chapter 7) demonstrated that, regardless of exercise timing, undertaking 4 x 30 seconds of "all out" intermittent star jumping, unsupervised, twice daily for three days per week across eight weeks improved cardiorespiratory fitness by a mean of 8% and reduced waist circumference by a mean of 1.3cm. However, no change in fasted nor postprandial glucose nor insulin were observed and there were no differences seen in other markers of metabolic health including fasted LDL-C, HDL-C, TC, TAG and HOMA-IR. Study 3a found no differences in body mass when high-intensity intermittent star jumping was undertaken twice daily on three days per week. There were also no differences in mean daily EI when exercise was undertaken within thirty minutes prior to a meal time compared with outside of one hour prior to a meal time. Mean daily EI on exercise days, however, was reduced by a mean of 426kcal a day compared with non-exercise days, although this was seen across both groups and was regardless of exercise timing. Postprandial subjective appetite increased following the intervention in those who had undertaken exercise within thirty minutes prior to meal times. Meanwhile, GLP-1 AUC concentration increased and postprandial subjective appetite decreased at 120 minutes following the standardised meal following the intervention in those undertaking exercise outside of one hour prior to a meal time. Additionally, study 3b found positive affective responses were seen post-exercise across the duration of the intervention, again regardless of exercise timing. Preferable enjoyment responses, however, were seen in weeks 6, 7 and 8 in the group undertaking exercise outside of 1 hour prior to meal times. Nonetheless, both groups deemed the intervention acceptable and had high intentions to continue. Together, the findings of both study 3a and 3b answered both aim 2 and objective 3.

8.3 Adapting low volume apparatus-free high-intensity intermittent exercise for optimal effectiveness

Findings of this programme of research show that meaningful acute responses and adaptations over 8 weeks can be achieved with HIIE and HIIT, respectively, without the requirement of apparatus. The findings of study 1 (Chapter 4) of this thesis demonstrated that similar physiological responses can be achieved with "all out" intermittent star jumping compared with traditional "all out" intermittent cycling, while study 3b showed that meaningful improvements in cardiorespiratory fitness and waist circumference are seen with eight weeks of "all out" star jumping, twice a day for three days per week. This aligns with recent systematic reviews and meta-analyses which demonstrate that HIIT is effective in inducing significant and meaningful improvements in cardiorespiratory fitness (Sloth *et al.*, 2013; Gist, Freese and Cureton, 2014; Weston *et al.*, 2014; Batacan *et al.*, 2017; Sultana *et al.*, 2019), and improvements in waist circumference (Wewege *et al.*, 2017). No changes in fasted nor postprandial glycaemic control and no changes in fasted cholesterol measures were seen in study 3b (Chapter 7), however, which is in contrast to many previous HIIT studies that have demonstrated improvements in insulin resistance in inactive and/or overweight/obese individuals (Musa *et al.*, 2009; Sandvei *et al.*, 2012; Jelleyman *et al.*, 2015; Stavrinou *et al.*, 2018).

In study 1 (Chapter 4) of this thesis, physiological responses immediately following the first 30-second interval of either "all out" cycling, star jumping or squatting did not appear to be a sufficient perturbation in metabolic stress on its own. Thus, it appeared single 30-second bouts of HIIE were unlikely to have the potential to induce the improvements in markers of metabolic health across an intervention. In turn, 4 x 30 seconds of "all out" star jumping, understandably, offered the largest perturbation in metabolic stress with an apparatus-free protocol, achieving 87±5% of HR_{max} and 7.3±2.4mmol·L^{-1} BLa upon cessation of the protocol compared with the smaller volumes of 1 x 30 and 2 x 30 seconds. That such improvements in cardiorespiratory fitness and waist circumference are achieved with 4 x 30 seconds of apparatus-free HIIT, twice a day on three days per week (Chapter 7) aligns with the posed time-efficiency of HIIT. Of note, across four weeks, reducing interval duration from 4-6 x 30 seconds to 4-6 x 15 seconds did not attenuate any improvements in cardiorespiratory fitness (Zelt *et al.*, 2014). However, findings by Nalçakan *et al.* (2018) suggest that reducing interval

duration from 2 x 20 seconds to 2 x 10 seconds did attenuate improvements in cardiorespiratory fitness over six weeks, although it is also noted that this also represents a lower training volume than of Zelt *et al.* (2014). Furthermore, a recent meta-analysis importantly demonstrates that improved cardiorespiratory fitness is not attenuated with fewer intervals (Vollaard, Metcalfe and Williams, 2017), although 1 x 20 seconds of "all out" cycling was insufficient to induce improvements in VO_{2max} over four weeks when performed on three days per week (Songsorn *et al.*, 2016), suggesting that the lower perturbation in metabolic stress seen with 1 x 30 seconds in study 1 (Chapter 4) would have been unlikely to induce the adaptations seen in study 3 (Chapter 7).

It is acknowledged that there were longer rest intervals within the 4 x 30 seconds of "all out" cycling compared with those of the star jumping and squatting protocols of study 1 (Chapter 4). Nonetheless, 4-6 x 10 seconds of high-intensity cycling induced similar improvements in aerobic and anaerobic performance regardless of whether the rest interval was of 2 minutes or 4 minutes duration (Hazell *et al.*, 2010). Furthermore, ranging rest intervals from 1-3 minutes between 6-10 x 2 minutes of high-intensity cycling over five weeks did not alter muscle adaptations nor VO_{2peak} improvements, despite modulating exercise-induced changes in metabolites and ions (Edge *et al.*, 2013). Therefore, the shorter rest interval of the "all out" star jumping protocol in study 1 (Chapter 4) is unlikely to have altered the adaptations at least seen in cardiorespiratory fitness when carried forwards into study 3b (Chapter 7) of this thesis. Furthermore, the perturbations in metabolic stress that were seen with 4 x 30 seconds of "all out" star jumping, despite its reduced rest intervals, only further promote its time efficiency as an exercise protocol.

It is not surprising that 4 x 30 seconds of "all out" star jumping elicited the greater EE compared with 2 x 30 seconds in study 2 (Chapter 5) and it is likely that increasing the number of intervals further would only induce a greater EE. Certainly, this is important within the context of energy balance and inducing a negative energy deficit for promoting effective reductions in body mass. Moreover, 4 x 30 seconds of "all out" star jumping induced a trend for a reduction of 121kcal in relative EI in study 2 (Chapter 5) of this thesis, although there were no differences in feeding latency nor subjective appetite following either 2 x 30 seconds or 4 x 30 seconds of "all out" star jumping compared with a resting control condition. Nonetheless, the reduction in relative EI following the 4 x 30 seconds condition was

considered meaningful, especially with such a low volume bout of apparatus-free HIIE. The magnitude of the reduction in relative EI was similar to that following both an acute moderate-intensity continuous cycling (-141±171kcal) and an acute HIIE cycling protocol (-87±174kcal) in overweight/obese individuals Martins *et al.* (2015) which is of interest given that both of these protocols induced a 250kcal EE. Given that a greater EE was achieved by Martins *et al.* (2015) compared with the EE of the exercise conditions in study 2 (Chapter 5) of this thesis, it is likely that the protocols of Martins *et al.* (2015) had only a small influence on absolute EI, whilst a greater time commitment would have been required to reach this EE compared with that of study 2.

A strength of study 2 (Chapter 5) was that participants were free to request to eat at any point they wished following the condition. Neither Beaulieu *et al.* (2015) nor Holliday and Blannin (2017a) demonstrated reductions in either EI or relative EI following 4 x 30 seconds of high-intensity running or cycling, respectively. However, both studies assessed such eating behaviour at pre-determined time points at least one hour following exercise which may not have been an optimal point of assessment, given the feeding latency and relative EI findings following 4 x 30 seconds of "all out" star jumping in study 2 (Chapter 5) of this thesis.

Given that 4 x 30 seconds of "all out" star jumping elicited the greatest physiological responses in studies 1 and 2 (Chapters 4 and 5, respectively), this was one contributing reason for this protocol to be carried forward to study 3 (Chapters 6 and 7). The findings of study 3 (Chapter 6), however, showed that such a low volume HIIT intervention did not induce changes in body mass, although did demonstrate improvements in waist circumference in both groups and in hip circumference in the 'anytime' group. Although this suggests that at least abdominal fat mass was lost (Clasey *et al.*, 1999), fat mass itself was not directly measured in study 3 (Chapters 6 and 7). Moreover, the lack of change in body mass in study 3 (Chapter 6) aligns with a recent systematic review where low volume HIIT as defined by Sultana *et al.* (2019), does not appear to induce improvements in total body fat mass, lean mass nor total body fat percentage, suggesting that low volume HIIT does not significantly modulate body mass nor body composition (Sultana *et al.*, 2019).

In turn, it has been noted that gait-based (or bodyweight-based) HIIT, as opposed to cycle ergometer-based HIIT, may be preferable in modulating body fat (Wewege *et al.*, 2017). This is likely a result of the larger volume of muscle fibres recruited with whole-body exercise

which would induce a greater EE for a given intensity. The significantly lower BLa following 4 x 30 seconds of “all out” star jumping compared with cycling in study 1 (Chapter 4) of this thesis, would align with the likely greater muscle mass recruited with star jumping given its “whole-body” nature, although as demonstrated this was not a sufficient intervention to demonstrate changes in body mass over eight weeks. Although fat mass was not assessed in the present study, it is likely that a larger weekly volume of HIIT than that of study 3 (Chapters 6 and 7) is required for improvements in body fat to be seen (Wewege *et al.*, 2017), such as a greater number of intervals that would induce a greater EE. Nonetheless, given the commonly-reported barriers to regular PA, as well as the posed negative affective responses of HIIE in inactive and/or overweight/obese populations (Biddle and Batterham, 2015; Hardcastle *et al.*, 2014), aims of this thesis included the acknowledgement of the affective and enjoyment responses to HIIE for inactive, overweight/obese individuals.

Findings in study 1 (Chapter 4) of this thesis showed that undertaking 4 x 30 seconds of “all out” star jumping did not induce changes in post-exercise affective response, however, post-exercise affective valence was greater than that immediately following 4 x 30 seconds of “all out” cycling. Furthermore, the “all out” cycling protocol also tended to be less enjoyable than either 4 x 30 seconds of “all out” squatting and “all out” star jumping in study 1 (Chapter 4). In study 2 (Chapter 5), positive affect was also significantly greater following both 4 x 30 seconds and 2 x 30 seconds of “all out” star jumping compared with a resting control condition. In turn, across both groups of study 3b (Chapter 7), post-exercise affective valence was increased from rest across the duration of the intervention while high levels of enjoyment were maintained across the intervention in the ‘anytime’ group. Importantly, previous studies have demonstrated that bodyweight interval exercise, which consisted of 5 x 2 minutes of bodyweight exercises (such as star jumps and tuck jumps) has been shown to be comparably enjoyable with moderate-intensity continuous exercise, while affective valence increased 20 minutes post-exercise compared with at rest (Greene, Greenlee and Petruzzello, 2018). However, it is acknowledged that participants in Greene, Greenlee and Petruzzello (2018) were recreationally active and mostly healthy weight as it is posed that affective responses to higher intensity exercise tend to decrease in overweight/obese individuals compared with healthy weight individuals (Ekkekakis and Lind, 2006; Ekkekakis, Lind and Vazou, 2009).

However, differences between the 'healthy BMI' and 'overweight/obese BMI' groups were not seen in affective nor enjoyment responses in study 1 (Chapter 4) of this thesis.

Nonetheless, as the number of intervals in low volume HIIE increase beyond 3-4, affective responses become less pleasant for participants classed as inactive compared with active counterparts (Frazão *et al.*, 2016). Therefore, increasing the number of intervals in the studies of this thesis may have induced less positive, or more negative affective and enjoyment responses. It is clear that it is possible to promote too many repetitions of intervals specifically in an inactive and/or overweight/obese population, which can induce negative affective responses (Frazão *et al.*, 2016). Astorino *et al.* (2019) demonstrated a consistent reduction in post-exercise affective valence during two HIIT interventions in an inactive, obese female population. Of note, more aversive affective and enjoyment responses were seen in bouts of HIIE that were of higher volume and where work intervals were of longer duration. Hence, authors again suggested that work intervals of shorter duration are preferable for this population (Astorino *et al.*, 2019). It should also be noted that Astorino *et al.* (2019), along with the majority of the discussed literature, used cycle ergometer-based interventions.

Given the differences in affective valence and enjoyment between cycle ergometer-based and apparatus-free HIIE in an inactive population demonstrated in study 1 of this thesis (Chapter 4), this highlights one of the novelties of this thesis, such that only 4% of HIIE protocols (2 out of 55) of the studies comprising the systematic review of Stork *et al.*, (2017) that have studied psychological responses to HIIE have been apparatus-free and/or bodyweight-based. This demonstrates a line of future research to urgently be further explored. Although, as discussed, a greater volume of HIIE and a greater number of intervals would induce a greater EE and may promote greater modulations in body fat (Wewege *et al.*, 2017), it is again important to consider such findings and discussions in the context of commonly-reported barriers to regular PA and the aims of this thesis included the acknowledgement of the affective and enjoyment responses to HIIE for inactive, overweight/obese individuals. It appears that fewer intervals are beneficial for affective responses, as well as improvements in cardiorespiratory fitness in this population (Vollaard, Metcalfe and Williams, 2017).

A common consideration in much of this literature, and the HIIT literature as a whole, is the apparent heterogeneity within HIIT parameters such as modality, interval duration and

number of intervals. It is evident that such heterogeneity could promote an extensive "list" of different modes and volumes of HIIE. However, at the same time this poses limitations with the generalisability of study findings to a free-living setting. A well-established "sub-section" of HIIT is occupied by SIT, which specifically (and traditionally) consists of 4-7 x 30 seconds of "all out" cycling (Weston *et al.*, 2014). It could therefore be argued that the protocols used in the studies of this thesis fit with the SIT model although, distinctly, not all protocols used within this thesis were cycle ergometer-based. Despite such heterogeneity between HIIE protocols and HIIT interventions and the potential combinations of manipulations of parameters of HIIE, viability of HIIE as an effective model of exercise is certainly emerging (Stork *et al.*, 2017). However, it is also firmly acknowledged that, inherently, with such heterogeneity across HIIE studies it is not possible to draw general conclusions about HIIE without considering the variability in exercise parameters and populations. Nonetheless, a recent meta-analysis has demonstrated that low volume HIIE bouts (≤5 minutes) consisting of intervals of ≤30 seconds duration are a time-efficient and effective strategy for improvement of cardiorespiratory fitness (Wen *et al.*, 2019). The findings of this thesis demonstrate that this is also the case, at least for cardiorespiratory fitness, when employing eight weeks of 4 x 30 seconds of "all out" apparatus-free, bodyweight-based star jumping in a free-living setting in inactive, overweight and obese females.

8.4 Is there ultimately a trade-off between time commitment to exercise and weight management?

Given the important physiological responses and health benefits seen over eight weeks with the 4 x 30 seconds of "all out" star jumping protocol and that it did not require any equipment or facility and only <10 minutes of time, this corroborates that low volume HIIE protocols can be time efficient. Factors (alongside the duration of time required for exercise) that have been demonstrated in determining such time efficiency primarily include markers of cardiorespiratory and metabolic health (Gillen and Gibala, 2014). For a given EE, exercise at a high-intensity favours a negative energy balance to a greater extent than that of a low to moderate-intensity (Tremblay, Simoneau and Bouchard, 1994) while what appears to be the greatest predictor of fat loss during an exercise intervention is, in fact, the EE of the exercise

that makes up the intervention (Deighton and Stensel, 2014). Given that a larger EE would require either a high-intensity or a greater duration of exercise, or both, it is to here that perhaps the posed time efficiency would not extend. This would essentially eliminate the "low volume" aspect of low volume HIIE, along with its associated time efficiency. This is a likely explanation as to why moderate-intensity continuous training is favoured for reduction of total body fat, compared with low volume HIIE (Keating *et al.*, 2017).

Similar to the findings of study 3 in this thesis (Chapter 6), multiple low volume HIIT interventions have demonstrated improved cardiorespiratory fitness amongst other markers of metabolic health, whilst seeing no improvements in fat mass nor body mass (Kong *et al.*, 2016; Phillips *et al.*, 2017; Ho *et al.*, 2018). Furthermore, the HIIT programme (~20 minutes duration) of Kong *et al.* (2016) was described as beneficial for maintenance of body mass, while reductions in body mass, fat mass and percentage body fat were seen only in a moderate-intensity continuous exercise programme. Importantly, the HIIT intervention used in study 3 of this thesis could have, similarly, prevented gain in body mass despite not inducing losses in body mass. However, although this is considered, it is somewhat difficult to determine with the absence of a non-exercising control group in study 3 of this thesis where a gain in body mass may have been demonstrated in an overweight/obese population in the absence of any exercise intervention and resulting perturbation in energy balance. Furthermore, it is noted that assessment of body composition itself would have shed further lights on any changes in body composition in study 3 of this thesis.

Importantly, programmes in Kong *et al.* (2016) were not controlled for EE, such that the moderate-intensity programme expended an estimated ~6244kcal across the five weeks whereas EE of the HIIT programme was an estimated ~3088kcal. Therefore, this corroborates that it is the magnitude of EE that is mostly responsible for the absence or presence of loss in body and/or fat mass, of which low volume HIIT alone is unlikely to achieve. Nonetheless, at this point it should also be considered that the EE of the hours and days following exercise should be assessed and included in calculating the total EE of an exercise bout, although it is acknowledged that this is difficult to assess and especially in a free-living setting. For example, post-exercise energy utilisation is increased immediately following repeated bouts of two minutes of moderate-intensity activity that interrupt sedentary time every thirty minutes, compared with not interrupting sedentary time at all (Fenemor *et al.*, 2018). Although there

was no evidence of raised excess post-exercise oxygen consumption following 4 x 30 seconds of high-intensity cycling compared with a resting control condition (Holliday, 2014, p. 195), the possibility of HIIE influencing non-exercise activity thermogenesis beyond the exercise bout should not be discounted in future studies, where this is possible to assess.

Despite an inherently small EE with low volume HIIE, study 3 of this thesis (Chapters 6 and 7) demonstrated a mean waist circumference reduction of ~1.3cm in both groups as well as a reduction of ~1.4cm in hip circumference (in the 'anytime' group only), all in the absence of change in body mass. Such findings are similar to those of Stavrinou *et al.* (2018) who demonstrated a ~1.4cm and a ~2.4cm reduction in waist circumference with 10 x 1 minute of high-intensity cycling either twice or three times per week, respectively, in inactive individuals. Therefore, it should be noted that modest improvements in body composition can occur in the absence of changes in body mass with HIIT interventions (Wewege *et al.*, 2017), while it has also been demonstrated by an additional recent meta-analysis that loss in total body mass does not always reflect modulations in visceral fat (Verheggen *et al.*, 2016). This is an important adaptation to consider in further determining the time efficiency and effectiveness of low volume HIIE. A reduction in waist circumference is a beneficial improvement as waist circumference can be seen as an accurate predictor of total abdominal fat and abdominal visceral fat (Clasey *et al.*, 1999) whilst increased trunk fat percentage is associated with a heightened risk of cardiovascular disease in females (Chen *et al.*, 2019). Thus, effects on visceral and abdominal fat mass should not be overlooked. However, the issue still remains that an EE greater than beyond what could be considered low volume HIIE is likely required for modulation in body mass itself.

Findings of the present studies agree that low volume HIIT is effective (and time efficient) for improving cardiorespiratory fitness and waist circumference, but inefficient for modulating body fat mass or body fat percentage (Sultana *et al.*, 2019); hence, unlikely having an effect on reducing overall body mass (unless lean mass is lost). Indeed, it is therefore perhaps unsurprising that current guidelines prescribe 200-300min·week^{-1} of moderate-intensity PA for long-term loss of body mass (Donnelly *et al.*, 2009). Thus, at present, despite the time efficiency of low volume HIIT for metabolic health, there appears to be a "trade off" between time commitment and inducing loss in body mass and body fat. With commonly-reported barriers to achieving these guidelines including a perceived lack of time (Trost *et al.*, 2002;

Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017) in particular, this still presents a number of public health issues.

While accepted that low volume HIIE has been shown to have minimal effect on body mass, the novel aspect of study 3 of this thesis was to strategically time low volume HIIE prior to meal times, given the tendency for a reduced relative EI following 4 x 30 seconds of "all out" star jumping in study 2 (Chapter 5) of this thesis. If such a strategy could potentially reduce energy intake, as well as modestly increase EE across a longer term, it was hypothesised that this dual effect of the exercise, on both EE and EI, may promote a meaningful energy deficit resulting in loss in body mass.

8.5 Does timing of low volume high-intensity intermittent exercise around meal times matter?

Given that in study 2 (Chapter 5) of this thesis there was a modest 121kcal reduction in relative EI ~30 minutes following 4 x 30 seconds of "all out" star jumping, it was hypothesised that exercising repeatedly in this "window" could lead to reductions in daily EI on exercise days. This rationale was further supported by the consensus that both dietary manipulation and exercise combined leads to greater body mass loss than caloric restriction alone, (Shaw *et al.*, 2006; Franz *et al.*, 2007) while exercise encourages the loss of fat mass and maintenance and promotion of lean mass which represent favourable changes in body composition (Parr, Coffey and Hawley, 2013).

Findings in study 3 (Chapter 6) of this thesis show that self-reported EI was reduced on exercise days (4 x 30 seconds "all out" star jumping, twice a day) compared with non-exercise days by ~420kcal per day. However, that this occurred in both the 'pre-meal' (exercise within 30 minutes prior to a meal time) and the 'anytime' group (exercise anytime outside of one hour prior to a meal time) demonstrates that timing exercise during the supposed transient period of appetite suppression following low volume HIIE does not influence eating behaviour or EI. It is possible that two differing manners were responsible for such similar EI responses between groups. Indeed, exercise within ~30 minutes prior to a meal may have reduced EI through a similar mechanism where there was a tendency for a reduction in relative EI in study 2 (Chapter 5). Nonetheless, although beyond the capacity of this thesis to measure, a

heightened satiety response upon feeding that lasts for beyond one hour following cessation of exercise could be responsible for such reductions in EI on exercise days in the 'anytime' group. This is partly supported by findings of study 2 (Chapter 5) too, whereby despite being inside of 1 hour following exercise and despite a tendency for a reduction in relative EI following 4 x 30 seconds of "all out" star jumping, there were no changes in subjective appetite from pre- to post-exercise with either 4 x 30 seconds or 2 x 30 seconds of "all out" star jumping, compared with a resting control condition. Such a reduction in relative EI may have occurred due to changes in satiety upon feeding rather than state of appetite prior to feeding, *per se*. Previously, when moderate-intensity exercise was performed in the postprandial state (two hours following a meal), compared with undertaking exercise in the fasted state one hour before a meal, exercise increased the satiety response to the meal although it is noted that this was 50 minutes of cycling at 60% VO_{2max} and so is physiologically different to the exercise modes employed in the present studies (Cheng *et al.*, 2009). It is therefore possible that performing low volume HIIE outside of one hour prior to a meal, but in the postprandial period of a previous meal (a plausible scenario for the 'anytime' group), also alternately reduced daily EI in the 'anytime' group by increasing the satiety response to the previous meal and perhaps reducing further EI in this group. Restrictions in the 'anytime' group did not extend to exercising within or outside a particular period following a meal.

Nonetheless, that there was a trend for mean daily EI on non-exercise days during week 8 to be greater than mean daily EI during baseline suggests that the difference in daily EI between exercise and non-exercise days may also be partly due to compensation on non-exercise days. This is in contrast with some previous findings that have demonstrated a delayed reduction in EI in inactive females, such that EI was reduced on the day following moderate-intensity exercise when compared with active participants (Rocha *et al.*, 2015). However, the findings of Rocha *et al.* (2015) were an acute occurrence and so the findings of study 3 in this thesis demonstrate that compensation may occur differently when monitored across repeated exercise days, and as a result of an intervention on both exercising and non-exercising days.

However, one means that may overcome such compensation in EI on exercise and non-exercise days may be to reduce the number of non-exercise days each week. Should this limit non-exercise day EI compensation, it would be of interest to explore whether this would induce any greater modulations in body mass than those seen in study 3 (Chapter 6) of this

thesis. Moreover, it may be that exercising every day in a way similar to that of study 3 in this thesis is required in order to eliminate compensatory EI responses on non-exercise days which again may give rise to meaningful reductions in body mass. Whether other compensatory responses in EE occur, or whether such a method would not induce such large modulations in EI are questions that must be answered. Nonetheless, exercising every day has been highlighted in the recent update of UK physical activity guidelines (Department of Health & Social Care, 2019). Due to monitor malfunctions and insufficient wear time adherence from participants during week 8 of study 3 (Chapter 6) of this thesis, it was not feasible to assess EE specifically on non-exercise and exercise days, however what effects exercising every day would have on daily EE and EI responses over the longer term is of interest to the current public health climate.

Moreover, in study 3 (Chapter 6) of this thesis, there appeared to be a heightened postprandial satiety response to the standardised breakfast meal (demonstrated by a greater GLP-1 AUC concentration) following the intervention, alongside a suppression in subjective appetite at 120 minutes following the meal, in the 'anytime' group only. This would align with previous findings that have demonstrated a greater satiety response to a standardised meal following an exercise intervention (Martins, Morgan and Truby, 2007; King *et al.*, 2009; Martins *et al.*, 2010; Caudwell *et al.*, 2013a). Meanwhile, in the 'pre-meal' group, no changes in appetite nor satiety hormones were observed, yet an increase in subjective appetite following the standardised breakfast was evident following the intervention.

Although such subjective appetite responses between groups in study 3 (Chapter 6) of the present study did evidently not appear to influence daily EI in a parallel manner between groups throughout the intervention, reasons for the discrepancies in subjective appetite responses to the standardised test meal post-intervention between the two groups are unclear. One possible explanation is related to the timing of food preparation around exercise in the "pre-meal" group who were likely required to plan and/or prepare meals ahead of undertaking the exercise. It has recently been demonstrated that participants served a greater energetic content of food when asked to serve themselves in anticipation of exercise (Sim, Lee and Cheon, 2018). It is noted that participants who demonstrated this in the study of Sim, Lee and Cheon (2018) were restrained eaters, whilst participants in this thesis were required to be classified as 'non-restrained eaters' (assessed using the DEBQ; van Strien *et al.*,

1986a; appendix 2). However, similarly, a greater EI was served following having read and thought about fatiguing exercise in a larger sample of 123 participants, compared with having listened to music and thought about exercise framed as "fun" (Werle, Wansink and Payne, 2011). It is noted, however, that dietary restraint was not assessed by Werle, Wansink and Payne (2011) and that EI response may have been confounded by how exercise is framed or perceived rather than the exercise session itself *per se*. Nonetheless, practically, participants in the 'pre-meal' group may have opted to, or could have had to, prepare or begin preparing the meal that would follow their bout of HIIE, before commencing exercise.

Participants in the 'pre-meal' group also perceived exercise as less enjoyable (and therefore possibly less fun) towards the end of the intervention. Hence, it is possible that this compensatory phenomenon seen in Sim, Lee and Cheon (2018) and Werle, Wansink and Payne (2011) occurred with participants of the 'pre-meal' group in study 3 (Chapter 6) of this thesis. Moreover, alongside possible greater occurrence of plate clearing tendencies in overweight and obese individuals (Robinson, Aveyard and Jebb, 2015), it could be that the originally prepared (compensatory) meal portion was consumed following exercise in the 'pre-meal' group regardless of either subjective or objective appetite sensations. It is plausible that some extent of desensitisation to appetite hormones occurred as a result of this, given the increased subjective appetite in the 'pre-meal' group following the intervention, without any accompanying changes in appetite or satiety hormones. Participants may have been eating to pre-determined portions, rather than to appetite and satiation at the time of eating, thereby overriding any hormonal signals to stop eating due to feeling full. This may explain the diverging responses in subjective and objective appetite responses in the 'pre-meal' group of study 3 (Chapter 6). Although this is speculating beyond the capacity of what this thesis assessed, such findings lead to other possible considerations and implications from the primary aims and objectives of this thesis.

At this point, it is noted that differences in eating behaviour are also likely to exist when assessed at a laboratory-prepared and presented *ad libitum* buffet and in a free-living setting in a population which already perceives a 'lack of time' for regular PA. Therefore, this should be considered with regards to practical implications of timing exercise around meal times in an inactive, overweight/obese female population. Indeed, with regards to optimising low volume HIIT in a free-living setting, it does not appear preferable to time exercise prior to

meal times in this way in this population, certainly for the regulation of energy balance within a weight management context. This is especially true given that remarkably similar effects on daily EI are seen in the group where timing in this way was not required, while enjoyment of the intervention in this group was preferable towards the end of the intervention.

Findings in study 3 (Chapter 7) of this thesis demonstrated that neither timing of exercise, nor the exercise intervention itself, induced differential responses in fasted TAG concentrations. However, benefits of exercise prior to feeding have been demonstrated at least for body fat regulation and lipid metabolism. For example, postprandial TAG response was 17% lower when one hour of moderate-intensity exercise was completed thirty minutes prior to a meal, compared with a resting control condition, while there was no significant reduction when exercise was completed thirty minutes following a meal (Farah and Gil, 2013). Although fat regulation was not assessed in the studies of this thesis, findings in study 3 (Chapter 6) of this thesis did demonstrate a trend for a reduction in proportion of calories from fat on exercise days compared with non-exercise days. However, that this response occurred across both groups again suggests that timing low volume HIIE in this way was not required to influence reductions in calories from fat intake.

Previous work has demonstrated that 6 x 1 minutes of high-intensity hill walking and/or resistance exercise undertaken thirty minutes prior to a meal reduced both 3-hour and 24-hour postprandial glucose concentrations compared with a moderate-intensity exercise condition (Francois *et al.*, 2014). It could therefore be hypothesised that repeated, low volume HIIE prior to meal times across the longer term may lead to beneficial metabolic health adaptations. However, no improvements were seen in fasted nor postprandial glucose nor insulin in either group of study 3 (Chapter 7) of this thesis. This suggests that 4 x 30 seconds of "all out" star jumping, twice a day for three days per week over eight weeks does not improve these markers of metabolic health, while timing exercise either within thirty minutes prior to a meal or outside of one hour prior to a meal has no additional effect. However, participants of the study by Francois *et al.*, (2014) were overweight/obese adults with insulin resistance, while markers of metabolic health in the populations of both groups in study 3 (Chapter 7) of this thesis were indicative of a reasonably healthy metabolism. This may have indeed been responsible for the lack of improvement in many of the markers of metabolic health in either group of study 3 (Chapter 7) of this thesis. Moreover, as the study

of Francois *et al.* (2014) employed a low volume HIIE protocol of a larger volume than that of study 3 in this thesis, it may be that a larger exercise stimulus is required to see improvements in these measures in a population similar to this, although further study is warranted. However, as discussed, the aims of this thesis were to utilise a time-efficient model of low volume, apparatus-free HIIE and consider commonly-reported barriers to regular PA which include a perceived lack of time (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017).

While, ultimately, a trade-off may exist between EE of exercise (and time commitment) and energy balance for weight management, it may be that a greater exercise stimulus (and EE) is also required for a population similar to that explored in this thesis to experience benefits to lipid, glucose and insulin regulation. Nonetheless, it was imperative to consider how optimising the timing of exercise for any benefits would be applicable, feasible and acceptable in a free-living setting in this population.

8.6 Optimising low volume high-intensity intermittent training for health in a free-living setting

The findings of this line of research provide some considerations for the optimal adoption of low volume HIIE in a free-living setting, which is important given the commonly-reported barriers to regular PA, as discussed. Timing low volume bouts of HIIE around meals did not appear important for manipulating daily EI, nor did it result in improved glycaemic control when assessed at baseline and post-intervention, as demonstrated in study 3 (Chapters 6 and 7) of this thesis.

Nonetheless, interestingly, interrupting prolonged sedentary time with any intensity of PA has been associated with a reduced waist circumference and BMI, as well as triglycerides and glucose concentrations (Healy *et al.*, 2008). Of note, the effects seen by Healy *et al.* (2008) were seen independent of both the total volume of sedentary time and total volume of MVPA, illustrating the importance of interrupting sedentary time alone for metabolic health. Furthermore, interrupting sedentary time every twenty minutes with two minutes of moderate-intensity activity following a meal reduces postprandial glucose, compared with uninterrupted sedentary time (Bailey *et al.*, 2017). Furthermore, a recent meta-analysis has

demonstrated that interrupting prolonged sedentary time with PA breaks attenuates postprandial glucose, insulin and TAG, while such postprandial glycaemic attenuation increases as BMI increases (Loh *et al.*, 2019). Hence, although no changes were seen in postprandial glycaemic control nor fasted TAG following the intervention in study 3 (Chapter 7) of this thesis, how interrupting prolonged sedentary time with low-volume HIIE, particularly if apparatus-free, given its feasibility and acceptability demonstrated in the findings of this thesis, may benefit postprandial metabolism following exercise bouts during an exercise programme in this population remains of interest. This would be of particular interest to study alongside accurate daily assessments of energy balance as it has been suggested that it may be the energy deficit as opposed to exercise *per se*, that is responsible for benefits to postprandial glycaemic control (Braun and Newman, 2019). Given the reduction in EI seen on exercise days in both exercise groups of study 3 (Chapter 6), it is therefore plausible that an energy deficit created by exercising in this way could induce postprandial metabolic benefits on such exercise days.

Although it appeared that percentage of sedentary time actually increased during week four in study 3 (Chapter 6) of this thesis, perhaps in place of reducing light-intensity activity, this did not seem to occur by week eight of the intervention. A dose response relationship exists between total sedentary time (above 7.5 hours·day^{-1}) and all-cause mortality (Ekelund *et al.*, 2019), while new UK PA guidelines promote minimising the total time spent sedentary and to break up prolonged bouts of sedentary time with any intensity of activity (Department of Health & Social Care, 2019), alongside the common recommendations to "sit less and move more" (Franklin, Brinks and Sternburgh, 2010; Piercy *et al.*, 2018).

Timing low volume bouts of HIIE around meals did not appear important for manipulating daily EI, nor did either group demonstrate any changes in dietary restraint, control of eating or food cravings, as demonstrated in study 3 (Chapter 6). However, interrupting sedentary time every hour with five minutes of moderate-intensity walking improved assessments of mood state and reduced food cravings assessed at the end of the testing day compared with uninterrupted sedentary time in inactive adults (Bergouignan *et al.*, 2016). Interestingly, the beneficial effects on mood and food cravings were not seen when one individual bout of thirty minutes of moderate-intensity walking was performed in the morning ahead of uninterrupted sedentary time, demonstrating the potential importance of interrupting sedentary time over

and above performing moderate-intensity activity, certainly for effects on mood state and food cravings. Of note, neither appetite nor EI were affected across two days of either prolonged sedentary time or interrupting sedentary time every thirty minutes with two minutes of walking in inactive adults (Mete *et al.*, 2018). However, that post-exercise energy utilisation is increased immediately following repeated bouts of two minutes of moderate-intensity activity that interrupt sedentary time every thirty minutes (Fenemor *et al.*, 2018) demonstrates that the post-exercise energy utilisation is important to consider in an energy balance context when exercise bouts are repeated across a day, although this is difficult to assess and was not able to be assessed in study 3 of this thesis. Nonetheless, whether accumulated bouts of low volume HIIE that could interrupt prolonged sedentary time across a day, could also lead to reductions in daily EI and possible increases in EE post-exercise is certainly of interest in the context of regulating energy balance and weight management in an inactive and overweight/obese population.

Certainly, the efficaciousness and effectiveness of "exercise snacking", as a novel approach to incorporating low volume HIIE into daily routine in a free-living setting, is gaining consideration in the literature. Interestingly, even when individual intervals of high-intensity cycling are separated by 1-4 hours of recovery, cardiorespiratory fitness is still improved to a similar extent (~4-6%) compared with when traditionally separating cycling intervals with a 3-minute rest interval (Little *et al.*, 2019). Similarly, cardiorespiratory fitness is modestly improved with high-intensity stair climbing when intervals are separated by 1-4 hours of recovery (Jenkins *et al.*, 2019). Therefore, this suggests that the time commitment for low volume HIIE can be further separated across the course of a day (whilst perhaps interrupting sedentary time) without any attenuation of effects on cardiorespiratory fitness, at least, which enhance its feasibility and effectiveness for some individuals with a lifestyle where a perceived lack of time is a barrier to regular PA. Furthermore, although it did not focus on nor include HIIT studies, a recent meta-analytic review concluded that accumulating exercise in multiple small bouts across a day induces benefits on cardiorespiratory fitness, lipids, insulin and glucose regulation similar to exercising continuously (Murphy *et al.*, 2019).

With apparatus-free HIIE in particular, this gives rationale for opening up low volume HIIE as 'incidental' PA that could be more practical to incorporate into daily living within such a lifestyle (Stamatakis *et al.*, 2019), given that PA guidelines in the UK have abandoned the

requirement for PA to be accumulated in bouts of at least 10 minutes (Stamatakis *et al.*, 2019; Department of Health & Social Care, 2019). Moreover, the new UK PA guidelines now acknowledge the inclusion of 'very vigorous-intensity activity' for health (Department of Health & Social Care, 2019). MET responses demonstrated a significant increase in %V-VIG when assessed during week eight of the intervention compared with baseline, across both groups in study 3 (Chapter 6) of this thesis. Such apparatus-free low volume HIIE seems to induce important benefits to health, as also demonstrated in study 3 (Chapter 7) of this thesis and appears to overcome many of the commonly-reported barriers to regular PA such as a perceived lack of time and a lack of access to apparatus and/or facilities (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017). Furthermore, considering these barriers it seems understandable to recommend low volume HIIE being whole body and/or bodyweight-based, especially as exercise requiring a greater proportion of muscle groups leads to a greater peak HR response to exercise (Karlsen *et al.*, 2017).

High-intensity functional training (HIFT) has recently been recognised as a mode of exercise training that "emphasises functional, multi-joint movements that can be modified to any fitness level and elicit greater muscle recruitment than more traditional exercise" (Feito *et al.*, 2019, p. 1). While the fairly new concept of HIFT typically varies in terms of type of exercises and duration of work and rest intervals, it does not tend to require apparatus (Feito *et al.*, 2019). Nonetheless, at present, HIFT studies have included 'CrossFit'-based activities of which some require specialist apparatus and facilities (Heinrich *et al.*, 2014; Feito *et al.*, 2018; Feito *et al.*, 2019) and therefore greater emphasis should be placed upon apparatus-free HIFT that does not require specialist facilities, based on the commonly-reported barriers to regular PA and the findings discussed in this thesis. Therefore, as high-intensity star jumping does require multi-joint movements and as blood markers confirm that it recruits large muscle groups (as demonstrated and discussed in study 1 (Chapter 4)), it could be that HIFT may provide similar benefits to metabolic health and energy balance in this population, although it is noted that a greater variety of additional functional, multi-joint high-intensity exercises would likely be required. Although research is lacking and although exercise is not always specifically characterised as HIFT as opposed to HIIT, possible exercises that could qualify also include "burpees" (Gist, Freese and Cureton, 2014), stair climbing (Allison *et al.*, 2017; Jenkins *et al.*, 2019), tuck jumps (Greene, Greenlee and Petruzzello, 2018), squat thrusts or static

sprints (Blackwell *et al.*, 2017). Nonetheless, future studies should explore alternative types and modes of HIFT that reach a sufficient intensity, are apparatus-free, elicit greater muscle recruitment than more traditional exercise and emphasise multi-joint movements without inducing aversive affective responses.

HIFT, specifically, has been shown to be effective in improving cardiorespiratory fitness by 7-17% (Buckley *et al.*, 2015; Cheema *et al.*, 2015), as well as insulin resistance (Nieuwoudt *et al.*, 2017), body fat percentage and systolic blood pressure in inactive, overweight adults over twelve weeks (Cheema *et al.*, 2015). Furthermore, HIIT that incorporates multimodal strength exercises also increased assessments of strength and muscle performance relative to a rowing-based HIIT programme over both six and twelve weeks (Buckley *et al.*, 2015; Brown *et al.*, 2018). This therefore highlights the potential benefits of including a functional and a strength focus in HIIT over and above an apparatus-based, non-weight-bearing mode of exercise such as rowing or cycling. Moreover, a greater proportion of participants intended to continue with HIFT compared with combined aerobic and resistance training, while enjoyment was maintained throughout the HIFT intervention compared with an aerobic and resistance exercise intervention (Heinrich *et al.*, 2014). Bodyweight-only strength exercise can reduce risk of all-cause mortality as well as mortality from cardiovascular disease and cancer to a similar extent to gym-based only strength exercise (Stamatakis *et al.*, 2018) while strength training is also as effective as moderate-intensity exercise for increasing cardiorespiratory fitness, metabolic health and aerobic work capacity (Schjerve *et al.*, 2008). Therefore, this gives strong rationale for promoting HIFT, as a development of HIIT, not only to improve cardiorespiratory fitness and metabolic health but also gains in strength and muscle performance which are often 'forgotten' in PA guidelines (Strain *et al.*, 2016). Moreover, activities to maintain or develop strength are again highlighted in the UK's most recent edition of PA guidelines (Department of Health & Social Care, 2019). HIFT can also be performed without the requirement of apparatus, while bettering exercise enjoyment and intentions to continue are all relevant to the commonly-reported barriers to regular PA in inactive individuals (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017). Certainly, benefits to lean muscle mass with HIFT would induce favourable modulations to body composition. As such, further research is required to explore the effects of HIIT and HIFT programmes on body mass and fat mass in overweight/obese individuals as

well as appetite, eating behaviour and energy balance. This is strongly warranted given the various benefits to health and the current climate of public health.

Given the commonly-reported barriers to regular PA, particularly for inactive and overweight/obese females, that include a perceived lack of time and a lack of access to apparatus and/or facilities (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017), there is strong rationale for apparatus-free low volume HIIT in a free-living setting. Nonetheless, a key consideration for optimising low volume HIIT for health in free-living setting includes increasing the variety and volume of options for an inactive population. As shown in study 3 of this thesis (Chapter 7), some individuals would have preferred to exercise in a group, rather than individually. Indeed, group-based, apparatus-free HIIT classes have also been demonstrated as effective in supporting autonomy, competence and relatedness such that the components of the self-determination theory are met to support behaviour change (Burn and Niven, 2019). Meanwhile, relatedness between group participants attenuated the negative affective responses associated with a cycle ergometer-based HIIT intervention in a workplace setting (Kinnafick *et al.*, 2018). Therefore, group-based HIIT should not be discounted, for its potential effectiveness in a free-living setting. Nonetheless, the option for HIIT to be home-based or free-living is still likely to overcome many of the commonly-reported barriers to regular PA.

Greater choice and flexibility with PA can induce greater autonomy support for inactive individuals (Beer *et al.*, 2017). Recent work by Holliday *et al.* (2018) demonstrated that a 'points-based' approach to physical activity, offering choice and flexibility in exercise mode and intensity, reduced body mass and fat mass in inactive, overweight/obese females to a greater extent than a structured moderate-intensity exercise programme. Moreover, the choice and flexibility that participants had in selecting exercise mode and intensity was hypothesised to contribute to the ~445kcal reduction in daily EI by the end of the intervention, given that both groups undertook similar volumes of PA per week across the twenty-four week intervention (Holliday *et al.*, 2018). Similar findings with mean daily EI did not occur in study 3 of this thesis (Chapter 6), although it is noted there was not a similar capacity for choice and flexibility compared with the programme of Holliday *et al.* (2018). Speculatively, a greater choice and flexibility within a HIIT programme, such as that in study 3 of this thesis, may therefore assist in contributing to a reduced mean daily EI.

Certainly, it is acknowledged that study 3 of this thesis provides a potential 'model' for an apparatus-free HIIT intervention, whereby further insight into other efficacious types and modes of apparatus-free HIIE would likely add choice and flexibility. In turn, this would likely avoid tedium and boredom which would likely otherwise occur in interventions of a longer duration that consist of one mode of exercise only. Either way, future apparatus-free low volume HIIT studies should allow for a greater choice and flexibility with options for HIIT which should include options for HIFT too, as previously discussed, as this is likely to be of great importance for adherence especially when investigated beyond the duration that this study employed.

Should future research explore HIIT or HIFT programmes with greater choice and flexibility for all parameters of exercise beyond the typical eight to twelve weeks that the majority of the discussed literature employs, contributions of "spill over" effects as a result of a greater consciousness for health-related behaviours should be increasingly considered. In the 'points-based' model of PA accumulation by Holliday *et al.* (2018), it was hypothesised that a prolonged consciousness for health-related behaviours was present as a by-product of encouraging PA accumulation throughout a day, leading to a "spill-over" effect of promoting another healthy behaviour: reducing energy and fat intake. Of note, encouraging the accumulation of HIIT or HIFT across a day would also allow for interruption of prolonged sedentary time which, as previously discussed, is of importance to metabolic health for inactive, overweight/obese individuals. Thus, it is also possible that encouraging low volume, apparatus-free HIIT in inactive, overweight females may itself act as a "gateway" or "stepping stone" behaviour to raise consciousness for health-related behaviours, such as a furthered increase in PA and/or an improved cardiorespiratory fitness (as demonstrated in study 3 of this thesis) for further PA.

Whilst, as discussed, it is moderate-intensity continuous exercise that is likely to induce a greater EE and energy deficit than low volume HIIE, it should not have to be the case where by this happens in isolation of HIIE. Following the possible "gateway" that low volume HIIT may provide, low volume HIIE could be used as an adjunct within a moderate-intensity continuous exercise bout. This would, theoretically, increase EE whilst also possibly inducing reductions in EI and/or relative EI as seen in findings of this thesis.

Furthermore, reframing individuals' perceptions of a 'lack of time' for exercise reduced exercise-related cognitive errors (that were leading to a negative, distorted view of PA and behaviour change) and induced greater PA levels (Locke, McKay and Jung, 2019). Therefore, given that a perceived lack of time is a common barrier for an inactive, overweight/obese female population, interventions that include reframing of such biased thoughts towards exercise, alongside a time-efficient, acceptable and feasible HIIT intervention may increase an individual's capacity to be more physically active, which future studies should consider.

Based on the findings of this thesis, timing of exercise around meal times *per se*, for the purpose of manipulating EI and energy balance is not required. Nonetheless, this means that, with a focus of promoting energy deficit in the context of weight management, exercise can be undertaken at a time that suits the individual. Although beyond the capacity of this thesis, it is posed that greater choice, flexibility and enjoyment of HIIT could be obtained, with future work. If the reduction in EI seen on exercise days in study 3 of this thesis (Chapter 6) could be maintained over a longer duration than within this thesis, this would likely encourage maintenance or gain of lean mass, alongside loss in fat mass, and thus favourable modulations in body composition. Therefore, this could promote a time efficient, apparatus-free PA strategy that is efficacious and effective in promoting favourable modulations in metabolic health, energy balance and body composition in an inactive, overweight/obese population.

8.8 Limitations

The findings throughout this thesis should be considered with acknowledgement of existing limitations. Although participants were female and inactive, which should be credited as it thereby provided a relevant population to study, sample sizes were small due to difficulties with recruitment within the allocated resources with which to complete this thesis. As a result, this increased the risk of type II error and likely meant that findings were more vulnerable to individual variability within some measures. Therefore, it is likely that some measures were underpowered to detect significant differences in all studies (in particular study 3) of this thesis.

Furthermore, difficulties with recruitment resulted in the lack of a non-exercise control group for study 3 (Chapters 6 and 7). This results in difficulty in concluding whether findings are attributable to either exercise intervention alone, or to other unrelated behaviour changes that may have occurred. Randomised controlled trial designs are, undoubtedly, the most preferable option for attenuating this limitation. Hence, some conclusions of the studies in this thesis may differ with greater sample sizes and/or the addition of a non-exercising control group. Additionally, a greater variety in types of exercise incorporated into the intervention would have been preferable, although it was beyond the scope and capacity of this thesis to explore further modes of exercise in study 1 of this thesis and did not seem appropriate to introduce new, unjustified modes of exercise during study 3. It should be noted that the model of "all out" star jumping used throughout this thesis was indeed a model for the purpose of scientific investigation. For the deployment of an intervention, other exercises would be incorporated.

It is likely, however, that a greater number of researchers, alongside greater capacity for laboratory time and resource, a larger timescale and greater financial resource would counteract many of these addressed limitations and, in turn, enhance the quality of findings of future studies. Otherwise, future studies should be powered appropriately to allow for the dropout rates observed in studies of this thesis, although difficulty in primarily recruiting a sufficient number of eligible participants is not underestimated. Furthermore, novel approaches which enhance recruitment in this population should be sought to allow for larger sample sizes and better-powered studies in this area.

A strength of all studies in this thesis was that stage of each individual's menstrual cycle was controlled for as best as possible, although it is noted that assessing concentrations of female hormones such as oestrogen and progesterone in each individual would have strengthened the study, in order to confirm stage of the menstrual cycle. However, this means that findings that occurred during this stage of the menstrual cycle are applicable only specifically to this stage of cycle. Limiting experimental trials to particular calendar windows also added difficulty to recruitment and adherence to study participation, which likely attributed to the dropout rates seen in all studies of this thesis. However, females were not excluded on the basis of contraceptive method nor screened for premenstrual symptoms (unless either resulted in an irregular menstrual cycle). Again, this was so as to not reduce the pool of

possible participants even further. However, it should be noted that women with premenstrual syndrome can experience greater food cravings during the luteal phase (Dye and Blundell, 1997), although assessments of EI were endeavoured to be taken during the follicular phase of the menstrual cycle for all studies in this thesis. Moreover, daily EI has been demonstrated to be greater in inactive females using oral contraceptives compared with inactive females not using oral contraceptives (Rocha *et al.*, 2018). However, in both study 2 (Chapter 5) and study 3 (Chapters 6 and 7) of this thesis, only 1 participant out of the 12 and 15 participants, respectively, were using oral contraceptives. Therefore, this was unlikely to have influenced overall EI, whilst assessment of EI was also undertaken within-subject in study 2. Nonetheless, given its potential to influence EI and also to reduce pools of eligible participants, future study should further clarify whether contraceptive method (or lack of) is likely to meaningfully influence appetite and eating behaviour assessments in a between-subject manner.

Studying human behaviour in a free-living setting, which was an aim of this thesis, creates a limitation whereby behaviours cannot be controlled as they can in a supervised setting. Although this should be considered as a strength in study 3, thereby exploring both efficaciousness and effectiveness, it does give rise to a greater influence of other uncontrolled, environmental and behavioural factors, while the reliance on self-reported behaviours should be considered alongside the findings. It is also acknowledged that eight weeks is, relatively, of fairly short duration. Given the high intentions to continue with the exercise intervention in study 3 (Chapter 7), a follow-up assessment of participants would give insight into the longer term effectiveness of the exercise intervention and any behaviour change in response to such intentions; this represents a possible avenue of future research amongst others.

8.7 Practical applications

The UK's most recent edition of PA guidelines now recognise that, although greater health benefits are achieved as PA levels increase, benefits are achieved at levels below the 150 minutes and 75 minutes of moderate-intensity and vigorous-intensity PA thresholds, respectively (Department of Health & Social Care, 2019). Moreover, these guidelines state

that PA bouts are no longer required to reach a 10 minute duration as well as promote the notion that some activity is better than no activity (Department of Health & Social Care, 2019). Although low volume HIIE protocols and low volume HIIT interventions such as the intervention used in study 3 (Chapters 6 and 7) of this thesis do not meet the recommended levels of weekly PA in these guidelines, findings of study 3 demonstrate important health benefits for an inactive population and therefore support this amendment in most recent guidelines. The potential practical application of the findings of this thesis are further underlined by the recognition of HIIT as a PA strategy in the new guidelines (Department of Health & Social Care, 2019). Moreover, as the interruption of prolonged sedentary time with PA is also promoted in these guidelines, encouraging the accumulation of low volume HIIT across a day would also allow for this which, as previously discussed, is of importance to metabolic health for inactive, overweight/obese individuals.

It is important that PA interventions are efficacious in inducing health benefits such as those demonstrated in study 3 (Chapters 6 and 7) of this thesis are also practically achievable by approaching commonly-reported barriers to regular PA, such as a perceived lack of time (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017), a lack of access to apparatus or facilities as well as limited access to apparatus at home (Trost *et al.*, 2002; Cerin *et al.*, 2010). That the developed HIIT intervention utilised in study 3 (Chapters 6 and 7) of this thesis was low volume and thus required a minimal time commitment (<10 minutes per session) alongside having no requirement for particular apparatus nor facilities again strengthens the practical application of the findings of this thesis. Although it is not discounted that such health benefits may have been greater if the intervention was supervised (Wen and Ang, 2019), it is important to note that they were seen in unsupervised and free-living conditions which thereby improves effectiveness and practical application of the findings. Moreover, that affective responses were positive in response to high-intensity star jumping across all studies of this thesis may benefit longer term adherence (Williams *et al.*, 2008; Kwan and Bryan, 2010a), further demonstrating potential effectiveness and practical application of the findings of this thesis.

It is likely that greater health benefits exist with a greater volume of PA than those demonstrated in this thesis, especially if meaningful reductions in body mass are the aim. However, findings of this thesis show that benefits can be observed with low volume HIIE with

little require of time nor apparatus, which are commonly reported as barriers to regular PA in inactive individuals (Trost *et al.*, 2002; Salmon *et al.*, 2003; Cerin *et al.*, 2010; Burgess, Hassmén and Pumpa, 2017). Therefore, apparatus-free, low volume HIIE is a viable option for public health, especially given its demonstrated effectiveness in a free-living setting in the findings of this thesis.

8.9 Future research

Although the initial focus of this thesis was solely appetite and energy balance-focused, its findings alongside the current public health climate has led to other, additional considerations throughout. Certainly, further study on manipulating the exercise parameters of apparatus-free HIIT for its optimisation in an inactive, overweight/obese population, whilst considering the commonly-reported barriers to regular PA, is warranted for the optimisation of low volume HIIT for health in a free-living setting. Following this, exploring the effects of home-based (or, at least, in a free-living setting) HIIT programmes that incorporate an optimised choice and flexibility with regards to exercise timing and mode of exercise on metabolic health, specifically in inactive populations, is warranted. How accumulation of bodyweight-based HIIT or HIFT across a day influences appetite, eating behaviour (on exercise days as well as overall behaviour), energy balance, body composition and weight management is also warranted. Moreover, how exercising in this way can interrupt prolonged sedentary behaviour, influence postprandial metabolism and raise consciousness for additional health-related behaviours is also merited across a longer duration. The twenty four weeks duration of the study by Holliday *et al.* (2018) where a raised consciousness for health behaviours was speculated saw greater reductions in fat mass than a moderate-intensity exercise intervention of equivalent duration, although losses in lean mass were also observed. Authors suggested that the manipulation of accumulated PA to include some activity specifically of a high-intensity would likely encourage the maintenance of lean mass. Therefore, another option for future research could consist of a similar 'points-based' approach to HIIT, which could also include specific strength-based (HIFT) options, as discussed.

Given the initial focus of this thesis and the findings obtained, a greater insight into the timing of low volume HIIT around meal times and its effects on appetite/satiety hormones and actual

eating behaviours should also be a research priority. Indeed, whether timing a meal within the supposed appetite suppression window following low volume HIIE has contrary effects to appetite control and eating behaviour to those originally hypothesised in this thesis requires critical attention. In particular, assessing whether exercising prior to planning or preparing a meal, rather than simply prior to eating a meal that may have already been planned or prepared is of interest. However, it is imperative that this is still explored in a free-living setting and in a relevant population to allow for effectiveness as well as efficaciousness to be examined. Indeed, although beyond the capacity of this thesis, the additional introduction of qualitative methodology should be considered as, previously, such a multidisciplinary approach allowed for further insight into participants' attitudes and views of an exercise intervention (Gram *et al.*, 2014).

In study 3 (Chapter 7), 9/15 participants disagreed that they would have preferred to exercise in a group, while barriers exist to females in joining exercise classes or groups (Withall, Jago and Fox, 2011). However, as receiving positive feedback whilst engaging in cycle ergometer-based HIIE improves enjoyment responses (Tritter *et al.*, 2013), this gives rationale to provide some basis or source of positive feedback with regard to bettering enjoyment responses for adherence. It may be that a web-based social networking platform as a source of support and feedback for exercisers is an effective adjunct to a free-living HIIT programme. It would also be of additional interest to explore the prospect of increasing exercise workload as an intervention progresses (Karlsen *et al.*, 2017) and if (and how) this influences affective and enjoyment responses.

Furthermore, despite the tendency for a lack of exercise research specifically in females (Costello, Bieuzen and Bleakley, 2014), such branches for future research should be explored in males as well as females. Although, at present, there is no evidence to suggest sex differences in appetite and eating behaviour responses to exercise (Thackray *et al.*, 2016; Dorling *et al.*, 2018), more well-controlled studies are required. Furthermore, studies with non-exercise control groups and those that study greater sample sizes across a longer duration than that typically explored in intervention studies are urgently required for this line of research. However, the difficulty in doing so with limited resources whilst working in free-living settings is not underestimated and certainly contributed to the limitations of the studies of this thesis.

8.10 Conclusion

In conclusion, this thesis provides a novel insight into the effects of apparatus-free HIIE and HIIT on appetite, EI, energy balance, weight management and metabolic health in inactive, overweight and obese females. Given the rising levels of physical inactivity and overweight/obesity, along with commonly-reported barriers to regular PA, there is growing interest in strategies to effectively increase PA and regulate energy balance for effective weight management.

Specifically, the main findings that this thesis presents are:

- Comparable physiological responses to "all out" cycling can be achieved with apparatus-free HIIE such as "all out" star jumping;
- Preferable affective and enjoyment responses can be achieved with apparatus-free HIIE compared with "all out" cycling;
- No changes in subjective appetite were observed following differing doses of "all out" star jumping, however there was a tendency for a meaningful reduction in relative EI at a meal ~30 minutes following exercise compared with the resting control condition;
- Undertaking "all out" star jumping within thirty minutes prior to a meal twice a day for three days over eight weeks reduced daily EI compared with non-exercise days to the same extent as undertaking "all out" star jumping outside of one hour prior to meal times;
- Despite daily EI on exercise days being lower than non-exercise days in both groups, it was not lower compared with daily EI on baseline days and no modulations in body mass were observed were found in either group following the intervention;
- The postprandial GLP-1 response and subjective appetite response to a meal was improved following the exercise intervention in the group performing "all out" star jumping outside of one hour prior to a meal time;
- The postprandial subjective appetite response was increased following the intervention in the group undertaking "all out" star jumping within thirty minutes prior to a meal, although no accompanying changes in appetite nor satiety hormones were observed in this group;

- Regardless of exercise timing, undertaking “all out” star jumping twice a day for three days over eight weeks improved cardiorespiratory fitness and reduced waist circumference, whilst hip circumference was reduced in those undertaking exercise outside of one hour prior to meal times;
- Undertaking “all out” star jumping within thirty minutes prior to a meal twice a day for three days over eight weeks reduces enjoyment of exercise towards the end of the intervention, although this did not seem to influence acceptability of, nor intentions to continue with, the exercise intervention;
- Acceptability of, and intentions to continue with, the intervention were strong in both groups of inactive, overweight/obese females.

This thesis contributes to the knowledge base for strategies to effectively increase PA and regulate energy balance for effective weight management in inactive, overweight/obese females, but further research and insight into how these strategies can be further optimised for improved weight management in this population is required.

8.11 Personal reflections

This thesis represents both a journey in my personal development as well as a fluid journey of research questions and ideas. I wanted this thesis to constitute a progressive body of work and that each individual experimental chapter is informed by the previous chapter is something that I am very proud of. Working with an inactive, overweight/obese female population was one of my favourite parts of this journey as it was during these times that I really discovered both the rationale and the practical application of my research (which had both positive and negative applications). Although recruitment of this population was challenging, I would still choose to study further research questions in this population for this reason and also to reduce the underrepresentation of this population in sport and exercise medicine research. However, I would endeavour to increase the amount of time that was available for recruitment by starting this process as soon as possible into the research journey. In turn, I have identified effective techniques and strategies for reaching potential pools of this population such as the use of social media and community groups. I have also developed skills in ensuring a good rapport is built with potential participants to gain interest and trust

from a population underrepresented in sport and exercise research, to ultimately improve participant recruitment and retention as best as possible. In future work, I would endeavour to continue to implement these techniques as soon as possible, in order to ensure that studying in this population is not compromised by an insufficient amount of time to produce a well-powered and well-controlled study.

Furthermore, although I believe that I started this journey with a positivist epistemological approach (Comte, 1974), it is without doubt that I gained appreciation in sharing this approach with critical realism (Bhaskar, 1975). In line with wishing to assess and demonstrate effectiveness as well as efficacy, I was directed by objective realities and interpretations that much of this thesis is based upon, however, I also learnt that positivist reasoning alone cannot be used to completely understand life science-based research aims and objectives. In working closely with an inactive, overweight/obese and female population, the complexity of such life-science based research aims and objectives were made clear and it is here that I realised the potential addition that qualitative research methodologies such as semi-structured interviews and the value of interdisciplinary research could offer. These techniques are something that I would wish to increase my competence in in the future, to allow for a deeper insight and understanding into the practical application and effectiveness of PA strategies in this population with more of a critical realist approach.

The journey of this thesis also represents a multitude of lessons learned which range from practical to personal. I have gained competence in skills ranging from bloodletting, ELISA analysis as well as a large range of laboratory techniques, to recruitment and community engagement techniques, to statistical techniques as well as making personal developments that only through persistence throughout this thesis have I discovered. I take pride in that I was responsible for each stage of the research journey and process, including shaping research questions, designing studies, recruiting participants, data collection and analysis, as well as producing this resultant thesis. It is now, upon reflection, that I feel sufficiently experienced, prepared and skilled to lead similar research projects independently. Alongside the original contributions to knowledge that this thesis provides, reflecting on the journey of this thesis demonstrates to me my earning of a license to become an independent researcher.

“Every new beginning comes from some other beginning's end” – Seneca

8.12 References

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APPENDICES

Appendix 1: International Physical Activity Questionnaire (IPAQ; Craig et al., 2003)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ ***Yes***

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

This is the end of the questionnaire, thank you for participating

Appendix 2: Dutch Eating Behaviour Questionnaire (DEBQ; van Strien et al., 1986a)

Please answer ALL questions below by circling the appropriate response for each question. Thank you!

When you have put on weight do you eat less than you usually do?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you try to eat less at mealtimes than you would like to eat?		**never**	**seldom**	**sometimes**	**often**	**very often**
How often do you refuse food or drink offered to you because you are concerned about your weight?		**never**	**seldom**	**sometimes**	**often**	**very often**
Do you watch exactly what you eat?		**never**	**seldom**	**sometimes**	**often**	**very often**
Do you deliberately eat foods that are slimming?		**never**	**seldom**	**sometimes**	**often**	**very often**
When you have eaten too much, do you eat less than usual the following day?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you deliberately eat less in order not to become heavier?		**never**	**seldom**	**sometimes**	**often**	**very often**
How often do you try not to eat between meals because you are watching your weight?		**never**	**seldom**	**sometimes**	**often**	**very often**
How often in the evenings do you try not to eat because you are watching your weight?		**never**	**seldom**	**sometimes**	**often**	**very often**
Do you take your weight into account with what you eat?		**never**	**seldom**	**sometimes**	**often**	**very often**

If food tastes good to you, do you eat more than usual?		**never**	**seldom**	**sometimes**	**often**	**very often**
If food smells good, do you eat more than usual?		**never**	**seldom**	**sometimes**	**often**	**very often**
If you smell something delicious, do you have a desire to eat it?		**never**	**seldom**	**sometimes**	**often**	**very often**
If you have something delicious to eat, do you eat it straight away?		**never**	**seldom**	**sometimes**	**often**	**very often**
If you walk past a baker, do you have a desire to buy something delicious?		**never**	**seldom**	**sometimes**	**often**	**very often**
If you walk past a snackbar or café, do you have a desire to		**never**	**seldom**	**sometimes**	**often**	**very often**

buy something delicious?						
If you see others eating, do you also have a desire to eat?		**never**	**seldom**	**sometimes**	**often**	**very often**
Can you resist eating delicious foods?		**never**	**seldom**	**sometimes**	**often**	**very often**
Do you eat more than usual, when you see others eating?		**never**	**seldom**	**sometimes**	**often**	**very often**
When preparing a meal, are you inclined to eat something?		**never**	**seldom**	**sometimes**	**often**	**very often**

Do you have a desire to eat when you are irritated?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you have nothing to do?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you are depressed or discouraged?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you are feeling lonely?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you somebody lets you down?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**

Do you have a desire to eat when you are cross?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you are something unpleasant is about to happen?		**never**	**seldom**	**sometimes**	**often**	**very often**
Do you get the desire to eat when you are anxious, worried or tense?		**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when things are going against you and when things have gone wrong?		**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you are frightened?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you are disappointed?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you are emotionally upset?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**
Do you have a desire to eat when you are bored or restless?	**not relevant**	**never**	**seldom**	**sometimes**	**often**	**very often**

Appendix 3: Study 1 participant information sheet

University of Worcester

Participant Information Sheet

Alice Burgin

a.burgin@worc.ac.uk

Name of Study:	Acute physiological responses to high-intensity gym and non-gym-based exercise

You are invited to take part in a research study. Before you decide whether to volunteer, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

Please do not hesitate to contact us (see below) if there is anything that is not clear, if you would like more information or if you wish to take part in the study.

Contacts for Study

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What is the purpose of this study?

High-intensity interval exercise is recently becoming a more popular mode of training based on its time-efficiency compared with traditional, continuous and moderate-intensity exercise. However, the time-efficiency becomes questionable if a time-consuming visit to the gym is required in order to undertake this training and so we are looking to investigate some non-gym based bouts of high-intensity interval exercise that may make this type of training more accessible and time-efficient, and whether similar responses occur in the body to previously studied gym-based bouts of high-intensity interval exercise. The purpose of this study is therefore to investigate the physiological responses to two non-gym-based bouts of high-intensity interval exercise (squats and star jumps) compared with a bout of gym-based high-intensity interval cycling. You will undertake a different 4x30-second bout of each of the following: squats, star jumps and paced maximal intervals on an exercise bike, on separate occasions in the laboratory.

Why have I been chosen?

You have been chosen to take part in this study because you have expressed an interest and your physical activity levels and BMI meet our inclusion criteria.

The **exclusion** criteria for this study are listed below:

- Any musculoskeletal, metabolic or cardiovascular disorders; including pain and/or swelling in your hips or knees
- Dieting or intent to diet
- Blood pressure > 140/90mmHg
- Any medication that may influence appetite
- Smoking
- Pregnancy or breast feeding

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and read and then be asked to sign a consent form if you are still happy to participate. Even after you have consented, you are free to withdraw at any time and without giving a reason. If you decide to withdraw from the study your data will be destroyed immediately. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

On your first visit to the laboratory, if you are still happy to partake in the study you will undergo relevant health screening procedures. You will also have your height and weight measured and data on your age and physical activity levels will be collected. You will undergo an incremental exercise test on an exercise bike and try out one bout of the gym and non-gym-based exercise modes to be undertaken on the following visits. This first visit to the laboratory will last approximately 2 hours. On each visit to the laboratory following this, each exercise bout will last around 8 minutes and will consist of 4x30 seconds of either: stand-to-sit squat exercises, star jumps or cycling intervals at maximal intensity (90 seconds recovery between each interval). Identical physiological, anthropometric and cognitive measures will be taken before and after each exercise/rest bout. Each testing visit following your first visit will last around 3 hours.

What specifically do I have to do?

The study will comprise of four visits to the physiology laboratory at the University of Worcester. On your first visit you will be given information regarding the study, undergo screening questionnaires,

try out some of the exercise bouts, an incremental fitness test on an exercise bike and sign informed consent for acceptance into the study.

Each of the next 3 visits will last around 3 hours. On each visit, you will be asked to arrive after an overnight fast but will be provided with breakfast within 15 minutes of arrival. After a 2 hour period you will undertake one of the following conditions: 4 x 30 seconds of squats, 4 x 30 seconds of star jumps, 4 x 30 seconds of intervals on an exercise bike with 90 seconds of recovery time between each 30-second interval. This will take around 8 minutes total. You will then be encouraged to slowly walk around the laboratory for a short period of time to help alleviate pain and soreness in the 24-48 hours following exercise, which is totally normal You will then rest for a further 30 minutes before you are free to leave the laboratory.

Your weight will be measured upon arrival to the laboratory for each visit. Five finger prick blood samples will be taken during each visit too, but these will cause only mild, short-lived pain. Other, non-invasive, measures such as your heart rate, perceived exertion, and mood affect will also be taken at three points across each testing session too. All visits will follow an identical protocol of measures.

What will happen to the samples I give?

Whole blood collected will be immediately analysed and then disposed of. There will be no storage of blood or cellular tissues.

What will happen to the other data collected from me?

You will not be identifiable from the data we record and your anonymity will be guaranteed in any publication from this study. Only your consent form will have a record of your name; the rest of the documents will be given a code to anonymise your responses, and will be stored separately from your consent form. All of the data will be held in locked cabinets, in locked offices and/or on password protected computers/memory sticks. All data will be stored for a maximum of 10 years (in line with University policy), after which they will be destroyed.

Expenses and payments

A contribution of up to £20 can be made for your total travel expenses for all visits to the laboratory.

What are the possible disadvantages and risks of taking part in this study?

The exercise performed is of a high-intensity and so you are likely to experience some discomfort, although this will be short-term and health screening procedures will be carried out beforehand to reduce the risk of injury or medical complications and twenty-four hour first aid will always be accessible. A short warm-down walking period will be encouraged to aid in alleviating subsequent pain and soreness in the 24-48 hours post-exercise. You may still experience some pain and soreness in the 24-48 hours following exercise, but this is totally normal. Finger prick blood samples involve a sharp, sterile lancet being inserted through the skin on the fingertip. This may cause mild, short-lived pain and may result in some small, minor bruising.

What are the possible benefits of taking part in this study?

This study is not a training intervention but it will enable you to try out a type of exercise that you have not tried before which may be something you wish to adopt as a regular activity to increase physical activity levels in a time-efficient manner.

What if there is a problem?

If you have any complaint about the way you have been dealt with during the study or any concerns about possible harm you might suffer, you may contact Dr John-Paul Wilson (Director of Research) on (01905) 54 2196.

Who has reviewed this study?

This study has been reviewed and approved by the University of Worcester Institute of Sport and Exercise Science Ethics Committee.

If you are interested in being a participant in this research study, please email a.burgin@worc.ac.uk to arrange a visit to the research laboratory.

Appendix 4: Study 2 participant information sheet

University of Worcester

Participant Information Sheet (12th December 2016, v1)

Name of Study: Physiological responses to high-intensity non-gym-based exercise

You are invited to take part in a research study. Before you decide whether to volunteer, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

Please do not hesitate to contact us (see below) if there is anything that is not clear, if you would like more information or if you wish to take part in the study.

Contact for Study

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What is the purpose of this study?

High-intensity interval exercise is recently becoming a more popular mode of training based on its time-efficiency compared with traditional, continuous and moderate-intensity exercise. However, the time-efficiency becomes questionable if a time-consuming visit to the gym is required in order to undertake this training and so we are looking to investigate a non-gym based bout of high-intensity interval exercise that may make this type of training more accessible and time-efficient, and whether similar responses occur in the body across the few hours afterwards, to previously studied gym-based bouts of high-intensity interval exercise. The purpose of this study is therefore to investigate the physiological responses to a non-gym-based bout of high-intensity interval exercise (star jumps). You will undertake either 2x30 seconds, 4x30 seconds or 6x30 seconds of star jumps or no exercise, on separate occasions in the laboratory.

Why have I been chosen?

You have been chosen to take part in this study because you have expressed an interest and your physical activity levels and BMI meet our inclusion criteria.

The **exclusion** criteria for this study are listed below:

- Any musculoskeletal, metabolic or cardiovascular disorders; including pain and/or swelling in your hips or knees
- Dieting or intent to diet
- Blood pressure > 140/90mmHg
- Any medication that may influence appetite
- Smoking
- Pregnancy or breast feeding

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and read and then be asked to sign a consent form if you are still happy to participate. Even after you have consented, you are free to withdraw at any time and without giving a reason. If you decide to withdraw from the study your data will be destroyed immediately. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

On your first visit to the laboratory, if you are still happy to partake in the study you will undergo relevant health screening procedures. You will also have your height and weight measured and data on your age and physical activity levels will be collected. You will try out one bout of the non-gym-based exercise mode to be undertaken on the following visits and then you will undergo a submaximal exercise test on an exercise bike. This first visit to the laboratory will last approximately 2 hours. If you are happy to participate, you will undertake 4 further visits to the laboratory starting at 8am. Identical physiological, anthropometric and cognitive measures will be taken across each of these visits. Each testing visit following your first visit will last around 7 hours, but for much of this time you will be allowed to occupy yourself with work or reading at a desk.

What specifically do I have to do?

The study will comprise of five visits to the physiology laboratory at the University of Worcester. On your first visit you will be given information regarding the study, undergo screening questionnaires, try out the exercise bout and sign informed consent for acceptance into the study. You will also undergo an exercise test on an exercise bike. This will start off very light and then every 3 minutes a bit more resistance will be added onto

the pedals. The test will continue until you reach 80% of your predicted maximum heart rate, a somewhat strenuous intensity which you may find uncomfortable but not painful.

The day before your first experimental visit, you will be asked to avoid alcohol and caffeine and record your food and drink intake in a food diary. You will then be asked to follow this diet the day before your remaining experimental visits. Each of the visits following your familiarization visit will last around 7 hours, starting at 8am. On each visit, you will be asked to arrive after an overnight fast but will be provided with breakfast within 15 minutes of arrival.

On the first visit, prior to breakfast your resting metabolic rate will be measured. This will involve you laying on a bed and wearing a small mask over your nose and mouth for 20 minutes. This will collect samples of the gases that you are breathing in and out and from this your energy expenditure can be calculated. Following breakfast on all 4 visits, after a 3 hour period you will undertake one of the following conditions: 4 x 30 seconds of star jumps, 2 x 30 seconds of star jumps (both with 90 seconds of recovery time between each 30-second interval) or you will undertake a control condition where you will remain resting. These conditions will each take around 8 minutes in total. If you have exercised, you will then be discouraged from sitting down straight away and will be encouraged to slowly walk around the laboratory for a short period of time to help alleviate any potential pain and soreness in the 24-48 hours following exercise, which is totally normal. You will then rest for approximately a further 2 hours before you are free to leave the laboratory. During this time, measurements will be repeated and you will be provided with lunch. After 2 hours, your resting metabolic rate will be measured again. Upon leaving the laboratory, you will be asked to keep wearing your activity monitor for the following 3 days and keep a food diary for the following 3 days.

Your weight will be measured upon arrival to the laboratory for each visit. Three finger prick blood samples will be taken during each visit too, but these will cause only mild, short-lived pain. Other, non-invasive, measures such as your heart rate, perceived exertion, and mood affect will also be taken at three points across each testing session too. All visits will follow an identical protocol of measures.

What will happen to the samples I give?

Whole blood collected will be immediately analysed and then disposed of. There will be no storage of blood or cellular tissues.

What will happen to the other data collected from me?

You will not be identifiable from the data we record and your anonymity will be guaranteed in any publication from this study. Only your consent form will have a record of your name; the rest of the documents will be given a code to anonymise your responses, and will be stored separately from your consent form. All of the data will be held in locked cabinets, in locked offices and/or on password protected computers/memory sticks. All data will be stored for a maximum of 10 years (in line with University policy), after which they will be destroyed.

Expenses and payments

Upon completion of the study, you will be offered a free month's gym membership at the McClelland Centre at the University of Worcester and be entered into a prize draw to win a prize up to the value of £150. A contribution of up to £20 can be made for your total travel expenses for all visits to the laboratory.

What are the possible disadvantages and risks of taking part in this study?

The exercise performed is of a high-intensity and so you are likely to experience some discomfort, although this will be short-term and health screening procedures will be carried out beforehand to reduce the risk of injury or medical complications and twenty-four hour first aid will always be accessible. A short warm-down walking period will be encouraged to aid in alleviating subsequent pain and soreness in the 24-48 hours post-exercise. You may still experience some pain and soreness in the 24-48 hours following exercise, but this is totally normal. Finger prick blood samples involve a sharp, sterile lancet being inserted through the skin on the fingertip. This may cause mild, short-lived pain and may result in some small, minor bruising.

What are the possible benefits of taking part in this study?

This study is not a training intervention but it will enable you to try out a type of exercise that you have not tried before which may be something you wish to adopt as a regular activity to increase physical activity levels in a time-efficient manner.

What if there is a problem?

If you have any complaint about the way you have been dealt with during the study or any concerns about possible harm you might suffer, you may contact Dr John-Paul Wilson (Director of Research) on (01905) 54 2196.

Who has reviewed this study?

This study has been reviewed and approved by the University of Worcester Institute of Sport and Exercise Science Ethics Committee.

If you are interested in being a participant in this research study, please email a.burgin@worc.ac.uk to arrange a visit to the research laboratory.

Appendix 5: Study 3 participant information sheet

University of Worcester

Participant Information Sheet (20th February 2018, v1)

Alice Burgin

a.burgin@worc.ac.uk

Name of Study: 8 week apparatus-free high-intensity intermittent exercise

You are invited to take part in a research study. Before you decide whether to volunteer, it isimportant for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

Please do not hesitate to contact us (see below) if there is anything that is not clear, if you would like more information or if you wish to take part in thestudy.

Contact for Study

Name: Alice Burgin

Research School Jenny Lind

Building

University of Worcester Farrier

Street Worcester

WR1 3BB

Email: a.burgin@worc.ac.uk

What is the purpose of thisstudy?

High-intensity intermittent exercise is recently becoming a more popular mode of training based on its time-efficiency compared with traditional, continuous and moderate-intensity exercise. However, the time-efficiency becomes questionable if a time-consuming visit to the gym is required in order to undertake this training. We are looking to investigate a non-gym based bout of high-intensity intermittent exercise that may make this type of training more accessible and time-efficient. Some of our research group's work so far suggests that similar responses occur in the body during exercise with 4x30 seconds of star jumps, to previously studied gym-based (or exercise bike-based) bouts of high-intensity intermittent exercise that we know are beneficial in the long term. However, non-gym-based bouts of high-intensity intermittent exercise are yet to be explored outside of the laboratory or as part of a longer-term exercise programme. Interesting responses also occur in the body in the short-term when short bouts of exercise like this are undertaken just before meal times, but again this hasn't been explored using non-gym-based high-intensity intermittent exercise in the longer term. The purpose of this study is therefore to investigate a range of health benefits to a non-gym-based high-intensity intermittent exercise intervention, based outside of the laboratory. You will undertake 8 weeks of an exercise programme (either undertaking the exercise bouts within 30 minutes of a meal or anytime that is not just before a meal) consisting of doing two lots of 4x30 seconds of star jumps on 3 days per week.

Why have I been chosen?

You have been chosen to take part in this study because you have expressed an interest and your age and physical activity levels meet our inclusion criteria. Prior to reading the rest of this information sheet, if you have not already done so, we advise you to check your BMI using self-measures of your height and weight using the following link: https://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm. An **inclusion** criterion for involvement in the study is a BMI of between 25 and35.

The remaining **inclusion** criteria for this study are listed below:

- Female
- Inactive (<150 minutes moderate-intensity exercise per week)
- Premenopausal
- Aged 18-45
- Regular menstrual cycle, or taking contraception to prevent menstruation

Please note that you will also require a smartphone with 'WhatsApp' (a free messaging service for smartphones) downloaded to take part in this research study. In order to participate, you will also be required to inform the main researcher your mobile number for purposes of the study, explained in detail in this information sheet. These contact details will be stored on a password-protected mobile phone under your study identification number, accessed only by the main researcher and deleted following completion of your participation. Access to kitchen desktop weighing scales will also be preferable for participation in this study, but these can be borrowed from the researcher if required.

The **exclusion** criteria for this study are listed below:

- Any musculoskeletal, metabolic or cardiovascular disorders or symptoms of these; including pain and/or swelling in your hips orknees
- Disability that precludes ability to undertake starjumps
- Cardiac arrhymias
- Dieting or intent to diet
- Blood pressure > 140/90mmHg or blood pressure medication
- Any medication that may influence lipid metabolism orappetite

- Smoking
- Pregnancy or breast feeding
- Irregular menstrual cycle and not taking contraception that preventsmenstruation
- Any known food allergies or intolerances, excluding lactoseintolerance

Your eligibility to take part will be confirmed during the formal screening procedure, which will take place during the initial laboratory visit if you remain interested in participating. If you are aware of any of the above exclusion criteria before entry into the study, or during the study, please inform the main researcher (Alice Burgin) appropriately.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and read and then be asked to sign a consent form if you are still happy to participate. Even after you have consented, you are free to withdraw at any time and without giving a reason. If you decide to withdraw from the study your data will be destroyed immediately. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part? What specifically do I have todo?

On your first visit to the laboratory, the study will be explained to you in full and any questions or queries you may have can be answered. We will then ask you to sign an informed consent form if you are still happy to partake in the study. You will then undergo relevant health screening procedures, including a health questionnaire, a blood analysis questionnaire and a measure of your blood pressure. You will also have your height and weight measured and data on your age and physical activity levels will be collected. Following this, and if you meet the inclusion criteria, we will ask you to choose from an envelope to decide which type of 'intervention' participant you will be (the only difference between these will be the timings of day that we would like you to undertake your exercise bouts). Your group will be fully explained to you after your selection. Following this, you will then try out one bout of the non-gym-based exercise mode to ensure you are happy with the exercise we will be asking you to do at home.

You will then undergo a continual, maximal exercise bout on an exercise bike in the laboratory. This will start of very easy but gradually more resistance will be provided to your cycling, making the test harder as it does so. However, this will only become uncomfortable towards the end of the test. We will ask you to carry on with the test until you no longer can, but you can end the test at any point you like. This test will give us an idea of your current fitness levels. During this, we will ask you to wear a mask over your nose and mouth as we will collect samples of the gases you are breathing in and out to inform the data that we will get throughout the study. We will also monitor your heart rate during this period. After this, we will answer any questions or queries you may have, before you are free to leave the laboratory. This first visit to the laboratory will last approximately 2-3 hours and can be done at any time.

Following this visit, if you are happy to continue participating, you will be given an activity monitor to begin wearing and a food diary to begin recording on an agreed date during your first visit to the laboratory. This date will be arranged for a time that suits you as well as taking into account the stage of your menstrual cycle, as we know that different stages of the menstrual cycle can affect these sorts of measures in some ways. We will ask you to wear the activity monitor and record your food diary for 5 days before starting the 8 weeks of continuing your normal lifestyle or starting either exercise intervention. We will then ask you to come back into the laboratory on an agreed day. The day before this visit, you will be asked to avoid alcohol and caffeine and record your food and drink intake in a food diary. You will then be asked to follow this 'pre-testing day' diet the day before your second experimental visit after 8 weeks. We will ask you to arrive fasted, having nothing but water from waking up to arriving at the laboratory. This visit will begin at approximately 8am and will be known as your 'baseline' visit. Upon arrival, a researcher will check over your food diary and activity monitor. We will then we will take a measurement of your resting metabolic rate which involves you laying down on a bed with a mask over your nose and mouth, as you did during the exercise test. We will ask you to lay relaxed for around 20 minutes, before we will then take a resting blood pressure measurement. After this, we will take a resting blood sample from your arm, by inserting a needle into one of your main veins in the forearm. We will then take measures of your height, weight, waist and hip circumference, followed by a resting finger prick blood sample. Following this, we will ask you to undertake a very short questionnaire and then give you a breakfast meal which will consist of porridge made with semi-skimmed milk, oats and brown sugar and a glass of orange juice. Please notify the main researcher of any food allergies or intolerances as these may be an exclusion criterion for the project for your own health and safety, as we cannot always guarantee the absence of some allergens in the kitchen where the breakfast meal isprepared.

15 minutes following your breakfast, we will take another blood sample from your forearm and will do this again at 30, 45, 60, 90 and 120 minutes following your breakfast. In total we will collect 70ml of blood from your arm per visit. To do this, this time we will insert a needle with what's called a cannula into your forearm. This is a small tube that will sit inside your vein so that we can easily take further blood samples later on in the visit without having to pierce your skin any more.

We will also ask you to undertake the some short questionnaires at each of these times. From the blood samples that we will collect, we will measure some of the things going on in your blood after you have eaten the breakfast meal. Between these times, you are welcome to bring something to occupy your time with, including work, a laptop, a book etc. Following this, you will then undertake the exercise bout on the exercise bike as you will have done on your very first visit. Once you have completed this, you are free to leave the laboratory (this will be approximately 12pm if arriving at 8am). Before leaving, the researcher will confirm with you when to start your 3 days per week of star jump exercises and answer any questions you mayhave.

You will then undertake the 8 weeks of undertaking the exercise programme. The intervention will consist of 4x30 seconds of star jumps - either within 30 minutes prior to meal times, or anytime that is not 1 hour prior to a meal, twice a day on 3 days per week. We will ask you to do a 1 minute walking warm-up and warm-down period before and after the 4x30 seconds. We will not ask you to come in the laboratory again until 8 weeks after your 'baseline' visit. We will, however, get in regular contact every week via text or email to check everything is going okay and answer any questions or queries you might have. We will also ask you to fill out a short set of scales and an enjoyment questionnaire at the end of every week, which we will ask you to send back to us in a stamped, addressed envelope that we will provide to you.

In the fourth as well as the eighth and final week, we will ask you to wear an activity monitor and record your food diary again for a 5 day period, similar to how you will have done before the start of the intervention. During these weeks, we will also ask you to send the main researcher text messages with the timings of your exercise and meals for that week to help us check that you are getting on ok with your intervention condition. We will then ask you to follow your 'pre-testing day' diet the day before coming into the laboratory for a third and final time on an agreed day for your 'post-testing' visit. This will be exactly the same structure with exactly the same measures as your 'baseline' visit.

If you have any questions or queries at all during your participation, please do not hesitate to contact the main researcher (Alice Burgin) using the contact details on the front of this information sheet.

What will happen to the samples Igive?

Capillary blood collected will be immediately analysed and then disposed of. Whole blood collected from your forearm will be immediately centrifuged to separate the plasma from the blood cells. The plasma will be stored for later analysis but there will be no storage of blood cells or cellular tissues.

What will happen to the other data collected fromme?

All data including personal data will be pseudo anonymized. Your anonymity will be guaranteed in any publication from this study. Only your consent form will have a record of your name; the rest of the documents will be given a code to anonymise your responses, and will be stored separately from your consent form. All of the data will be held in locked cabinets, in locked offices and/or on password protected computers/memory sticks. All data will be stored for a maximum of 10 years (in line with University policy), after which they will be destroyed.

Expenses and payments

Upon completion of the study, a contribution of up to £20 can be made for your total travel expenses for all visits to the laboratory.

What are the possible disadvantages and risks of taking part in thisstudy?

The exercise performed is of a high-intensity and so you are likely to experience some discomfort, although this will be short-term and health screening procedures will be carried out beforehand to reduce the risk of injury or medical complications, as your exercise bouts will not take place in a lab supervised by medically-trained staff. We encourage you to undertake both the short warm-up and warm-down walking period, to avoid musculoskeletal injury and aid in alleviating subsequent pain and soreness in the 24-48 hours post-exercise. You may still experience some pain and soreness in the 24-48 hours following exercise, but this is totally normal. Finger prick blood samples involve a sharp, sterile lancet being inserted through the skin on the fingertip. This may cause mild, short-lived pain and may result in some small, minor bruising. Inserting the needles and cannula into the vein in your forearm also involves a sharp, sterile needle being inserted through the skin on the forearm, where the cannula will then be put into place. This may also cause mild, short-lived pain and may result in some small, minorbruising.

We understand that not everyone will be comfortable with the blood measurements, so if you have concerns about these, this may not rule you out of the study as if you remain interested and fit the inclusion criteria for everything else we may be able to do all measures apart from these blood measures. Please don't hesitate to contact Alice Burgin if you have any questions or queries regardingthis.

What are the possible benefits of taking part in thisstudy?

This study will enable you to try 8 weeks of exercise that you may not have tried before. We hope that this 8 week programme has the potential to increase cardiovascular and cardiorespiratory fitness and promote a number of other important health benefits. Following completion of the study, the programme may be something you wish to adopt as a regular activity to increase physical activity levels in a time-efficient manner.

What if there is a problem?

If you have any complaint about the way you have been dealt with during the study or any concerns about possible harm you might suffer, you may contact the secretary of the ethics committee, Michelle Jellis, on michelle.jellis@worc.ac.uk or 01905 542767.

Who has reviewed this study?

This study has been reviewed and approved by the University of Worcester Ethics Committee under a full review.

PLEASE NOTE: In line with new EU General Data Protection Regulation legislations, we are required to inform you of the contact details of the Data Controller (the University of Worcester) and the Data Protection Officer for you to direct any questions or queries to (Helen Johnstone, Head of Information Assurance, h.johnstone@worc.ac.uk). The new law demands that data processing is lawful, fair and transparent. If you have any questions regarding this please feel free to ask them. The purpose of processing your personal data will enable us to assign the research data we collect from you to you. The lawful basis for using your data and your special category data in this publicly funded research will be 'task in the public interest' and such processing is necessary for scientific research in accordance with safeguards' (in accordance with permission from the University of Worcester Ethics Committee). Your personal data will only be shared within the research team (with pseudo anonymity still guaranteed) and will not be shared outside of the EU. We will not carry out automated decision making. Data will be retained for 10 years, aligning with the University of Worcester policy. After this, all data will be destroyed. Under data protection law, you can request access to and correction and erasure of your personal information, or object, suspend or request the restriction of processing your personal data. Please note that all pseudo anonymised data will no longer remain as personal data for the purpose of presentation or publication of data.

If you are interested in being a participant in this research study, please email a.burgin@worc.ac.uk to arrange a visit to the researchlaboratory.

Appendix 6: Study 1 participant consent form

Participant consent form

Project Title: Physiological responses to high-intensity gym and non-gym-based exercise

Please read this form, initial each statement below if you have no objections, and sign it once the investigator has fully explained the aims and procedures of the study to you.

- ☐ I voluntarily agree to take part in this study.
- ☐ I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.
- ☐ I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous.
- ☐ I authorise the investigators to disclose the results of my participation in the study but not my name.
- ☐ I confirm that I have been given a full explanation by the investigators and that I have read and understood the Participant Information Sheet (version 1, 16th May 2016) given to me. I am aware of any possible risks or discomfort.
- ☐ I agree to inform the researcher immediately if I feel uncomfortable during the study.
- ☐ I have been given the opportunity to ask questions on all aspects of the study and to discuss the study with the investigators, and I have understood the advice and information given as a result.
- ☐ I understand that I can ask for further information or explanations at any time.
- ☐ I understand that I will not receive any money for taking part in this study.

Name (participant): .....

Date: .....

Signature: .....

To be completed by the Investigator:

The above named participant has been informed of the protocol and procedures for the above study and has received a copy of the Participant Information Sheet.

Name (investigator): .....

Date: .....

Signature: .....

Appendix 7: Study 2 participant consent form

Project Title: Physiological responses to high-intensity non-gym-based exercise

Please read this form, initial each statement below if you have no objections, and sign it once the investigator has fully explained the aims and procedures of the study to you.

- ☐ I voluntarily agree to take part in this study.
- ☐ I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.
- ☐ I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous.
- ☐ I authorise the investigators to disclose the results of my participation in the study but not my name.
- ☐ I confirm that I have been given a full explanation by the investigators and that I have read and understood the Participant Information Sheet (version 1, 12th December 2016) given to me. I am aware of any possible risks or discomfort.
- ☐ I agree to inform the researcher immediately if I feel uncomfortable during the study.
- ☐ I have been given the opportunity to ask questions on all aspects of the study and to discuss the study with the investigators, and I have understood the advice and information given as a result.
- ☐ I understand that I can ask for further information or explanations at any time.
- ☐ I understand that I will not receive any money for taking part in this study, but I will be offered a free month's gym membership at the University of Worcester McClelland centre and be entered into a prize draw to win a prize up to the value of £150.

Name (participant): .....

Date: .....

Signature: .....

To be completed by the Investigator:

The above named participant has been informed of the protocol and procedures for the above study and has received a copy of the Participant Information Sheet.

Name (investigator): .....

Date: .....

Signature: .....

Appendix 8: Study 3 participant consent form

Project Title: 8 week high-intensity apparatus-free exercise intervention

University of Worcester

Agreement to participate

Please read this form, initial each statement below if you have no objections, and sign it once the investigator has fully explained the aims and procedures of the study to you.

- ☐ I voluntarily agree to take part in this study.
- ☐ I understand that I am free to withdraw my participation in data collection at any time, without having to give a reason for withdrawing.
- ☐ I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous.
- ☐ I authorise the investigators to disclose the results of my participation in the study but not my name.
- ☐ I confirm that I have been given a full explanation by the investigators and that I have read and understood the Participant Information Sheet (version 1) given to me. I am aware of any possible risks or discomfort.
- ☐ I understand that I am free to cease exercising at any point if I feel unwell.
- ☐ I agree to inform the researcher immediately if I feel uncomfortable during the study. I have no known food allergies or intolerances (excluding lactose intolerance).
- ☐ I understand the requirement of the use of my mobile phone number for the purposes of the study but that it will be saved under my identification number, and authorise the main researcher to store this contact number on a password-protected mobile phone, accessed only by the main researcher and deleted from the mobile phone storage upon my completion of the study.
- ☐ I have been given the opportunity to ask questions on all aspects of the study and to discuss the study with the investigators, and I have understood the advice and information given as a result.
- ☐ I understand that I can ask for further information or explanations at any time. I understand that I will not receive any money for taking part in this study.
- ☐ I have read and understood the privacy notice with regard to the new GDPR legislations:

In line with new EU General Data Protection Regulation legislations specifically regarding personal data, we are required to inform you of the contact details of the Data Controller (the University of Worcester) and the Data Protection Officer for you to direct any questions or queries to (Helen Johnstone, Head of Information Assurance, h.johnstone@worc.ac.uk). The new law demands that data processing is lawful, fair and transparent. If you have any questions regarding this please feel free to ask them. The purpose of processing your personal data will enable us to assign the research data we collect from you to you. The lawful basis for using your data and your special category data in this publicly funded research will be 'task in the public interest' (see article 6(1)(e) and such processing is 'necessary for scientific research in accordance with safeguards' (in accordance with permission from the University of Worcester Ethics Committee; see article 9(2)(j)). Personal data will be handled subject to the exemptions of Article 9(2)(j) as processing is necessary for archiving purposes in the public interest, scientific or historical research purposes or statistical purposes in accordance with Article 89(1) based on Union or Member State law which shall be proportionate to the aim pursued, respect the essence of the right to data protection and provide for suitable and specific measures to safeguard the fundamental rights and the interests of the data subject. Your personal data (pseudo anonymised) will only be shared within the research team and will not be shared outside of the EU. We will not carry out automated decision making. Please note that all pseudo anonymised data will no longer remain as personal data for the purpose of presentation or publication of data. Upon completion of the study, all data will be fully anonymised and no longer retained as personal data.

Name (participant):

.....

Date: .....

Signature: .....

To be completed by the Investigator:

The above named participant has been informed of the protocol and procedures for the above study and has received a copy of the Participant Information Sheet.

Name (investigator):..... **Date:** .....

Signature: .....

Appendix 9: Physical Activity Readiness Questionnaire (PAR-Q; Thomas, Reading and Shephard, 1992)

University of Worcester
Institute of Sport and Exercise Science

STRICTLY CONFIDENTIAL

Module: _____

PRE-TEST QUESTIONNAIRE

Name: _____ Age: _____ D.O.B.: _____

As you are to be a participant in this laboratory please complete the following questionnaire truthfully and completely. The purpose of this questionnaire is to ensure that you are in a fit and healthy state to complete an exercise test. Data will be treated in accordance with the UW Data Protection Code of Practice.

Tick or * delete as appropriate

1. How would you describe your present level of activity?
   sedentary ☐ moderately active ☐ highly active ☐
2. How would you describe your current level of fitness?
   very unfit ☐ moderately fit ☐ trained ☐ highly trained ☐
3. How would you consider your present weight?
   underweight ☐ ideal weight ☐ slightly overweight ☐ very overweight ☐

		Yes	No
4. Smoking habits			
Are you a current smoker?		☐	☐
If *yes* a regular smoker	of.....per day		
an occasional smoker	of.....per day		
Are you a previous smoker?		☐	☐
If *yes*	of.....per day		
how long since stopping?	..... months/years *		
5. Consumption of alcohol			
Do you drink alcoholic drinks?		☐	☐
If *yes* do you have:	the occasional drink	☐	
	a drink every day	☐	
	more than one drink a day	☐	
6. Have you had to consult your doctor within the last 6 months?		☐	☐
If *yes* please give brief details:			
7. Are you presently taking any form of medication?		☐	☐
If *yes* please give brief details:			
8. Have you suffered from a bacterial or viral infection in the last 2 weeks?		☐	☐
9. Do you suffer, or have you ever suffered from any form of heart complaint?		☐	☐
10. Is there a history of heart disease in your family?		☐	☐

STRICTLY CONFIDENTIAL

	Yes	No
11. Do you suffer, or have you ever suffered from:		
Asthma	☐	☐
Diabetes	☐	☐
Bronchitis	☐	☐
Epilepsy	☐	☐
High blood pressure	☐	☐
Low blood pressure	☐	☐
12. Do you currently have any form of muscle or joint injury?	☐	☐
If *yes* please give brief details:		
13. Have you had to suspend training in the last two weeks for a physical reason?	☐	☐
If *yes* please give brief details:		
14. Is there anything to your knowledge that may prevent you from successfully completing the tests that have been outlined to you?	☐	☐

INFORMED CONSENT

Please read the following statements carefully. Please sign only when you have agreed with the statement and when you have had any relevant questions answered.

- I have read the information sheet for the above study and the full details of the tests have been explained to me (verbally and written). I am clear about what will be involved and I am aware of the purposes of the tests, the potential benefits and the potential risks.
- I know that I am not obliged to complete the tests. I am free to stop the test at any point and for any reason.
- I am responsible to report promptly any unusual feelings or discomfort during the exercise test.
- Tests results will only be used for the purposes of this module
- I have no injury or illness that will affect my ability to successfully complete the tests.
- I hereby give my full consent to take part in the study.

Signature of participant: _____ Date: _____

Name of Test Supervisor: _____

Signature of Test Supervisor: _____ Date: _____

Appendix 10: Pre-participation health questionnaire, based on ACSM pre-screening guidelines (American College of Sports Medicine, 2013)

University of Worcester

Pre-participation Health Screening Questionnaire

Participant ID _____ Date _____

Please mark all *true* statements:

SECTION1:

History:
You have had:
__ a heart attack
__ heart surgery
__ cardiac catheterization
__ coronary angioplasty (PTCA)
__ pacemaker/implantable cardiac defibrillator/rhythm disturbance
__ heart valve disease
__ heart failure
__ heart transplantation
__ congenital heart disease

Symptoms:
__ Experienced chest discomfort with exertion.
__ Experienced unreasonable breathlessness.
__ Experienced dizziness, fainting, blackouts.
__ Currently taking heart medications.

Other health issues:
Do you have any musculoskeletal problems? _____
Do you have concerns about the safety of exercise? _____
Do you takes prescription medication(s)? _____
Are you pregnant? _____

If any statements in this section are marked, a physician or appropriate health care provider should be consulted before engaging in exercise and documentation of this consultation should remain on file.

SECTION 2: CARDIOVASCULAR RISK FACTORS

Are you a woman older than 55 years? ___ Have you had a hysterectomy or are you post-menopausal? _____
Do you smoke? _____
Is your blood pressure > 140/90mmHg? _____
Do you take blood pressure medication? _____
Has your doctor previously told you that your blood cholesterol level is high (> 240 mg/dl)? _____
Do you have a close blood relative who had a heart attack; before age 55 if father or brother or before age 65 if mother or sister? _____
Are you physically inactive (< 30 minutes of physical activity on at least 3 days per week)? _____
Is your BMI >30kg•m²? _____

If 2 or more statements in this section are marked, a physician or appropriate health care provider should be consulted before engaging in exercise and documentation of this consultation should remain on file.

SECTION 3: NO HISTORY, SYMPTOMS, HEALTH ISSUES, OR CARDIOVASCULAR RISK FACTORS

__ None of the items in sections 1 and 2 above are true.

Participant should be able to exercise safely without consulting their healthcare provider.

Study Team Member Completing Form: _____

Appendix 11: Physical Activity Readiness Questionnaire for Everyone (PAR-Q+; Warburton et al., 2011)

2018 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it ***does not limit your current ability*** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

☑ **If you answered NO to all of the questions above, you are cleared for physical activity.**
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION
If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness centre may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

01-11-2017

2018 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?
If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?
If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*
If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you have High Blood Pressure?
If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*
If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2018 PAR-Q+

6. Do you have any Mental Health Problems or Learning Difficulties? *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*
If the above condition(s) is/are present, answer questions 6a-6b — If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. Do you have a Respiratory Disease? *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*
If the above condition(s) is/are present, answer questions 7a-7d — If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. Do you have a Spinal Cord Injury? *This includes Tetraplegia and Paraplegia*
If the above condition(s) is/are present, answer questions 8a-8c — If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. Have you had a Stroke? *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*
If the above condition(s) is/are present, answer questions 9a-9c — If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?
If you have other medical conditions, answer questions 10a-10c — If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2018 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact

www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2)3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

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Appendix 12: Pre-bloodletting and analysis questionnaire (according to University of Worcester laboratory guidelines)

University of Worcester
Institute of Sport and Exercise Science

STRICTLY CONFIDENTIAL

Participant number: _____

BLOOD ANALYSIS QUESTIONNAIRE

The purpose of this questionnaire is to ensure that you are in a fit and healthy state for blood analysis. Please complete the questionnaire truthfully and completely. Data will be treated in accordance with the UW Data Protection Code of Practice.

Tick or delete as appropriate

	Yes	No
1. Are you receiving any medicines, dental treatment, had a recent illness or attending hospital outpatients?	☐	☐
If yes, full details should be on the pre-test questionnaire		
2. Have you had a piercing, acupuncture or tattoo in the last 6 months?	☐	☐
3. Have you ever been advised by a doctor not to give blood?	☐	☐
4. Do you suffer, or have you ever suffered from:		
Allergy from latex	☐	☐
Diabetes	☐	☐
Epilepsy (fits)	☐	☐
Hepatitis (jaundice) or been in contact with a case in the last 6 months	☐	☐
Tropical disease especially malaria	☐	☐
Sexually Transmitted Infections?	☐	☐
5. Have you travelled outside Europe within the last 6 months and/or received travel vaccinations?	☐	☐

If shaded boxes have been ticked DO NOT PROCEED

DECLARATION

I have had testing explained to me and fully understand the reasons for blood analysis during exercise testing. To the best of my knowledge I am fully eligible to undertake blood testing and do so of my own free will.

Signature of participant: _____ Date: _____

Signature of Parent/Guardian: _____ Date: _____
(if applicable)

Name of Test Supervisor: _____

Signature of Test Supervisor: _____ Date: _____

Appendix 13: Food diary instructions

Food Diary

PLEASE READ THROUGH THESE PAGES BEFORE STARTING YOUR DIARY

We would like you to keep this diary of everything you eat and drink over the 24 hours before your next laboratory visit. Please include all food consumed at home and outside the home e.g. work, college or restaurants. It is very important that you do not change what you normally eat and drink just because you are keeping this record. **Please eat as you normally would do.**

Day
Please circle the study day (1,2,3,4,5) and write the day of the week at the top of the page each time you start a new day of recording. Please try to cover a sample of both weekend and weekday days.

Time Slots
Please note the time of each eating occasion into the space provided.

What did you eat?
Please describe the foods you eat in as much **detail** as possible. Be as **specific** as you can. The example day shows the level of detail needed such as –
Cooking methods: (fried, grilled, baked, micro-waved etc).
Any additions (sugar/sweeteners, sauces, pepper, salt, mustard, etc).
Type and amount of fat / oil used for cooking, e.g. teaspoon peanut oil, 15g butter.
What brands, e.g. Kellogg's corn flakes, places food bought, fresh, frozen, tinned, raw.
What variety – e.g. semi skimmed milk, low fat pro-biotic yogurt, reduced salt, reduced sugar?
Whether soft drinks were low calorie (diet) or decaffeinated?
Were fruit juices UHT, pasteurised or freshly squeezed, made from concentrate
Products such as cheese, fish and meat were they smoked or not
Meats – what part of the animal? Chicken breast? Legs? Wings? back bacon, streaky bacon, extra lean beef mince, value beef mince.

E.g. a cheese sandwich is really 3 foods – Bread, Margarine and Cheese. What type / amount of bread? Type / amount of spread? Type / amount of cheese?

Remember to record all snacks and drinks throughout the day.

Portion sizes
Please use the kitchen weighing scales provided to weigh every item of food / drink consumed – Remember to Zero after each item of food, and check it's in grams. Eat the amount you would normally eat – don't use the scales to determine when to stop!
When weighing is not possible, food quantities can be described using:
• household measures, e.g. one teaspoon (tsp) of sugar, two thick slices of bread, 4 tablespoons (tbsp) of peas, ½ cup of gravy, large portion of takeaway chips. Be careful when describing amounts in spoons that you are referring to the correct spoon size.
• use weights from labels, e.g. 4oz steak, 420g tin of baked beans, 125g pot of Yoghurt – but only if everything is eaten – did you eat the fat /skin **/ bones?**
• number of items, e.g. 4 fish fingers, 1 Rich Tea biscuit, 1 king size mars bar
With foods such as fruit, remember to record the weight of the skin/core/stone in the weight leftover column

For drinks, quantity can be described using:
• weight (grams), the size of glass, cup etc (e.g. large glass) or the volume (e.g. 300ml, 1 pint).
• volumes from labels (e.g. 330ml can of fizzy drink, 500ml strawberry Innocent smoothie).

We would like to know the amount that was actually eaten which means taking into account leftovers. You can do this in two ways:
1. Record what was served and note what was not eaten e.g. 30g of peas, only 12g eaten; 1 Weetabix, ate ½
2. Only record the amount actually eaten i.e. 18g of peas; ½ Weetabix

Homemade dishes
If you have eaten any homemade dishes e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe serves, and the cooking methods used. Write this down in the recipe section at the end of the diary. Record how much of the whole recipe you have eaten in the portion size column.

Take-aways and eating out
If you have eaten a take-aways or eaten dishes not prepared at home such as at a restaurant or a friend's house, please record as much detail about the ingredients as you can e.g. vegetable curry containing chickpeas, aubergine, onion and tomato. Please also record the name of the restaurant, takeaway, and the name of the meal, e.g. Domino's pizza, 16inch meat feast, thin base.

Brand name
Please note the brand name (if known). Most packed foods will list a brand name, e.g. Bird's eye, Hovis, or Supermarket own brands. For ready-made meals or for less well known brands, please keep the packet's nutritional information in the bag provided.

Supplements / Medications
Please also provide information about any supplements you took. Please record the brand name, full name of supplement, strength and the amount taken should be recorded, e.g. Maximuscle cyclone powder – 40g (2 scoops), Holland and Barret Cod Liver Oil and Glucosamine Capsules (500mg) – 1 capsule.
If you take any new medicines or stop taking ones we know about please record it here.

Was it a typical day?
After each day of recording please tell us whether this was a typical day or whether there were any reasons why you ate and drank more or less than usual. E.g. Drank 4 pints of Guinness as it was St Patricks day, day 2 ate very little as not feeling well.

When to fill in the diary
Please record your eating as you go, **not from memory at the end of the day**. Use written notes if you forget to take your diary with you and fill out your diary ASAP. Each diary day covers a 24hr period, so please include any food or drinks that you may have had during the night. Remember to include foods and drinks between meals (snacks) including water.

Overleaf you can see an example day that have been filled in. These examples show you how we would like you to record your food and drink, and how to record a homemade dish.

Please document what you ate & drank in as much detail as possible, Remember if it has passed your lips record it! ☺

It only takes a few minutes for each eating occasion!

We thank you for your efforts in filling out this diary.
Recipes / Takeaways

Write in recipes of ingredients of homemade dishes or take-aways, an example is below:

Name of Dish Big Joe's 16" Meat Feast pizza	**Serves:** 2 equal portions
Ingredients	**Amounts**
Deep pan pizza base	16 inch, weight unknown
Tomato Sauce based	1 ladle
Green peppers	Half green bell pepper
Spicy salami	~ 12 large slices
Pepperoni	~ 20 small slices
Tandoori chicken pieces	~ half chicken breast
Beef meatballs	6 small meatballs
Onion	Half
Garlic mayo dip	75ml pot
Description of cooking method Oven cooked	

Appendix 14: Example of weighed food diary – additional copies and instructions given regarding number of and which days to record (see individual chapters for details)

DAY (Date:)					
TIME	**ITEM/ DESCRIPTION**	**BRAND/ VARIETY**	**AMOUNT**	**PREPARATION**	**LEFT-OVERS**

Appendix 15: Rating of Perceived Exertion Scale (Borg, 1982)

Rating	Descriptor
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix 16: Feeling Scale (Hardy and Rejeski, 1989)

Feeling Scale (FS)
(Hardy & Rejeski, 1989)

While participating in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasant. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. Scientists have developed this scale to measure such responses.

+5 Very good

+4

+3 Good

+2

+1 Fairly good

0 Neutral

-1 Fairly bad

-2

-3 Bad

-4

Appendix 17: Positive and Negative Affect Scale (Watson, Clark and Tellegen, 1988)

This scale consists of a number of words that describe different feelings and emotions. Read each item and then circle the choices that best describe how you are feeling right now. Indicate to what extent you currently feel this way.

	1 Very slightly or not at all	2 A little	3 Moderately	4 Quite a bit	5 Extremely
Interested	1	2	3	4	5
Distressed	1	2	3	4	5
Excited	1	2	3	4	5
Upset	1	2	3	4	5
Strong	1	2	3	4	5
Guilty	1	2	3	4	5
Scared	1	2	3	4	5
Hostile	1	2	3	4	5
Enthusiastic	1	2	3	4	5
Proud	1	2	3	4	5
Irritable	1	2	3	4	5
Alert	1	2	3	4	5
Ashamed	1	2	3	4	5
Inspired	1	2	3	4	5
Nervous	1	2	3	4	5
Determined	1	2	3	4	5
Attentive	1	2	3	4	5
Jittery	1	2	3	4	5
Active	1	2	3	4	5
Afraid	1	2	3	4	5

Appendix 18: Physical Activity Enjoyment Scale (Kendzierski and DeCarlo, 1991)

Please rate how you feel at the moment about the physical activity you have been doing:

I enjoy it	1	2	3	4	5	6	7	I hate it
I feel bored	1	2	3	4	5	6	7	I feel interested
I dislike it	1	2	3	4	5	6	7	I like it
I find it pleasurable	1	2	3	4	5	6	7	I find it unpleasurable
I am very absorbed in this activity	1	2	3	4	5	6	7	I am not at all absorbed in this activity
It's no fun at all	1	2	3	4	5	6	7	It's a lot of fun
I find it energising	1	2	3	4	5	6	7	I find it tiring
It makes me depressed	1	2	3	4	5	6	7	It makes me happy
It's very pleasant	1	2	3	4	5	6	7	It's very unpleasant
I feel good physically while doing it	1	2	3	4	5	6	7	I feel bad physically while doing it
It's very invigorating	1	2	3	4	5	6	7	It's not at all invigorating
I am very frustrated by it	1	2	3	4	5	6	7	I am not at all frustrated by it
It's very gratifying	1	2	3	4	5	6	7	It's not at all gratifying
It's very exhilarating	1	2	3	4	5	6	7	It's not at all exhilarating
It's not at all stimulating	1	2	3	4	5	6	7	It's very stimulating
It gives me a strong sense of accomplishment	1	2	3	4	5	6	7	It does not give me any sense of accomplishment at all
It's very refreshing	1	2	3	4	5	6	7	It's not at all refreshing
I felt as though I would rather be doing something else	1	2	3	4	5	6	7	I felt as though there was nothing else I would rather be doing

Appendix 19: Respiratory exchange ratio-specific caloric equivalent of oxygen (Frayn, 1983)

RQ / RER	kCal Equiv	% kCal from		grams per LO_2	
	kCal. LO_2	CHO	Fat	CHO	Fat
0.71	4.686	0.0	100.0	0.000	0.496
0.71	4.690	1.1	98.9	0.012	0.491
0.72	4.702	4.8	95.2	0.051	0.476
0.73	4.714	8.4	91.6	0.090	0.460
0.74	4.727	12.0	88.0	0.130	0.444
0.75	4.739	15.6	84.4	0.170	0.428
0.76	4.750	19.2	80.8	0.211	0.412
0.77	4.764	22.3	77.2	0.250	0.396
0.78	4.776	26.3	73.7	0.290	0.380
0.79	4.788	29.9	70.1	0.330	0.363
0.80	4.801	33.4	66.6	0.371	0.347
0.81	4.813	36.9	63.1	0.413	0.330
0.82	4.825	40.3	59.7	0.454	0.313
0.83	4.838	43.8	56.2	0.496	0.297
0.84	4.850	47.2	52.8	0.537	0.280
0.85	4.862	50.7	49.3	0.579	0.263
0.86	4.875	54.1	45.9	0.621	0.247
0.87	4.887	57.5	42.5	0.663	0.230
0.88	4.899	60.8	39.2	0.705	0.213
0.89	4.911	64.2	35.8	0.749	0.195
0.90	4.924	67.5	32.5	0.791	0.178
0.91	4.936	70.8	29.2	0.834	0.160
0.92	4.948	74.1	25.9	0.877	0.143
0.93	4.961	77.4	22.6	0.921	0.125
0.94	4.973	80.7	19.3	0.964	0.108
0.95	4.985	84.0	16.0	1.008	0.090
0.96	4.998	87.2	12.8	1.052	0.072
0.97	5.010	90.4	9.6	1.097	0.054
0.98	5.022	93.6	6.4	1.142	0.036
0.99	5.035	96.8	3.2	1.186	0.018
1.00	5.047	100.0	0.0	1.231	0.000

Appendix 20: Hedonic scale for ad libitum buffet

Please rate how much you like each of the foods available in the buffet from 1 to 9.
Thank you!

Food item	Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
Cheese & onion sandwiches	1	2	3	4	5	6	7	8	9
Egg mayonnaise sandwiches	1	2	3	4	5	6	7	8	9
Tuna sweetcorn mayonnaise sandwiches	1	2	3	4	5	6	7	8	9
Ready salted crisps	1	2	3	4	5	6	7	8	9
Cheese & onion crisps	1	2	3	4	5	6	7	8	9
Salt & vinegar crisps	1	2	3	4	5	6	7	8	9
Cranberry cereal bars	1	2	3	4	5	6	7	8	9
Chocolate cereal bars	1	2	3	4	5	6	7	8	9
Apples	1	2	3	4	5	6	7	8	9
Bananas	1	2	3	4	5	6	7	8	9
Oranges	1	2	3	4	5	6	7	8	9
Water	1	2	3	4	5	6	7	8	9

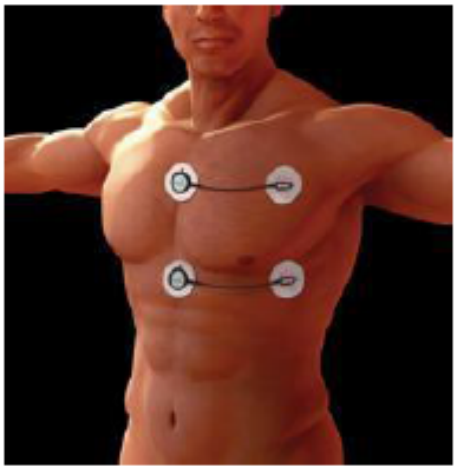
Appendix 21: Activity monitor wearing instructions

University of Worcester

Wearing the Actiheart

The Actiheart continuously monitors your heart rate and activity levels. It is extremely important for our study that you wear the monitor properly for the 5 day period agreed by you and the researcher. Please follow these instructions carefully and if you have any questions or queries please don't hesitate to contact me. Thank you very much! ☺

- Once a day, please remove the Actiheart and clean the skin where the strap/electrodes are placed. This can easily be achieved by using warm water and soap or other detergent.
- The positioning of the electrodes can be seen below. It is just to the left of the sternum, ideally below the bra, with the position of the activity monitor in line with that of the position below the bra on the image. However, if this is uncomfortable please align the position of the activity monitor in line with the upper position on the image:



- You have been given some electrodes as well as a strap, in case these are more comfortable to sleep in – please feel free to use these overnight but adhere to the wearing instructions above as best as you can.
- Position the chest electrode/strap on the prepared skin area, then attach the Actiheart (press the top button in, then attach it to the clip). If using the chest strap, attach the Actiheart to the second clip onto the outer side of the strap. If using the electrodes, then attach the 2nd electrode to the Actiheart (before placing on skin), keeping the wire stretched to its full length and as horizontal as possible (a mirror will be needed!) attach the electrode to the prepared skin area.
- Keep the Actiheart on all day and night and do not let anyone else wear it.
- Note on the next page if anything was unusual, for example, much less active than normal as travelling in car for long periods, and also any details on planned exercise/training (not your star jump bouts).
- Please remember to wear the activity meter for all the consecutive days. There is no "ON" or "OFF" switch that you need to worry about.

Thank you very much for your adherence, if you have any questions or queries please don't hesitate to contact me using the details below if you would like any assistance.

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Appendix 22: Visual Analogue Scale (Hill and Blundell, 1982)

Participant number: Condition: Date:

Visual Analogue Scales

How hungry do you feel at the present moment?

Not hungry at all — *The hungriest I have ever been*

How full do you feel at the present moment?

Not full at all, empty — *Very, very full*

How strong is your desire to eat at this moment?

Very weak — *Very strong*

How much would you expect to eat if provided with a meal right now?

Nothing — *Lots*

How nauseous do you feel at this moment?

Not nauseous at all — *Extremely nauseous*

Appendix 23: Control of Eating Questionnaire (Hill, Weaver and Blundell, 1991)

Please read each question carefully and put a mark through the line at the point that best represents your experience. Answer all questions according to your experience over the last 7 days.

1. How hungry have you felt?

Not at all hungry — *Extremely hungry*

2. How full have you felt?

Not at all full — *Extremely full*

3. How strong was your desire to eat sweet foods?

Not at all strong — *Extremely strong*

4. How strong was your desire to eat savoury foods?

Not at all strong — *Extremely strong*

5. How happy have you felt?

Not at all happy — *Extremely happy*

6. How anxious have you felt?

Not at all anxious — *Extremely anxious*

7. How alert have you felt?

Not at all alert — *Extremely alert*

8. How contented have you felt?

Not at all contented — *Extremely contented*

A food craving is a strong urge to eat a particular food or drink

9. During the last 7 days how often have you had food cravings?

Not at all — *Very often*

10. How strong have any food cravings been?

Not at all strong — *Extremely strong*

11. How difficult has it been to resist any food cravings?

Not at all difficult — *Extremely difficult*

12. How often have you eaten in response to food cravings?

Not at all — *After every one*

How often have you had food cravings for the following types of food/drink?

13. Chocolate or chocolate flavoured foods

Not at all — *Extremely often*

14. Other sweet foods (cakes, pastries, biscuits, etc)

Not at all — *Extremely often*

15. Fruit or fruit juice

Not at all — *Extremely often*

16. Dairy foods (cheese, yoghurts, milk, etc)

Not at all — *Extremely often*

17. Starchy foods (bread, rice, pasta, etc)

Not at all — *Extremely often*

18. Savoury foods (French fries, crisps, burgers, pizza, etc)

Not at all — *Extremely often*

19. Generally, how difficult has it been to control your eating?

Not at all difficult — *Extremely difficult*

20. Which one food makes it most difficult for you to control eating?

.....

21. How difficult has it been to resist eating this food during the last 7 days?

Not at all difficult — *Extremely difficult*

Appendix 24: Intentions to Exercise Scale using methods previously described by Focht (2013) and Krinski et al. (2017)

Intentions to Exercise Scale

Please rate the probability that you would intend to participate in 4x30 seconds of star jumps twice a day, three days per week:

Tomorrow:

No intention at all — *Moderate intention* — *Strong intention*

For the next week:

No intention at all — *Moderate intention* — *Strong intention*

For the next 2 weeks:

No intention at all — *Moderate intention* — *Strong intention*

For the next month:

No intention at all — *Moderate intention* — *Strong intention*

Appendix 25: Acceptability of the Intervention Scale (using methods previously described by Boereboom et al., 2016)

Acceptability of the HIIT intervention

Please rate how you feel about the physical activity programme you have been doing:

	1 – Strongly agree	2	3	4	5 – Strongly disagree
HIIT was well explained	1	2	3	4	5
I enjoyed HIIT	1	2	3	4	5
I would recommend HIIT to others	1	2	3	4	5
HIIT was more demanding than expected	1	2	3	4	5
I would do HIIT again	1	2	3	4	5
The physical strain interfered with my life	1	2	3	4	5
I believe my fitness has improved	1	2	3	4	5
I would like to have exercised in a group	1	2	3	4	5