The effects of

an 8-week supervised exercise program

on appetite and appetite-regulating gut hormones

in overweight and obese adults

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Abstract

Background and Purpose

All types of physical activities are associated with some kind of health benefits. These include weight gain prevention, weight loss and weight regain prevention after weight loss and maintaining healthy body composition and fitness. However, the long term effects of exercise on energy homeostasis vary according to the exercise duration, mode, intensity and volume; and the characteristics of the participants studied and whether the exercise is accompanied with dietary restrictions. Therefore, the aim of this research was to determine the effects of 8-week supervised and structured exercise training programs – of different modes, intensities and volumes – on appetite and appetite-regulating gut hormones, as well as effects on body weight and waist circumference in overweight and obese adults.

Methods

A longitudinal randomised controlled pilot trial was conducted at the Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders at the University of Sydney in the period September 2012 to November 2013. Twenty-eight eligible overweight and obese adults were randomly allocated to one of 4 intervention groups or a control group as follows: High-intensity/low energy expenditure aerobic exercise training (HI:LO) (n=7), Low-intensity/high energy expenditure aerobic exercise training (LO:HI) (n=8), Low-intensity/low energy expenditure aerobic exercise training (LO:LO) (n=4), Progressive resistance training (PRT) (n=4), and the control group (n=5). Subjective appetite was assessed using the Electronic Appetite Ratings System (EARS), and the plasma concentrations of total ghrelin and peptide tyrosine tyrosine (PYY) hormones were assessed at baseline and after intervention in the fasted state; and anthropometric measurements were also recorded. Participants were not prescribed any dietary restriction and were asked not to change their usual diet. Statistical analyses were performed using SPSS. The baseline anthropometric measurements were analysed using ANOVA according to the type of exercise training intervention. For all other parameters, non-parametric tests were adopted. To test the significance of changes after intervention, a Paired Sample t test was used for continuous variables and a Wilcoxon signed-rank test for non-parametric variables. P values of ≤ 0.05 were accepted as being statistically significant.

Results

None of the exercise interventions had any effect on body mass index (BMI), but HI:LO and LO:HI resulted in statistically significant reductions in waist circumference when comparing pre- to post-intervention values; however, this change was not statistically significant after adjusting for baseline values. There were no statistically significant differences in the subjective appetite responses among the studied groups, either pre- or post-intervention. Plasma ghrelin and PYY concentrations were associated with similar findings for subjective appetite, as there were no statistically significant changes after all interventions.

Conclusions

Eight weeks of different intensities and volumes of regular aerobic exercise and progressive resistance training did not significantly influence subjective appetite or the plasma levels of the gut-derived appetite-regulating hormones, total ghrelin and PYY in overweight and obese adults who participated in four interventional supervised programs. This result is contrary to what might have been expected from several acute studies, which showed an "acute exercise-induced anorexia" effect. The "no change" effect of chronic exercise on appetite seen in this trial indicates the need for future studies that define the potential roles of different modes and intensities of chronic exercise in weight regain prevention programs, particularly in those subjects on diet-induced weight loss program who have persistent changes in appetite that may ultimately stimulate food intake and obesity relapse.

Statement of originality

I declare that the work presented in this thesis, to the best of my knowledge and belief, is original except as acknowledged in the text and is entirely my work. I also declare that this thesis has not been published elsewhere or submitted for a degree in this or any other universities.

Signature:

Date:

Friday, April 4, 2014

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List of abbreviations

1RM	1-repetition maximum
ACSM	American College of Sports Medicine
AE	Aerobic exercise
AG	Acylated ghrelin
AgRP	Agouti-related protein
ARC	Arcuate nucleus
AUC	Area under the curve
BAL	Energy balance
BBB	Blood brain barrier
BMI	Body mass index
CART	Cocaine and amphetamine-related transcript
CCK	Cholecystokinin
CNS	Central nervous system
CON	Control
CRH	Corticotropin-releasing hormone
CT	Computed tomography
DEF	Energy deficit
DMN	Dorso-medial nucleus
DVC	Dorsal vagal complex
DVN	Dorsal motor nucleus of vagus
EARS	Electronic Appetite Ratings System
EI	Energy intake
GABA	Gamma-aminobutyric acid
GHS-R	Growth-hormone- secretagogue receptor
GI	Gastrointestinal
GIT	Gastrointestinal tract
GLP-1	Glucagon- Like Peptide-1
GP	General practitioner
HI:LO	High-intensity/low energy expenditure aerobic exercise training
ICV	Intracerebroventricular

LHA	Lateral hypothalamic area
LO:HI	Low-intensity/high energy expenditure aerobic exercise training
LO:LO	Low-intensity/low energy expenditure aerobic exercise training
WC	Waist circumference
MC	Melanocortin
MET	Metabolic Equivalent
MRI	Magnetic resonance imaging
NPY	Neuropeptide Y
NTS	Nucleus of the tractus solitarius
OA	Osteoarthritis
OXM	Oxyntomodulin
PA	Physical activities
PA	Area postrema
POMC	Pro-opiomelanocortin
PP	Pancreatic Polypeptide
PRT	Progressive resistance training
PVN	Paraventricular nucleus
PYY	Peptide tyrosine tyrosine
RT	Resistance training
T2DM	Type 2 diabetes mellitus
VAS	Visual analogue scale
VAT	Visceral adipose tissue
VMN	Ventromedial hypothalamic nucleus
WHO	World Health Organisation

1 Introduction

1.1 Obesity

Overweight and obesity are conditions in which excess body fat accumulates in adipose tissue depots around the body and impairs health [1-4]. The distribution of this additional fat varies between men and women: in men, fat mainly accumulates around the waist (android distribution), and in women it accumulates around the buttocks (gynoid distribution) [4]. Various methods to measure body fatness have been used including cadaver analysis, computed tomography (CT), magnetic resonance imaging (MRI), dual-energy X-Ray abosorptiometry, bioimpedance analysis and body mass index (BMI) [4]. However, the most widely used index of obesity is BMI, which is calculated by body weight in kilograms (kg) divided by height in square meters (m^2), BMI = kg/m^2. The desirable or healthy BMI ranges between 18.5 to 24.9 kg/m^2 [1].Overweight ranges between BMI of 25 to 29.9 kg/m^2, and a BMI equal or above 30 kg/m^2 is defined as obesity [1, 4].

1.1.1 World overweight and obesity prevalence rates

The prevalence of obesity has doubled since 1980 [1]: between 1980 and 2008, worldwide mean BMI increased by 0·4 kg/m^2 and 0.5 kg/m^2 per decade for men and women, respectively [5]. In 2008, the World Health Organisation (WHO) estimated that more than 1.4 billion adults aged 20 and older were overweight; and of these, over 200 million men and nearly 300 million women were obese [1]. It is in fact estimated that more than one in ten of the world's adult population is obese [1]. Among the high-income countries, people in the United States had the highest mean BMI [5]. In 2007-2008, 68% of Americans were overweight or obese and approximately 34% were obese [2]. Similarly high rates of overweight and obesity were reported in Oceanic countries [5]: both female and male BMIs increased to 33·9 kg/m^2 (32·8—35·0) for men and 35·0 kg/m^2 (33·6—36·3) for women in Nauru in 2008 [5]. In nine Oceanic countries female BMI increased by more than 2.0 kg/m^2 per decade [5].

1.1.2 Australian overweight and obesity prevalence rates

The prevalence of overweight and obesity has increased in Australia, from 56.3% in 1995 to 61.2% in 2007–08 [6]. In the 2011-2012 National Australian Health survey, about $\frac{2}{3}$ (63.4%) of adult Australians were overweight (35.0%) or obese (28.3%) [6]. More males than females were overweight or obese – 70.3% and 56.2%, respectively [6].

1.1.3 Diseases associated with obesity

Overweight and obesity are associated with high rates of morbidity and mortality [2]. Raised BMI negatively affects most of the body systems and is related to several non-communicable diseases or states of greater health risk: e.g., type 2 diabetes, dyslipidaemia, coronary artery diseases, hypertension, sleep apnoea, cognitive dysfunction, non-alcoholic fatty liver, and some malignancies such as breast, colon and prostate cancer [1, 2]. WHO records indicate that overweight and obesity are the fifth leading cause of death worldwide, and 2.8 million adults die annually as a direct result of overweight and obesity [1].

1.1.4 Obesity prevention

The increasing prevalence of overweight and obesity indicates the need for improving current intervention strategies. Several types of interventions for obesity management have been used, including healthy diet, regular exercise, behavioural modification, pharmacological treatment and bariatric surgery [3]. Among all the interventions, bariatric surgery provides substantial and sustained weight loss compared to the other conventional interventions. In the large majority of cases (>90%), bariatric surgery is effective, safe, and well tolerated [7]. Data suggest that gastric bypass surgery results in greater weight loss than other procedures; but this is based on a very limited number of trials, and there are still safety and cost concerns regarding it in comparison with the others [8].

Lifestyle programs are multifactorial interventions tailored to the needs of an individual or of a group of subjects according to their risk factors status [3]. Lifestyle modification includes dietary modification and physical exercise [7]. However, food restriction alone may not solve the problem of excess weight unless it is accompanied by physical training [7]. Lifestyle modifications can be effective in the prevention and treatment of obesity in the mid- to long-term, leading to significant reductions in blood pressure, blood lipids and blood glucose levels [3].

1.2 Exercise

Physical activity (PA) is recommended as one of the important components of any weight management program that may also include energy restriction, pharmacological and surgical interventions. Physical activity in the context of weight management aims for weight gain prevention, weight loss, and the prevention of weight regain after weight loss [9-12]. These positive impacts are achieved fully or partially in all types of exercise, such as the

unstructured regular lifestyle activities and the structured exercises that include various forms of aerobic exercises (AE) and progressive resistance training (PRT) [12]. However, the extent of the influence of physical activity on body weight, metabolism and body fitness relates to the extent of reduction in energy intake [11], the mode and the intensity of exercise, which have dose-dependent effects [9]. The next section reviews the health benefits of physical activity, discusses the various types of exercise, and defines their various roles in weight loss, weight maintenance, body composition and fitness. It also discusses its benefits for some kinds of common chronic illnesses that relate to increasing body weight – namely, diabetes, hypertension and osteoarthritis.

1.2.1　The health benefits of physical activity

There is wealth of information available showing that increasing levels of all types of physical activities provide health benefits, which are attributed largely to the positive effects of physical activity on weight gain prevention, weight loss and prevention of weight regain after weight loss [9, 10, 13, 14]. No randomised controlled trials were published on physical activity as a cause of obesity, and there are significant scientific data to support a causative link between lack of physical activity and increasing BMI [10]. Other positive effects of various types of physical activity include effects on obesity-related chronic illnesses, such as atherosclerotic cardiovascular diseases [13], type 2 diabetes mellitus [15], hypertension [16], hyperlipidaemia [13], osteoarthritis [17] and some cancers [9, 10]. Moreover, exercise training improves body composition [16], cardio-respiratory fitness [18] and cardiovascular risk profile [13], and all translate to better health outcomes [15]; while other benefits include positive effects in managing asthmatics [9, 18]. Some of these health benefits are achieved independently of weight loss, and, perhaps, even if weight is gained [13]. Progressive resistance training has not traditionally been promoted in weight loss interventions, possibly because the energy expenditure associated with progressive resistance training is limited [13]. However, progressive resistance training may increase muscle mass, which may in turn increase 24-h energy expenditure [13].

1.2.2　Types of physical activity

Numerous categorizations of types of physical activity are used in the literature [13, 19], which leads to difficulties in assessing and comparing results of various physical activity studies [20]. So in this study, physical activities are categorized into two main types: unstructured lifestyle activities and structured exercises – the latter including various forms of

aerobic exercise and progressive resistance training. Published data indicate that the intended effects of physical activity depend on sound understanding of the mode of exercise performed, and its intensity – as discussed below [20].

1.2.2.1 Lifestyle activities

This term refers to all activities an individual performs in the course of daily life that can contribute to significant energy expenditure [21]. Lifestyle activities may be defined, therefore, as all forms of unstructured PA not intended to constitute part of structured exercise [13]. Levine et al. (2007) have developed the concept of non-exercise activity thermogenesis (NEAT) which they define as "all energy expenditure that is not from sleeping, eating, or planned exercise programs" [22]. Examples of these activities include occupational activity, commuting, work around the home and leisure activities [14]. Several methods have been used to measure this type of physical activity and its resultant energy expenditure, including subjective self-reported physical activity questionnaires such as the International Physical Activity *Questionnaire* IPAQ [23], and more objective measurements using pedometers, inclinometers, accelerometers, or doubly-labelled water assessments [14, 20, 22, 24].

Numerous studies have hypothesized that higher levels of lifestyle activity prevent initial weight gain as well as assisting in weight loss and weight loss maintenance after weight loss [14]. A systematic review of the literature shows that an increase in the number of steps walked by 2100 per day, as measured by a pedometer, can decrease BMI by a modest yet significant 0.38 kg/m^2 [21]. However, methodologies of numerous studies conducted on lifestyle physical activity are inconsistent; because researchers use a wide variety of assessment methods, diverse populations and are without clear definitions of lifestyle physical activity [12]. Despite these limitations of published studies and the reported modest effect of lifestyle physical activity, it is reasonable to recommend its increase as a strategy for weight management [12].

1.2.2.2 Structured exercises

The lack of precise definition of lifestyle physical activity and its modest effects indicates the need for more planned, supervised and structured types of physical activity. In this regard, the scientific literature refers to two main categories of structured physical activity, namely:

1. aerobic exercise that includes aquatic physical activity; and

2. progressive resistance training.

Each type has relatively different goals and benefits, as outlined later in this section [12, 19]. According to the Australian National Health Survey (2012), 74% of respondents reported that they were not involved in any type of structured physical activity [6].

1.2.2.2.1 Aerobic exercise

Aerobic exercise is any brisk exercise that increases the circulation of oxygen and is accompanied by increased breathing rates [12]. Forms of aerobic exercise include walking, jogging, running, cycling, swimming and some endurance game activities: in all, large muscle groups are utilized in a rhythmic fashion [18]. Benefits of aerobic exercise include reduced risk of acute coronary ischaemia, of stroke and of overall cardiovascular mortality, as well as improved blood lipid profiles, blood pressure and inflammatory markers [25]. Aquatic exercise has been recommended as a more comfortable form of physical activity for overweight or obese individuals who have potential functional limitations [9]. One of the few studies published in this area aimed to assess the effects of combined supervised aquatic-exercise and walking compared to walking alone on the body weight of 44 obese women over a 16-week period. The study suggested that aquatic exercise in combination with walking can improve functional status and is a better alternative than walking alone for overweight women [26].

1.2.2.2.2 Progressive resistance training

'Resistance training' or 'progressive resistance training' refers to all forms of training that cause muscles to contract against resistance, thus aiming to increase muscle strength, mass, tone and/or endurance [27]. Progressive resistance training is well known as an effective method for developing musculoskeletal strength, muscular fitness, and the prevention as well as the rehabilitation of musculoskeletal injuries [20, 28]. In progressive resistance training, multi joints and large muscle groups are involved in rhythmic fashion [19]; and training programs usually consist of working on all the major muscle groups in order to strengthen them [19] (in older adults, it is advised that these training programs should focus on multi-joint exercises [27]). Progressive resistance training usually stimulates skeletal muscles to synthesize new muscle protein and undergo hypertrophy [19]: to improve its effects on increasing muscle mass, it is recommended to do 1-2 sets of 8 to 12 repetitions per set with an intensity more than 60% of 1-repetition maximum (1RM), with 8 to 10 exercises per session and 2-3 sessions weekly [20, 28]. Evidence suggests that maintenance of a large lean

body mass may decrease metabolic risk factors or disease including obesity, type 2 diabetes mellitus and dyslipidaemia [15, 19]. In addition to the muscular benefits of progressive resistance training, it can improve insulin-stimulated glucose uptake, may be an alternative to aerobic exercise in modifying metabolic risk factors [29], and may increase bone mineral density [30]. However, further evidence is needed to support the potential benefit of progressive resistance training in weight loss and weight management [12, 19].

1.2.3   Benefits of physical activity for weight loss and subsequent weight maintenance

Available data support the benefits of increasing daily activity in prevention of the global epidemic of obesity. The benefits on long-term weight loss have been reported in overweight individuals, in patients with severe obesity (BMI $\geq$ 35kg/m^2), and even in patients who have undergone bariatric surgery [9]. Physical activity seems to be an important component of lifestyle interventions for weight loss and maintenance. The 2009 stand of the American College of Sports Medicine [12] stresses the importance of increasing the levels of physical activity for better weight loss and preventing weight regain after weight loss [13, 14].

1.2.3.1   Benefits of physical activity on weight gain prevention in non-obese

Evidence is accumulating regarding the role of an active lifestyle in prevention of substantial weight gain and obesity with age; but also indicating early promotion of physical activity in non-obese adults who aim to maintain their normal weight throughout life [31]. In the consensus statement of the American College of Sports Medicine, researchers indicated a moderately strong relationship between the risk of developing obesity and decreasing levels of physical activity [32]. Furthermore, another prospective study conducted on 6223 men and women indicated a positive relationship between increasing physical activity duration and weight maintenance in a study group that had weight, peak VO$_2$, and other parameters measured at each of three visits. The study reported that for each 1-minute improvement in exercise on the treadmill – as an indication of increased physical activity and fitness – there was 21% reduction in the odds of gaining 10 kg or more in both sexes over the 7.5 years study period [31].

According to the most recent stand by the American College of Sports Medicine, it is now evident that physical activity of 150 to 250 min$^.$wk^{-1} with an energy equivalent of 1200

to 2000 min.wk^{-1} (e.g., brisk walking of approximately 20-32 kilometres per week) is effective to prevent weight gain greater than 30% in most adults [13].

1.2.3.2 Benefits of physical activity on weight loss

It is already evident that a negative energy balance generated by physical activity will result in weight loss, and this effect is greater the more the negative energy balance [13]. The earlier (2001) American College of Sports Medicine guidelines [14] indicate that a minimum of 150 min.wk^{-1} of moderate intensity physical activity for overweight and obese adults is recommended for health improvement; however, for long-term weight loss more physical activity is recommended – 200–300 min.wk^{-1} [14]. The updated guidelines from 2009 [13] indicate that 150 min.wk^{-1} physical activity promotes only initial minimal weight loss and stresses greater amounts of physical activity (more than 250 min.wk^{-1}) being needed to achieve clinically significant weight loss [13]. It also indicates a dose-response relationship between physical activity and loss of weight, as physical activity of >150 min.wk^{-1} results in modest losses of 2 to 3 kg, whereas >225–420 min.wk^{-1} results in losses of 5 to 7.5 kg over a year [13]. Thus, higher doses of physical activity may provide a loss of 3% or more of initial weight [13].

The effects of physical activity on body weight depend not only on the duration, but also on the intensity of the physical activity [9, 13, 19]. Intensity is usually measured by a unit termed 'Metabolic Equivalent' (MET), which describes the energy expenditure of a certain activity where 1 MET equals 3.5ml O_2/kg/min [33]. For example, a 4 MET activity expends 4 times the energy used by the body fat at rest (1MET), and so on [33]. According to the Physical Activity Guidelines for Americans [33], light intensity is defined as 1.1 to 2.9 METs, moderate intensity activity is 3.0 to 5.9 METs, and vigorous activity is 6 METs or more [33]. For example, walking at 4.8 kilometers per hour requires 3.3 METs of energy expenditure and is therefore considered a moderate intensity exercise.

Conflicting findings are reported on the effects of physical activity on weight loss in both sexes [13, 34, 35]. In a randomised controlled trial over 16 months, involving 225 min. of moderate intensity physical activity per week, Donnelly et al. (2003) find a minimal but significant difference of 0.4 kg between males and females in favor of men, who lost 5.2 kg versus the women, who lost 4.8 kg compared with controls [34]. However, Stefanick et al. (1998) have not found any similar gender difference [35].

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When it comes to the mode of exercise in weight loss, most studies focus on the role of various forms of aerobic exercise rather than on progressive resistance training [10], although it is now evident that the latter is associated with loss of fat mass and increase of fat-free mass, as well as decreasing health risks [13, 19]. Evidence also indicates that progressive resistance training even without concomitant weight loss may reduce health risks and improve metabolic factors in overweight and obese subjects [16]. These modifications include positive effects on blood lipids profiles [36], improving insulin sensitivity [37], decreasing glycosylated haemoglobin HbA1c [38] and reducing glucose-stimulated circulating insulin concentrations [13, 19]. All of these effects may lead to reduction in the complications of metabolic disorders [19].

However, physical activities alone may not be sufficient to promote weight loss, unless accompanied by energy restriction. Studies that investigate the chronic effects of different doses of exercise on weight change in adults *without* associated reduction of energy intake are few. In this regard, Jakicic et al. (2011) study the effect of different intensities of physical activity (moderate PA, high PA and a self-help control group) on weight change in 278 overweight adults, with no reduction of energy intake, over 18 months: participants who reduce their body weight by more than 3% increase physical activity by 162 minutes per week above baseline levels, compared with only 78.2 and 74.7 minutes per week in those participants who maintain their body weight within 3% of their initial weight or gain more than 3%, respectively [39]. When physical activity is added to the energy-restricted diet, the resultant weight loss after 6 months is 8.2 kg, which improves by 25% to 10.9 kg after combining both strategies in 130 severely obese subjects [40]. This effect of PA to enhance weight loss is greater when moderate, compared with severe dietary restriction [40]; possibly because the metabolic adaptations to severe energy restriction [41] may prevent any further benefit from physical activity on weight loss. In any case, these studies demonstrate overall the importance of adding physical activity programs to any dietary restriction program to maximize weight loss and improve the related health benefits [13].

Increasing the levels of physical activity and other behavioral therapies such as self-monitoring, goal-setting, problem-solving, meal replacements and food provision [42] are recommended in conjunction, also, with pharmacological and surgical interventions for further weight loss and weight maintenance as demonstrated in various studies [13, 14].

Finally, it is already noticed that individual responses to exercise vary, as some people do not experience the beneficial effects of exercise on body weight [43]. This finding is explained based on the various compensatory behavioural and metabolic responses in some individuals, which include changes in feelings of hunger or fullness and associated changes in food intake [43]. This inter-individual variability in the effects of PA on body weight needs further research to identify those at risk of not benefiting and the reasons behind this drawback in some subjects [43]. Regardless of the variability in effects of physical activity on body weight, regular physical activity should be recommended as an important component of any weight loss program, due to other benefits to health and weight maintenance, as outlined below.

1.2.3.3 Benefits of physical activity for weight maintenance

Physical activities are crucial for primary prevention of weight gain, promotion of long-term weight loss and the prevention of weight regain after weight loss. The maintenance of an initially normal weight, not weight reduction, should be the primary goal for exercise, because weight loss in already overweight or obese individuals is notoriously difficult. In this regard, many studies invariably point to the role of regular physical activities as a cornerstone of primary prevention of excess weight in early life as well as for weight maintenance throughout life [10].

1.2.3.4 Benefits of physical activity on weight regain prevention in post-obese

Physical activity is a critically important behavior for promoting long-term weight loss and prevention of weight regain [9]. Indeed, physical activity is often promoted as the best predictor of weight maintenance after weight loss [44, 45]. This observation is supported by the recommendations in 2009 of the American College of Sports Medicine, which recommends more than 250 minutes per week of physical activity for maintenance of lost weight [12]. It also indicates that several studies support the value of 300 minutes or more per week of physical activity to reduce weight regain [12] – which recommendation is consistent with earlier empirical studies [9]. However, the positive effects of physical activity on weight maintenance are reported largely in cross-sectional and prospective studies but not in well-designed randomised controlled trials [12]. Several studies indicate that the long-term effect of physical activity on prevention of weight regain is better when physical activity is accompanied by dietary restriction and behavioural changes, as almost $\frac{1}{3}$ of those who have

lost weight regain the initial weight within one year [46]. Interestingly, physical activity is also recommended in post-bariatric surgery patients to prevent weight regain. In this regard, greater weight loss has been reported at 6 and 12 months after obesity surgery amongst patients who participated in 150 minutes or more of exercise per week, compared with those participating for fewer than 150 minutes per week [47]. Overall, these data demonstrate the importance of physical activity for preventing weight regain after a variety of weight loss interventions.

1.2.3.5 Benefits of physical activity on body fitness and composition

To judge the effectiveness of physical activity by its direct effect on the weighing scale is insufficient and possibly even misleading [10], as physical activity has many health benefits in addition to its major role in weigh management. Physical activity improves both body fitness and fatness (i.e., body composition) [9]. Benefits to body fitness are related to the positive effects of PA on the functions of several systems – e.g., the cardio-respiratory and musculoskeletal systems – in addition to its positive effects on various metabolic processes. The Aerobics Center Longitudinal Study and numerous other studies indicate that cardio-respiratory fitness is a predictor of all-cause mortality and cardiovascular disease, independent of measures of body fatness [9]. Cumulative data in the literature suggest that physical activity improves cardio-reparatory functions in overweight and obese patients, and this is achieved regardless of whether weight is lost or not [9]. Similar positive effects were not observed when only dietary restriction was used. It has also been reported that improving fitness is associated with lower mortality rates, decreasing by 7.9% for each minute of improvement on a treadmill test [48]. Nocon et al. 2008 [49], systematically review 33 studies that involve 883,372 participants, finding that the majority of studies report significant risk reduction of mortality in active subjects. Physical activity is associated with marked risk reduction of cardiovascular mortality of 35% and all-cause mortality by 33%, even after adjusting for the other relevant risk factors in both men and women [49]. Available published data also suggest that both cardio-respiratory fitness and body fatness may influence selective risk factors for diabetes and cardiovascular disease [9, 19]. Therefore, interventions for overweight and obese adults that focus on both weight loss and improvements in cardio-respiratory fitness may provide the most significant improvements in health outcomes [9].

Besides benefits of fitness in reducing mortality rates, physical activity improves body composition: this effect varies according to the mode of physical activity used. For example, progressive resistance training increases fat-free mass when performed alone or in combination with dietary restriction [13]; and this effect also increases when combined with aerobic exercise, compared with progressive resistance training alone [9, 13]. The favourable effects of progressive resistance training with or without aerobic exercise on body fat distribution are described as changes in waist circumference, waist to hip ratio, and waist to thigh ratio in large observational studies [10]. Findings also demonstrate that progressive resistance training may be an effective alternative to improving body composition and maintaining reduced fat mass in obese patients, as it preferentially mobilises the visceral and subcutaneous adipose tissue in the abdominal region [19]. Recent data suggest that aerobic exercise is important in exercise programmes aimed at reducing visceral adipose tissue (VAT) [50].

1.2.4 Benefits to chronic illnesses of physical activity

The benefits of physical activity in the management of several chronic illnesses – or what have been recently referred to as the "chronic diseases of physical inactivity" [51] – have already been acknowledged in the literature [12, 15-19]. The list of these illnesses is long, and most are related to the increasing body weight seen in inactive populations [9]. Health and fitness professionals should be aware of these benefits, and consider prescribing various types of physical activity for the affected patients [20].

Next are discussed three of the common illnesses that may benefit from physical activity.

1.2.4.1 Diabetes mellitus

There is growing evidence that the increasing global prevalence rates of type 2 diabetes mellitus (T2DM) and pre-diabetes conditions (namely, impaired glucose tolerance and impaired fasting glucose) are linked to the steadily increasing rates of overweight and obesity [13, 15, 19]. This association has been attributed to recent changes in lifestyle, particularly lack of physical activity. Regular physical activity, in association with other lifestyle changes – dietary restriction – is effective in improving glycaemic control amongst people with pre-diabetes, as well as for the prevention and treatment of T2DM [15, 19]. In an observational study on 3,757 participants, standing or walking around the home for two hours daily was associated with a 9% and a 12% reduction in obesity and T2DM rates respectively, compared

with 24% and 34% reductions in obesity and T2DM rates respectively when brisk walking of one hour per day was performed [52]. In a more recent randomised controlled trial on a group of people with T2DM, Karstoft et al. (2012) report the importance of using physical activity in diabetics for improving physical fitness, body composition and glycaemic control [38]; so physical activity program should be a core component of any management plan for diabetic or pre-diabetic patients [15]. In this regard, and as with recommendations for the general, non-obese population, a minimum of 210 min per week of moderate intensity exercise, or 125 min per week of vigorous intensity exercise with no more than two consecutive days without training, are recommended for both diabetic and pre-diabetic patients [15]. Two or more progressive resistance training sessions per week are also recommended, within the total 210 or 125 min of moderate or vigorous exercise [15]. Ideally, the exercise should consist of a combination of some aerobic and some progressive resistance training; however, if only one modality is feasible, then either modality alone is effective [15, 19].

1.2.4.2   Hypertension

High blood pressure or hypertension is a common clinical condition that is a leading contributor to premature death and cardiovascular co-morbidities [16]. Lifestyle modification including increasing physical activity is one of the important first-line treatments for hypertensive patients. Aerobic exercise is regarded as a first approach in the primary prevention and treatment of hypertension. In contrast, evidence is lacking for benefits of progressive resistance training in lowering high blood pressure [16, 19], although resistance training will still provide health benefits to this population (as to the general populace). Patients with hypertension will thus benefit from a mix of moderate to vigorous aerobic activity in addition to progressive resistance training [16]. However, patients should be cautious when they perform certain types of vigorous progressive resistance training – such as lifting heavy weights – as it may have a pressor effect on blood pressure [16].

1.2.4.3   Osteoarthritis

Osteoarthritis is a chronic joint disease that affects mainly the lower body joints, particularly the hip and knee joints [17]: patients can suffer from severe joint pain, swelling, stiffness, joint instability and weakness [17]. Various types of exercise are recommended for improving the function and symptoms of osteoarthritis, including aerobic, resistance activities and aquatic exercises [17]. For the majority of patients a combination of physical activity such as aerobic and local strengthening activities is optimal [17]. These benefits of physical

activity are maximised when associated with other lifestyle modifications that reduce body weight.

1.3   The neuroendocrine control of appetite

The studying of the effect of different types of exercise on appetite and energy intake was the subject of several publications over the last decade. These aim to investigate the basic mechanisms responsible for exercise-related changes in appetite and why certain types of exercise increases hunger and others reduce it – as will be discussed later in section 4 – which are not yet well understood [53-56]. Therefore it is important to understand the physiology of normal appetite control and its role in the regulation of body energy homeostasis, particularly during the performance of various acute and chronic physical activities.

The last decade has witnessed great advances in understanding of the complex integration between peripheral and central neuroendocrine mechanisms that regulate food intake and energy expenditure through diverse hormonal, neuronal and metabolic signals [53, 57, 58]. Peripheral hunger and satiety signals require central integration to achieve efficient energy homeostasis [59]. Hormones released from the gut (the largest endocrine organ in the body [60]) and the adipose tissue play a key role in the regulation of food intake and energy homeostasis [53, 57]. In addition to these peripheral hormonal signals, the afferent neuronal vagal signals constitute feedback mechanisms that ultimately regulate appetite and subsequently food intake centrally at higher brain centres, including the hypothalamus and brainstem [61, 62]. This gut-brain axis has been described as the communication line between the gastrointestinal tract and the central nervous system (CNS) in modulating food intake [57, 58, 61].

In the next section, the various roles of the central nervous system on appetite regulation and energy homeostasis are discussed – particularly the hypothalamus and its important nuclei such as the arcuate nucleus that plays an important role in appetite regulation. In addition, the roles of the brainstem and the gastrointestinal vagal afferents are briefly reviewed. This is followed by discussion of five appetite-related gut hormones and their roles in appetite regulation. Towards the end of the section, the use of appetite regulating gut hormones in obesity treatment is discussed.

1.3.1 The role of the central nervous system in the regulation of appetite

Appetite regulation is controlled by complex neuronal pathways that have reciprocal connections between the hypothalamus, brainstem and higher cortical areas [61]. These central nervous system sites interpret and integrate the various signals to provide coordinated feeding and energy expenditure responses to prevailing conditions [62]. In this regard, the hypothalamus and brainstem are the main central nervous system areas that regulate body energy homeostasis, as they receive peripheral neural and hormonal signals that relay information about nutritional state and adiposity [57]. Signals from the periphery – e.g., gut hormones – may influence food intake at three sites:

- the vagus nerve,
- brainstem and
- hypothalamus [58].

Signals from higher cortical centres are integrated with peripheral signals within hypothalamic nuclei [61]. Clearly, there is a complex neuronal network within the central nervous system that controls energy homeostasis [58].

1.3.1.1 The role of the hypothalamus

The hypothalamus is located at the base of the brain, around the third ventricle [63]. It is an important region of the central nervous system that integrates signals from peripheral and central pathways, and plays a significant role in the regulation of a number of fundamental biological processes [63] – e.g., regulation of energy homeostasis [64] and appetite regulation [59]. Early experiments on the hypothalamus indicated that the lateral hypothalamic area is the "hunger centre" and that the ventromedial hypothalamic nucleus is the "satiety centre" [65]. However, later studies demonstrated that *more* nuclei and neuronal circuits are involved in the process of appetite control, and that these interact with brain stem and other higher cortical areas in the CNS [61]. In addition to this indirect access to the hypothalamus, there is an incomplete blood brain barrier at the median eminence of the hypothalamus and the area postrema of the brainstem, allowing direct access of peripheral circulating factors to the hypothalamus through fenestrated capillaries [57, 66, 67].

1.3.1.1.1   The arcuate nucleus

The arcuate nucleus (ARC) of the hypothalamus is an important one, that acts as the site of integration of a number of neurological and blood-born signals [61] and therefore plays major roles in the regulation of food intake and energy homeostasis [57]. It integrates afferent inputs of different endocrine and neuronal signals – often derived from the periphery – and forwards these messages to other brain centres, which send the appropriate efferent response signals [53]. Also, the arcuate nucleus communicates with other nuclei in the hypothalamus, such as the lateral hypothalamic area and dorso-medial nucleus [61]. In the arcuate nucleus there are two major neuronal populations with opposing effects on food intake and weight gain: namely, the orexigenic or appetite-stimulating neurons, and the anorexigenic or appetite suppressing neurons [57, 63]. Both of these neuronal populations project into the adjacent paraventricular nucleus [61]. The balance between activities of these neuronal circuits is critical to body weight regulation [59]. In this regard, there are many neuropeptides that influence short- or long-term changes in body weight and associated factors when they are administered centrally [41]. These include orexigenic signals such as galanin, melanin concentrating hormone, g-aminobutyric acid, hypocretins/orexins, and the anorexigenic oxytocins and the corticotropin-releasing hormone family of peptides as well as neurotensins [41]. However, the following will focus on major neurotransmitters whose expression is known to be altered by negative energy balance.

1.3.1.1.1.1   The orexigenic neurons

Orexigenic neurons are those containing neuropeptides that stimulate appetite [63] and are medially located in the arcuate nucleus of the hypothalamus. These neurons co-express neuropeptide Y (NPY) and Agouti-related protein (AgRP) [57, 68]. The two are commonly discussed in the literature.

1.3.1.1.1.1.1 NPY and AgRP

NPY, a 36-amino acid peptide expressed in the central and peripheral nervous systems, is one of the most powerful orexigenic agents known [69]. NPY and the other members of the NPY family exert their effects via at least five known Y receptors – Y1, Y2, Y4, Y5 and Y6 [69]. Of these five, NPY is thought to stimulate food intake through interactions with target neurons at the hypothalamic Y1 and Y5 receptors particularly [69]. A recent study on mice indicates that both are needed in mediating hyperphagic effects, hence dual deletion of Y1

and Y5 was needed to reduce food intake [69]. In contrast, AgRP increases food intake through antagonism of melanocortin (MC3) and (MC4) receptors [61, 70]. NPY and AgRP form a feedback loop with pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART) neurons through the release of CART and of the neurotransmitter gamma-aminobutyric acid (GABA), and via interactions with MC3 and Y1 receptors expressed on POMC/CART neurons [70-72]. Also, NPY/AgRP neurons project extensively to other nuclei within the hypothalamus, including the paraventricular nucleus LHA and dorso-medial nucleus [61]. A single intra-cerebroventricular injection of NPY acutely stimulates feeding in rodents [70]; while injection of NPY antagonists centrally reduces their food intake, and chronic administration of NPY into the PVN induces sustained hyperphagia and weight gain [70].

In addition to the central mechanisms that affect the function of NPY/AgRP neurons, these neurons are influenced by signals from peripherally-secreted, appetite-related hormones such as leptin, which is an adipose-derived satiety hormone, and insulin, which has similar effects on appetite. NPY/AgRP are also influenced by gut-derived, appetite-regulating hormones including the satiety hormones peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1); and the hunger-promoting hormone ghrelin [70]. Therefore, the NPY/AgRP secreting neurons are activated by ghrelin and inhibited by PYY and GLP-1 and the other appetite-related hormones that suppress food intake such as insulin and leptin [70]. Leptin is secreted by white adipose tissue cells at concentrations proportional to fat mass, with a relatively long half-life. Leptin administered to rodents peripherally or centrally reduces food intake and body weight and increases energy expenditure [62]. In contrary, low circulating levels of appetite-suppressing hormones such as leptin, insulin and PYY_{3-36}, and/or raised levels of ghrelin similar to those observed during fasting, lead to increase in NPY/AgRP neuronal activity and increased appetite [70].

1.3.1.1.1.2 The anorexigenic neurons

The arcuate nucleus of the hypothalamus also contains anorexigenic neurons that inhibit appetite and oppose the orexigeneic neurons [63]. These neurons express a-melanocyte stimulating hormone (a-MSH), derived from pro-opiomelanocortin, and cocaine and amphetamine-regulated transcript [57, 61, 73].

1.3.1.1.1.2.1 alpha-melanocyte stimulating hormone (a-MSH)

a-MSH is produced from POMC and binds to MC4 receptors to suppress food intake [74]. More than 70 different mutations of MC4 have been associated with obesity, and about 6% is found in cases of severe early-onset diabetes [75]. However, Cancell et al. 2012 debate the prevailing idea that the neighbouring neurons, namely the orexigenic appetite-stimulating AgRP/NPY, increase feeding by opposing the anorexigenic actions of POMC neurons; and instead state that only AgRP neuron activation – not POMC neuron inhibition – is sufficient to promote feeding [76].

1.3.1.2   Role of the brainstem and gastrointestinal vagal afferents

Numerous studies indicate the pivotal roles of signals from the periphery in transmitting information via afferent vagal fibres to the caudal brainstem or directly to the hypothalamus [57, 58, 60, 62, 63, 70, 77, 78]. Within the brainstem, the dorsal vagal complex is crucial in relaying and interpreting these signals prior to reaching the hypothalamus [61]. The DVC consists of the dorsal motor nucleus of the vagus, the area postrema and the nucleus of the tractus solitarius [61]. Hormonal and neuronal signals from the gastrointestinal tract that relate to meals are received via the blood in the area postrema [58] and through vagal afferent fibres, and converge on the NTS of the brainstem [57]. In the NTS, the neuronal projections carry signals to the hypothalamus: these brainstem centres are reciprocally connected with hypothalamic nuclei controlling energy balance [61]. Evidence suggests that some appetite-modulating hormones affect appetite through the vagus nerve [63, 78], as afferent neurons of the vagus nerve provide a pathway for gut peptides by triggering ascending pathways from the brainstem to the hypothalamus [77]. There are various mechanisms that operate at the level of vagal afferent neurons and may modulate the effect of gastrointestinal satiety signals [77]. This pathway offers a potential advantage in that hormones are able to have an influence at a site distant from the central nervous system [78] such as the gastrointestinal. This may explain why vagotomy – transaction of the vagus nerve – abolishes some of the centrally mediated effects of the peptide gut hormones on appetite regulation [63].

1.3.2   Gut hormones and appetite regulation

The gastrointestinal tract (GIT) is described as the largest endocrine organ in the body, as it releases more than 20 different regulatory bioactive peptide hormones [57, 61]. The concept of the gut as an endocrine organ is not new, as one of its hormones, cholecystokinin (CCK),

which has appetite inhibitory actions, was reported over 35 years ago [59]. Currently, the neuroendocrine role of the gut is one of the rapidly expanding fields of multidisciplinary scientific research [59]. Over the last two decades, the physiological influence of gut hormones on appetite regulation and energy homeostasis has been a dynamic area of research [57, 60, 78]. After a meal, nutrients pass into the gastrointestinal tract and this triggers the release of a number of gastrointestinal peptides and signals to optimise the digestive process [59]. Some of these gut peptides function as short-term satiety signals and possibly long-term regulators of body weight [59].

Gut hormones contribute to short-term changes in feelings of satiety and hunger [57, 59, 62]. These peptides may reduce food intake by decreasing hypothalamic orexigenic appetite-stimulating signals and increasing anorectic appetite-suppressing signals [60]. Another effect of these peptides is to mediate inhibitory feedback on intestinal transit contributing to prolonged gastric distension and increased satiety between meals [79]. These combined central nervous system effects and 'intestinal brake' mechanisms [79] mediated by gut peptides facilitate the control of food intake and the transit of food through the GIT after meals [57]. The functions of appetite-regulating gut hormones vary: e.g., ghrelin is referred to as the appetite-stimulating hormone, or hunger hormone, whereas PYY, PP, GLP-1 and CCK act as satiety or appetite-suppressing hormones. Research indicates that gut hormones can be manipulated to regulate energy balance and that obese individuals retain sensitivity to the actions of gut hormones [59]. The positive effects achieved by bariatric surgery are partially related to its effects on modulating secretion of several gut-derived hormones including decreasing ghrelin secretion and increasing PYY or other satiety-inducing gut hormones [60].

1.3.2.1 Ghrelin

Ghrelin is the only known appetite-stimulating gut-derived hormone, and is thus sometimes called "the hunger hormone" [60]. Ghrelin was first discovered as an endogenous ligand for the growth hormone secretagogue receptor; and is released into the circulation from the stomach [80, 81]. It is produced and released mainly by the gastric oxyntic cells, with the remaining circulating ghrelin being released by parts of the small intestine (duodenum and ileum) and the large intestine (caecum and colon) [62]. Ghrelin is composed of 28 amino acids and is modified by the addition of an octanoyl group to the serine residue at position three [81]. This acylation is necessary for ghrelin to bind to the GHS-R and to cross the blood brain barrier [81].

Ghrelin stimulates appetite and increases food intake, with circulating levels rising during fasting and falling after nutrient ingestion, in both rodents and humans [60]. In rats, central or peripheral administration of acylated ghrelin stimulates food intake and growth hormone release acutely, while chronic administration causes weight gain [82]. In humans, intravenous infusion or subcutaneous injection of ghrelin increases feeling of hunger and food intake [64, 83, 84]. Ghrelin release is not related to gastric distension, but to food ingestion, as it is observed that its release is not inhibited following water ingestion [82].

Circulating ghrelin concentrations exhibit a diurnal rhythm that gradually rises throughout the day, reaching a zenith between 0100 and 0200 h [83]. Ghrelin levels also change in response to fasting and meals; rising 1–2 h before the initiation of a meal and falling to trough levels 1–2 h after a meal [83]. Considerable evidence in the literature implicates the involvement of ghrelin in short-term energy homeostasis through its effects on mealtime hunger [83, 84]. Data also suggest that ghrelin plays a role in meal initiation [60, 83]. High plasma concentrations of ghrelin occur amongst individuals initiating meals voluntarily without time- or food-related cues, and also before every meal, in subjects who follow fixed feeding schedules [84]. It is also reported that ghrelin injections stimulate food intake rapidly and transiently by increasing the number of meals and meal initiations [84]. Wren et al. (2001) indicate that ghrelin infusion at physiological concentrations stimulate food intake by 28% at a subsequent free-choice meal and increase overall food intake over a 24-hour period compared with saline infusion [85]. Spontaneous changes in endogenous circulating ghrelin levels are probably triggered by sympathetic nervous output [83, 86]. In addition to these short-term effects of ghrelin on energy homeostasis and its probable role in meal initiation [83], ghrelin is involved in long-term weight regulation, as it fulfills the criteria for an adiposity-regulating hormone [54, 83, 84]. For example, circulating ghrelin levels are elevated in individuals with anorexia nervosa [86], but fall after weight regain [54, 86]. In contrast, low levels of plasma ghrelin rise after weight loss in obese subjects, which indicates an inverse correlation with adiposity [54, 59, 87, 88]. [59, 87, 89]. Circulating levels of ghrelin are also negatively correlated with body fat, fat mass [54, 59] and BMI [84], as well as circulating concentrations of leptin [86, 89] and insulin [54]. In obese subjects, plasma ghrelin decreases in direct proportion to the degree of insulin resistance [89].

1.3.2.2 Peptide tyrosine tyrosine (PYY)

PYY is a 36-amino acid appetite-suppressing gut peptide hormone [64]. Like similar peptides, NPY and Pancreatic Polypeptide (PP), PYY belongs to the 'PP-fold' family of peptides [57]. These are all 36 amino acids in length and share a common tertiary structural shape known as the PP-fold [57, 64]. Members of the PP-fold family mediate their effects via Y-receptors [90], a family of receptors comprising different receptor subtypes (Y1, Y2, Y4, Y5 and Y6). PP-fold peptides exhibit varying affinities for Y receptor subtypes [63].

PYY was first isolated in 1980 from porcine lower intestinal mucosa [91] and so named due to the presence of a tyrosine residue (amino acid abbreviation, Y) at both ends of the 36-amino acid polypeptide [92]. The L cells of the gastrointestinal tract are the major source of PYY, with the highest levels found in the rectum [59, 64]. PYY is present in two endogenous forms: PYY_{1-36} and PYY_{3-36}. The latter is the major circulating form that crosses the blood brain barrier freely [90]. PYY_{3-36} is also more potent in suppressing hunger than PYY_{1-36} [93].

In contrast with ghrelin, PYY circulating concentrations are lowest during fasting [71]. PYY plasma concentrations start to elevate 15 minutes after a meal, reaching a peak at one to two hours after food intake and remaining high for several hours [92]. Secretion of PYY is most likely via the vagus nerve, through a neural reflex, as its secretion from the lower GIT occurs before foods reaches the distal gut [94, 95]. PYY_{3-36} inhibits food intake by acting through Y2 receptor in the arcuate nucleus [94]. Circulating levels of PYY_{3-36} are influenced by many factors; and these include caloric intake and meal composition [60], with higher levels being seen following fatty meals compared with meals containing high protein or carbohydrate levels [94]. Furthermore, plasma levels of PYY increase in malabsorption states [94] as well as following bariatric surgical procedures, and this may contribute to the decrease in appetite and food intake after such surgery [96]. In contrast, circulating PYY levels are suppressed in patients with morbid obesity [94]. In humans, PYY levels correlate negatively with BMI and waist circumference, and postprandial PYY levels may predict the pattern of long-term weight changes [92]. Compared with a saline infusion, the infusion of PYY leads to an approximately 30% reduction in food intake in humans at a subsequent meal [97]. All of these findings indicate that PYY plays a significant role in reducing food intake in humans [59].

1.3.2.3 Pancreatic polypeptide (PP)

Similar to the PYY, PP is another 36-amino acid anorexigenic peptide gut hormone from the 'PP-fold' family of peptides [57, 94]. However, it is released from PP cells – sometimes called F cells – in the endocrine pancreatic islets of Langerhans [61]. PP has great affinity for the Y4 receptor in the brainstem and hypothalamus [61]. It is thought that PP's central actions may be mediated via the Y4 receptor in the area postrema of the brainstem, an area with a 'leaky' blood brain barrier [94]. It is also thought that PP exerts its satiety actions via several central and peripheral mechanisms that include delayed gastric emptying [94]. This observation may explain the different effects of PP on appetite and food intake according to the route of administration. Indeed, a satiety effect of PP is observed after peripheral administration, whereas food intake is stimulated after central administration of PP [61].

The role of PP in appetite regulation has been investigated for over 30 years [59]. Circulating PP level rises after a meal in proportion with the caloric content of that meal – PP level remains elevated for up to six hours after food intake [61, 94]. In mice, acute PP administration via a peripheral route leads to a prolonged decrease in food intake as it delays gastric emptying [94]; and chronic administration of PP reduces body weight of obese mice [98]. It is also noticed that transgenic mice over-expressing PP are lean [99]. In humans, an intravenous infusion of PP has been shown to result in a 25% reduction in 24-hour food intake in healthy lean subjects [100]. Similarly to PYY_{3-36}, lower PP levels are reported in obese compared with lean subjects [94], although some studies report similar levels in both obese and lean [94]. Some researchers link the satiety effects of PP to the secondary effect of delayed gastric emptying that occurs after intravenous administration of PP [94]. In view of this conflicting data, researchers recommend long-term studies to define the full potential of PP as an anti-obesity target [95].

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

GLP-1, GLP-2, oxyntomodulin (OXM) and glucagon are proglucagon-derived satiety hormones [61]. Proglucagon is expressed in the pancreas, L-cells of the small intestine and in the NTS of the brainstem [101]. Glucagon is produced in the pancreas, whereas GLP-1, GLP-2 and OXM are produced in the intestine, mainly by the L-cells of the GIT, the same endocrine cell type that releases PYY [63, 64]. GLP-1 is a potent incretin gut hormone i.e. a hormone that stimulates insulin secretion and is secreted from enteroendocrine cells into the blood stream within minutes of eating [102]. One of the main physiological roles of GLP-1 is

to regulate the amount of insulin that is secreted after eating [102]. Insulin secretion is released post-prandially in proportion to energy ingested [63, 64, 102]. Secretion of GLP-1 is mediated by vagal stimulation, acetylcholine and gastric inhibitory polypeptide (GIP) [103]. Circulating GLP-1 levels rise after a meal and fall in the fasted state [61]. However, GLP-1 is rapidly inactivated in the circulation, resulting in a short half-life of 1-2 minutes [61].

In rodents, food intake is inhibited when GLP-1 is injected into the CNS [64]. Similarly, peripheral administration of GLP-1 also inhibits food intake in animals [104]. Similar findings are reported in humans, as GLP-1 reduces food intake, and also suppresses glucagon secretion and delays gastric emptying [61, 104]. These effects of intravenous administration of GLP-1 in normal and obese humans occur in a dose dependent manner [105]. The satiety effect of GLP-1 is attributed to a delayed gastric emptying rate [70], and is thought to be mediated through vagal and brainstem pathways [57].

1.3.2.5 Cholecystokinin (CCK)

CCK is the prototypical satiety gut hormone as it was the first gut hormone for which effects on appetite were systematically researched [63]. It still remains one of the most intensively studied appetite regulating hormones [59]. Derived from a 115-amino-acid precursor, CCK is secreted from I cells of the small intestine, mainly in the duodenum and jejunum [59] in response to nutrients in the gut, particularly fat and protein-rich meals [59]. Circulating CCK levels rise approximately 5-fold in response to a meal [59, 106]. Two G-protein-coupled receptors for CCK (the CCK-1 and CCK-2 receptors, previously known as the CCK-A and CCK-B receptor, respectively) have been identified [63]. The effects of CCK on gallbladder contractility, gut motility and GI secretion are mediated predominantly by the CCK-1 receptor [107]. CCK-1 receptors are distributed peripherally in the gastrointestinal tract, on vagal afferent nerve fibres, and in areas within the CNS that are involved in the regulation of food intake [57], whereas CCK-2 is found in the cortex, hypothalamus, vagal afferents and gastric mucosa [57].

The mechanisms by which CCK alter appetite are debatable [63]. The inhibitory effect of CCK on food intake is thought to be mainly related to its effects on gastric emptying and GIT motility [108]. High doses of CCK, in common with many gut peptides, also induce nausea [63]. However, a satiety effect of CCK is seen at circulating levels below those that cause nausea [63]. CCK alone has a very short term effect on appetite and has a 1-2 minutes half-life [59, 109]. Furthermore, chronic administration of CCK alone does not result in

weight loss [59]. All of the available data demonstrates that the actions of CCK on food intake are short-lived [63, 109], as its anorectic effect disappears after 24 hours of infusing CCK continuously [110]. There are also concerns about the role of CCK in the development of pancreatitis and pancreatic carcinoma [63]. All of these findings cast doubts on the future use of CCK as a potential novel obesity therapy [59] and make it unlikely that manipulating CCK signalling mechanisms will prove a promising therapeutic strategy for obesity [63].

1.3.3 The potential use of appetite regulating hormones in the treatment of obesity

The search for an anti-obesity drug that produces sustained weight loss with minimal side effects is rife with difficulties, in part because of the significant built-in redundancy in the mechanisms that control energy balance and food intake [95, 111-113]. These mechanisms are influenced by other physiological mechanisms, as well as social and psychological factors that limit the effectiveness of all past and currently available anti-obesity drugs [112]. Anti-obesity drugs aim to increase metabolic rate, reduce the absorption of nutrients, or increase satiety [111]. Drugs used in the pharmacological therapy of obesity include thyroid hormone, dinitrophenol, amphetamines and their analogues, e.g. fenfluramin [111]. At present, only orlistat, a pancreatic lipase inhibitor is available for the long term treatment ($\geq$ 24 weeks) of obesity in some countries such as the United Kingdom and the United States [111], as sibutramine [114] and rimonabant [115] were withdrawn from the international market due to adverse cardiovascular and psychiatric events, respectively [111, 114, 115].

Recent research on the appetite-stimulating hormone ghrelin, as well as appetite-suppressing hormones such as PYY and GLP-1, have opened up new possibilities [57, 112] based on recent evidence that these hormones can influence appetite in several animal and human studies [63, 94, 97, 116]. Growing evidence is accumulating regarding the potential of gut hormones or their pathways of action in the treatment of obesity, as they may offer an alternative to centrally acting drugs [57, 59]. For example, PYY may play an important role in reducing food intake, although its mechanism of action remains to be fully elucidated [95], and there are reports of nausea as a side effect when given in high doses to humans [59]. However, researchers are still interested in exploring its potential use as an anti-obesity drug in both lean and obese individuals, with novel drugs designed to mimic the action of PYY itself, or targeted against the Y2 receptor [94]. Also, numerous studies suggest that longer-acting GLP-1 mimetic peptides such as Exedin-4 (which is isolated from the venom of a

lizard native to southwestern American states), and its synthetic version, Exenatide, are approved for the treatment of type 2 diabetes mellitus due to their incretin properties [57, 102]. Recent data suggest additional use of a human GLP-1 analogue, Liraglutide, as a promising anti-obesity drug, albeit currently with a 50% chance of nausea and vomiting [116]. Therefore, gut peptides may only have modest benefits as an obesity treatment, a major limitation appearing to be numerous side effects, as well as the limited weight lowering effects that last only as long as the drug is being taken, as well as weight regain after discontinuation [113].

To overcome some of the limitations of gut hormones as a therapy for obesity mentioned above, researchers and pharmaceutical companies are investigating dual administration. The concept of administering more than one gut hormone or gut hormone mimetic in order to produce a better additive effect on food intake inhibition was based at least partly on the fact that appetite regulating gut-hormones are co-released [57]. Examples of those combinations are PYY and GLP-1 [117], and PYY and OXM [118]. Evidence suggests that such combinations are more effective than individual administration [112]. In future, the poly-therapeutic strategy will hopefully rival surgery in terms of efficacy, safety and maintaining weight loss [112]. However, time will tell if promising pre-clinical data translates into clinical benefit [57]. Currently, liraglutide, a GLP-1 analogue currently marketed for the management of diabetes, is the most promising new anti-obesity drug nearing market launch [111]. However, it is uncertain if it will meet the strict regulations needed for licensing as a new anti-obesity drug in people who do not have diabetes [112].

Based on emerging understanding of the physiological role of gut hormones in the regulation of energy homeostasis and food intake, researchers are currently interested in exploring the effects of exercise, which is one of the best approaches for both prevention and treatment of obesity, on the circulating concentrations of gut hormones, both in the short and long term. Such an understanding may help in defining the role of exercise in combination with future strategies for weight management in overweight and obese subjects.

1.4   The effects of exercise on appetite and appetite regulating gut hormones

In recent years, researchers have become more interested in exploring the various effects of physical activity on body weight through better understanding of the impact of structured exercise programs on different aspects of appetite, as a step to defining their role in the

management of obesity. In this regard, investigation of the effects of acute and chronic structured exercise interventions on the motivation to eat, food intake, appetite regulation and appetite-regulating gut hormones is an area that has received great consideration over the last decade [119-121]. The recent understanding of the metabolism and effects of various appetite-regulating gut-derived hormones such as ghrelin, PYY and PP, in addition to other appetite regulating hormones such as leptin and insulin, has enhanced the interest in using these hormones as additional measures for the assessment of the effects of exercise on appetite and appetite control [119, 122, 123]. Therefore, several studies have recently correlated alterations in appetite with changes in plasma levels of the appetite-regulating gut-derived hormones following either acute [124-129] or chronic exercise [130-132]. However, it is still controversial as to whether acute or chronic exercise increases, decreases or has no effect on appetite, and whether these changes are affected by other factors such as age [119, 123, 132], sex [122, 133], and body mass index [124, 133]. The effects of exercise on appetite control were also correlated with the various type of exercise (aerobic versus resistance training) [124, 125, 128, 130, 131], as well as the duration [133], frequency [119, 120, 122], intensity [93], timing of the exercise relative to food intake (fasted or in the postprandial state) [134, 135], and ingestive behaviour before and after exercise [136].

In this section, the effects of exercise on appetite and appetite-regulating gut hormones will be discussed at length. This will help in better understanding the role of exercise in long-term weight maintenance, and, potentially, how the effects of exercise could be enhanced.

1.4.1 Exercise and appetite

Beneficial effects of exercise on appetite regulation have been suggested in the literature, but the exact mechanisms are not yet well understood [120, 121]. At the physiological level, the regulation of appetite is under neuroendocrine control involving both centrally and peripherally mediated signals and has already been discussed (section 1.4.). In this regard, growing interest has developed in studying the effects of various types of exercise on the circulating concentrations of appetite-regulating gut hormones such as the orexigenic hormone ghrelin, anorexigenic hormones such as PYY, PP, and GLP-1, in addition to other anorexigenic hormones such as leptin and insulin [120, 122].

Understanding the exact mechanisms of eating behaviors in general and appetite in particular are highly complex as they involve strong external influences that affect food

intake in free-living humans that can easily overcome normal physiology [120], such as environmental, psychological, social and cultural stimuli [137]. This interaction between external stimuli and internal stimuli may explain the conflicting results on the effects of exercise on appetite and related-hormones. Moreover, the need for reliable objective tests that assist in measuring the effects of exercise on appetite is essential for better understanding of the complexity of appetite regulation.

1.4.1.1 Appetite assessment methods in appetite research

Appetite is defined as the internal driving force for the search, selection and ingestion of food [138]. Appetite is usually linked to hunger, which is typically defined as the need or desire for food [138]. In appetite research, various methods have been used to assess alterations in appetite including visual analogue scales (VAS) [128, 139], food intake [119, 140], and more recently the circulating concentrations of various appetite-regulating hormones [129, 130], either in the fed and / or fasted state. In various studies, researchers used all or some of these methods to assess the effects of exercise on appetite. The reliability and objectivity of the various methods vary [138, 140-143]. For example, visual analoug scales are subjective scales that rate appetite changes indirectly [139]. Visual analogue scales consists of questions and answers about appetite such as 'How hungry are you?', 'Not at all hungry' vs. 'As hungry as I have ever felt' [119, 139]. When used appropriately, subjective appetite ratings have been shown to have good reproducibility and are considered reliable for appetite research [139]. Appetite level was recently assessed using the Electronic Appetite Ratings System (EARS), which is a validated electronic version of the visual analogue scale [144].

Another method used for assessing the influence of exercise on appetite is the measurement of food intake. In this regard, the *ad libitum* buffet meal is widely used for research purposes [61, 142]. In this method a selection of everyday palatable foods are offered to participants during the study. Participants are allowed to select freely and eat the foods and amounts they would like [142]. Despite inherent limitations of the *ad libitum* buffet meal, research indicates that it is a reproducible method that can be used to assess differences in energy intake driven by differences in appetite [141]. However, large variations in two separate occasions may occur in some subjects despite similar feeding conditions, and this phenomenon may be minimised by standardisation of the dietary intake prior to the assessment [141]. Another limitation of using food intake as an indicator of alterations in

appetite is that changes in appetite do not necessarily lead to changes in food intake in all individuals [141, 142].

An additional measure that has been used by several researchers in appetite research recently is the measurement of plasma levels of appetite-regulating gut-derived hormones, particularly ghrelin, the "hunger hormone" [122], the satiety hormone PYY [123], in addition to other satiety hormones such as leptin [120, 145]. These appetite related hormones have been in addition to other methods of appetite assessment as mentioned above [128, 131], or sometimes alone to assess possible alterations in appetite regulation [125, 126, 130]. Several studies recently demonstrated that moderate to intense exercise may influence some or all of the appetite-regulating gut-derived hormones [93, 119, 128, 130]. In the following section, nine identified studies that investigated the effects of acute and chronic exercise on appetite and appetite regulating gut hormones in adults and adolescents will be discussed. These studies are listed in Table 1.1.

1.4.2 The effects of acute exercise on appetite and appetite regulating gut hormones

Numerous studies have measured appetite-regulating gut hormones to investigate the effects of acute exercise on appetite [124-126, 128, 129, 133]. However, these studies were inconsistent as they had different aims, enrolled diverse populations, and used different protocols to investigate different types, forms and intensities of exercise. All of these variables make interpretation difficult and sometimes contradictive. For example, a cross-over randomised control study was conducted by King et al. [126] on a group of 9 lean healthy males (mean BMI 23.6 ± 0.4) to assess the effects of one bout of 90 minutes of running at 68.8 ± 0.8 of maximum oxygen uptake on appetite and energy intake. Appetite was assessed using visual analogue scale and measurements of one of the peptide gut hormones, namely the appetite-stimulating hormone, acylated ghrelin. Energy intake was assessed by an *ad libitum* buffet meal. Results of the study clearly indicated that exercise transiently suppressed appetite and circulating acylated ghrelin hormone levels for a few hours after exercise compared to the control group [126]. A similar finding of suppression of appetite and the circulating levels of ghrelin, as well as a corresponding elevation in levels of the satiety hormone PYY, was observed by Broom et al. (2012) [128] during and after acute exercise in a cross-over randomised control study on 11 healthy normal weight young male students who undertook three 8-hours trials of either resistance training that involved a 90-

minute free weight lifting session followed by a 6.5 h rest period, or aerobic exercise of a 60-minute run followed by a 7 h rest period; or a control group who rested for 8 hours. This observation was noticed after both aerobic and resistance training [128], and the researchers indicated significant interaction effects for hunger, ghrelin and PPY that lead to suppressed hunger in both of the interventions groups. However, in that study the appetite-suppressing hormone PYY increased only during aerobic exercise [128]. As noted above, these studies [126, 128] enrolled only lean young males, and these are possible limitations as later studies referred to the importance of obesity and increased BMI as important factors in hormonal response.

In this regard, Thomas et al. (2012) investigated the impact of body weight on the hormonal response to exercise [124]. In their study, they examined the effects of an acute high-volume whole-body resistance exercise program on plasma levels of the appetite-stimulating hormone ghrelin in sedentary obese (n=10) and lean young men (n=9). The obese group included 5 class 1 obese (BMI = 30.00-34.99 kg/m2), 5 class 2 / class 3 obese (BMI = 35-39.99 kg/m2) / (BMI ≥ 40), in addition to 9 lean men (BMI < 25 kg/m2). All participants completed an acute resistance exercise testing protocol (6 exercises, 3 sets of 10 repetitions at 85%-95% of 10-repetition maximum weight, with 120- and 90-second rest periods). Blood samples were collected pre-, mid-, and immediately post-exercise and during recovery. In this study, ghrelin levels increased in class 2/3 and lean groups but not in class 1 group; however only people in the class 2/3 obese group exhibited significantly greater ghrelin levels than those in the class 1 obese and lean groups [124]. There are a few limitations of this study as addressed by the authors, such as reporting total ghrelin levels instead of acylated ghrelin, making comparison with similar studies difficult. Additionally, and similar to other studies, only young men were included.

Unlike previous studies, the randomised crossover study of Martins et al. (2007) [129] enrolled both sexes (6 lean young males and 6 lean young females), aiming to investigate the acute effects of exercise on the postprandial levels of appetite-related hormones (namely: ghrelin; PYY, GLP-1, PP), energy intake and subjective measures of appetite. Participants had a standardised breakfast that was followed an hour later by either 60 minutes of cycling at 65% of maximal heart rate, or rest. Their appetite was assessed using visual analogue scale and subsequent energy intake at a buffet meal was measured. The study showed that exercise significantly increased mean levels of the appetite-suppressing hormones PYY, GLP-1 and

PP, and that this effect was sustained at three hours after exercise completion only for GLP-1 and PP but not PYY. However, postprandial levels of the appetite-stimulating hormone ghrelin were not significantly affected by the acute exercise. Hunger scores were significantly decreased during the exercise period, but this effect disappeared in the post-exercise period. This study therefore showed that hunger sensations were temporarily reduced in response to acute exercise of moderate intensity, and that this effect was not explained by the changes in the postprandial levels of ghrelin. Therefore, the researchers attributed the observed 'acute exercise-induced anorexia' to the increased levels of GLP-1, PP and probably also PYY, albeit the latter was obviously transiently elevated by exercise [129]. These positive effects of acute exercise on appetite suppression may explain the utility of exercise for weight loss and weight regain prevention on the long-term.

The above-mentioned study did not refer to any sex differences amongst participants. However, sex differences were reported in another study, with an indication of a lesser exercise-induced anorexia in women than in men [127]. That study investigated the appetite and hormonal responses to short-term exercise training programs in a group of 18 overweight and obese subjects (9 men and 9 women). Participants completed four bouts of exercise in conjunction with increased food intake to maintain energy balance, and four bouts of exercise without additional food intake, to induce an energy deficit [127]. The study found that in men, the area under the curve for acylated ghrelin levels was not different between conditions. Also, leptin concentrations were not different across conditions in either sex. However, in men but not in women, appetite was suppressed after exercise in energy balance compared to exercise during energy deficit. The study therefore demonstrated a clear sex difference in the effects of acute exercise on appetite and appetite-related hormones. The data indicate that women are less likely to lose body fat in response to exercise compared to men [127]. Therefore, the authors suggested that women may need to both decrease energy intake and increase energy expenditure in order to lose body fat [127]. These potential sex differences in hormonal responses to exercise have potentially important implications for exercise and dietary guidelines and should therefore be explored further.

Some research has investigated the effects of different modalities of acute exercise on appetite and appetite regulating hormones. A recent randomized controlled trial studied ten active men who completed 3 trials: 45-min of resistance exercise (free and machine weights), aerobic exercise (running), or resting control (CON) [125]. The aim of this study was to

investigate the acute effects of these two types of exercise on appetite-regulating hormones and subsequent energy intake [125]. Circulating ghrelin concentrations were lower following resistance exercise compared with both aerobic exercise and CON. In contrast, fasting circulating PP concentrations were significantly higher after both aerobic exercise and resistance exercise compared with CON. These results indicate that aerobic exercise may be preferable for achieving short-term negative energy balance than resistance training [125]. The researchers also indicated that the acute responses and alterations observed in their study may provide a solid basis for future studies on the long-term effects of regular aerobic and resistance training on appetite and appetite regulating hormones.

The aims and protocols of the above-mentioned studies are different, so it is difficult to draw solid recommendations out of them. However, three [126, 128, 129] of the six studies reviewed above reported decreases in the feeling of hunger after acute exercise, whereas the remaining three studies [124, 125, 133] did not find a significant difference in the feeling of hunger. With regards to the type of acute exercise, studies indicated that high intensity aerobic exercise such as running or cycling is followed by hunger suppression that lasts for few hours following cessation of exercise [127, 129]. In contrast, an appetite-suppressive effect was not reported after acute progressive resistance training in two studies despite the observed low plasma concentration of ghrelin hormone in these two studies [127, 129]. However, one study does report appetite suppression after acute progressive resistance training, and that study was limited to lean young males only [127].

1.4.3 The effects of chronic exercise on appetite and appetite regulating gut hormones

Interest in exploring the long-term effects of regular or chronic exercise on perceived hunger was observed recently [43, 130-134, 146]. However, only very few studies used the circulating levels of appetite-related hormones as an additional measure to assess the effects of regular exercise on appetite [130-132], in addition to the already known indicators such as ingestive behavior and food intake that were used in earlier studies [43, 133, 134, 146] (Table 1.1.).

In 2009, Jones et al. published an uncontrolled study on the effect of long-term aerobic training on plasma levels of five appetite-related hormones in overweight or obese adolescents [132]. After 32 weeks of exercise, there were decreases in both blood triglyceride levels and percent body fat. In addition, there was an increase in the plasma concentrations of

total PYY. This was considered a favourable outcome as PYY is a hormone that suppresses appetite and food intake [59]. In contrast, circulating levels of the appetite-stimulating hormone, ghrelin, were not affected by this type of long-term exercise training [132]. This study was limited to a specific age group (adolescents) and did not compare aerobic exercise with other modes of exercise, such as progressive resistance training.

In contradiction, a longitudinal study in adults produced somewhat different results [131]. This study involved a group of 22 sedentary, overweight or obese men and women (age 36.9 ± 8.3 years; BMI 31.3 ± 3.3 kg/m^2) [131]. Participants took part in a 12-week supervised aerobic (walking or running) exercise program (five times per week, 75% maximal heart rate). Researchers found differences between results determined in the fasting state or after meal ingestion. Exercise resulted in an increase in fasting plasma levels of acylated ghrelin as well as increased fasting hunger sensations. However, in the post-prandial state, there was an increase in the meal-induced rise in plasma GLP-1 levels (90-180 min), as well as a significantly greater suppression of acylated ghrelin levels. These changes would be expected to contribute to a greater feeling of fullness after exercise due to the increase in GLP-1 levels and the decrease in levels of the hunger hormone, ghrelin, later in the day. The authors therefore stated that the observed exercise-induced weight loss in the studied sample was not affected by the observed increased drive to eat in the fasting state, but seems to be related to the improved satiety response to a meal after chronic exercise [131]. Again, a few limitations were noted in this study, such as the small sample size as only 15 subjects completed the study and the investigation of one type of regular exercise, namely aerobic exercise.

There is some evidence that aerobic exercise has differential effects on appetite regulation compared to progressive resistance training. In a more recent longitudinal controlled study that enrolled 33 inactive overweight and obese middle aged men (BMI 30.8 ± 4.2 kg/m^2, age 49 ± 7 years), Guelfi et al. (2013) [130] investigated the effects of 12-weeks of aerobic exercise compared with resistance training on hunger and fullness, together with appetite-related hormones in both the fasted state and postprandially. People randomised to the aerobic exercise and resistance training arms completed 12-weeks of training, while those in a control group continued their usual daily routine. Both aerobic exercise and resistance training elicited a decrease in fat mass, while CON did not. However, no differences in hunger, either in the fasted state or in response to the energy intake, were observed in any

groups. In contrast, both fasting and postprandial feelings of fullness were higher following aerobic exercise, but not with resistance training or CON. No changes in plasma concentrations of ghrelin, PYY or PP were observed. The researchers therefore concluded that only aerobic exercise training but not resistance training is associated with an increase in satiety, and that these appetite changes were not accompanied by any observed alterations in the measured appetite-regulating hormones. The strength of this study is that it appears unique and well designed in comparing the effects of chronic aerobic and resistance exercise, in addition to the measurement of appetite regulating gut hormones [130]. However, the study had limitations such as recruitment of only men and using a liquid glucose drink rather than a mixed solid meal [130].

Two reviews on the effects of exercise on appetite were found upon searching the literature in this field [120, 122]. The mini-review by Kaemer and Castracne (2007) was not comprehensive and was limited only to investigation of effects of exercise on circulating ghrelin and adiponectin levels [122]. In contrast, the review by Martins et al. (2008) was more comprehensive as it reviewed ten studies conducted between 1980-2008 on the effects of acute exercise (7 studies) and chronic exercise (3 studies) on appetite regulation and appetite hormones [120]. Overall, the authors reinforce the critical role of exercise in body weight management and indicated the need for more long-term studies to establish the role of chronic exercise on appetite control in obese individuals [120]. They also indicated other limitations in the reviewed studies with regards to the methods used in subjective and objective measures of appetite [120].

The current review includes nine recent studies [124-126, 128-133] published on the effects of exercise on appetite and appetite regulating hormones since the above-mentioned reviews were published in 2007 and 2008. As discussed above, only three studies investigated the effects of chronic exercise on appetite and appetite-regulating hormones [130-132], compared to six studies on acute exercise [124-126, 128, 129, 133]. Only one of the three studies on chronic exercise was controlled and compared aerobic versus progressive resistance training [130]. However, that study was limited to men. It did report a positive effect of aerobic exercise but not progressive resistance training on the feeling of fullness, and this effect was not accompanied by relative alterations in the plasma levels of appetite-regulating hormones, namely ghrelin, PYY or PP. This paucity of data, and the conflicts of

existing data, indicate the need for future well-designed studies in this emerging area of research.

Table 1-1: Summaries of the studies published on the effects of acute and chronic exercise on appetite and appetite-regulating gut hormones

Reference	Study design	Participants	Type	Exercise protocol	Appetite	Gut Hormones	Notes
King et al., 2010, [126]	Cross-over randomised control	9 M; young (22.2 ±0.8yr); leans BMI (23.6±0.4)	Acute	Only AE, running for 90 ms, VO2=70%, Blood is taken 10 h after Ex., used VAS	↓ Hunger; After Ex.	↓ Ghrelin, After Ex.	Positive effects, but limited to young lean males and did not compare AE vs .RT
Broom et al., 2009, [128]	Cross-over randomised control	11 M; young (21.1 ± 0.3yr); leans BMI (23.1 ±0.4)	Acute	3 groups: AE (running), RT (weight lifting) , C; VO2= 68.8±0.8, used VAS and energy intake	↓ Hunger; During Ex. ↔ after meal	↓ Ghrelin (AE &RT) ↑ PYY (AE) ↔ PYY (RT)	Positive effects in AE, but limited to young lean males
Thomas et al., 2012, [124]	Cross-over randomised control	19M; young; lean group(n=9), obese C1(n=5), obese C2/3 (n=5)	Acute	High volume, whole body RT; blood samples collected pre- mid- and postprandial	N/A	↔ Ghrelin (in leans & C1), but ↑ Ghrelin (in C2/3)	Ghrelin response to RT differs according to BMI; limited to young males
Martins et al., 2007, [129]	Cross-over randomised control	12 (6M+6F); young (25.9 ±4.6yr); leans BMI (22.0±3.2);	Acute	Subjects either cycled for 60 mins at 65% of their mxHR or rested 1 hour, used VAS and energy intake	↓ Hunger; During Ex.	↔ Ghrelin ↑ PYY, GLP-1, PP (During and after Ex.)	The 'exercise-induced anorexia' after Ac.ex. is linked to ↑ PYY, GLP-1, PP, not ghrelin
Hagobian et al., 2008, [133]	Cross-over randomised control	18 (9M+9F); young (M26.8 ±8yr), (F23.3 ±8yr); overweight/obese BMI (M25.7 ±2.3), (F28.0 ±3.5)	Acute	Four bouts of Ex. (treadmill at 50-65%Po2) with energy added to the baseline diet and four bouts without; appetite questionnaire used	↔ Hunger (F) ↓ Hunger (M)	↑ Ghrelin (F) ↔ Leptin	Effect of Ac.ex. on appetite is mainly on M, F need both ↑ energy expenditure and ↓ intake
Balaguera-Cortes et al., 2011, [125]	Cross-over randomised control	10 M, young (21.3 ±1.4yr); leans BMI (23.7±2.0)	Acute	3 groups: AE (running, VO2= 70%), RT (45ms, 3 sets of 12 repetitions) , C; used energy intake	↔ food intake	↓ Ghrelin (RT) ↑ PP (AE &RT) ↔ PYY ↑ insulin	Positive effects of both AE & RT; However, AE has better long term effect on energy balance

Jones et al., 2009, [132]	longitudinal study	12, adolescents & adults; overweight/obese; BMI (31.8±0.4)	Chronic	32 wks, 3 times/wk free choice of AE, VO2= 60-85%	-	↔ Ghrelin ↑ PYY	Weak positive effect of AE on satiety but was limited to a specific age group; did not compare AE to RT
Martins et al., 2010, [131]	longitudinal study	22 but 15 completed (M8, F7); adults (36.9 ±8.3yr); leans BMI (31.3±3.3)	Chronic	12 wks, supervised AE (walking or running, five times/wk, 75% mxHR; used VAS; blood was taken fasting, pre- and post intervention	↑ Hunger; ↓ Fullness	↑ Ghrelin ↔ PYY, GLP-1 ↓ insulin	Small size, conflicting results, limited to AE
Guelfi et al. 2013, [130]	longitudinal controlled study	33 M; different ages (49±7yr); overweight & obese BMI (30.8±4.2)	Chronic	12 wks, 3 times/wk; 3 groups: AE(n=12), RT(n=13), C(n=8); AE (cycling), blood was taken 10 hrs after fasting and at the end	↑ Fullness (in AE)	↔ in appetite hormones ↓ leptin	Limited to men, used a liquid caloric diet and not a mixed one

(Abbreviations: Ac.ex., acute exercise; AE, Aerobic Exercise; BMI, body mass index; C, control; Ex., exercise; F, female; GLP-1, glucose like-peptide-1; M, male; mins, minutes; mxHR, maximum heart rate; N/A, not applicable or mentioned; PYY, polypeptide YY (peptide tyrosin tyrosine); PP, pancreatic polypeptide; RT, Resistance Training; wks, weeks; Visual analogue scale, VAS; VO2, measured maximum oxygen uptake; yr, year.)

1.4.4 Aims and hypothesis

The long term effects of exercise on body homeostasis vary according to the exercise type, duration and intensity/volume, as well as characteristics of the studied participants. It is also suggested that exercise increases energy expenditure, but this effect alone may not be sufficient to achieve the intended goals of exercise on body weight as it is thought that regular exercise alone may increase appetite, and therefore it must be combined with dietary restriction if weight loss is the desired outcome. These concerns have triggered a growing interest in exploring the effects of supervised exercise interventions on food intake and appetite. To measure the effects of exercise on appetite, several methods were used including measurements of plasma concentrations of several appetite stimulating and/or suppressing gut-derived hormones.

Based on the current review of the literature, nine studies were recently published on the effects of acute (n=6) or chronic (n=3) exercise on appetite and appetite-regulating hormones in participants who were not under energy restricted dietary regimes. However, these few studies had their own limitations and some conflicting results. Overall, the study protocols were inconsistent and therefore it is quite difficult to compare results (Table 1.1.). Limitations also included the fact that the majority of studies involved males only, or certain age group or lean participants. This has indicated the need for further research that bridges this gap in the published literature by overcoming the limitations of the previous works on the effects of chronic exercise on appetite and appetite regulating hormones, including the enrolment of both men and women and using a mix of measures that assess the long term effects of various modes of exercise on appetite.

Thus, the current longitudinal randomised controlled study aims to investigate the effects of different doses of chronic aerobic exercise and progressive resistance training on appetite and appetite regulating hormones in previously sedentary overweight, or obese males and females who are not under a prescribed regime of energy restriction. Based on the currently available literature, it is hypothesised that long term structured exercise will not increase subjective feelings of hunger and may increase subjective feelings of fullness, as measured with the Electronic Appetite Rating System. It is also hypothesised that the subjective appetite alterations will be

accompanied by a decrease or no change in the plasma concentrations of the appetite-stimulating hormone ghrelin, and an increase or no change in the concentrations of the satiety hormone PYY.

Insights from this study could help in improving prescriptions for exercise in future strategies for weight management in overweight and obese men and women. The expected findings may also influence dietary advice that is given to overweight or obese participants embarking on exercise regimes for health and weight management based on the effects of different types of exercise on appetite and potentially subsequently on food intake. Such information will help the concerned professional in prescribing the best dietary advice regarding the necessity for food restriction based on scientific evidence.

2 Methods

2.1 Overview

A longitudinal randomised controlled pilot trial was designed to assess the effects of various exercise training programs of aerobic and resistance exercise on appetite and appetite regulating hormones. The primary outcome of this research was to determine the effects of 8-week supervised exercise training programs of different exercise intensities, volumes and modalities on appetite and appetite regulating hormones. Secondary outcomes were to determine the effects of various interventions on body weight and waist circumference.

The study was conducted at the Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders at the University of Sydney in the period September 2012-November 2013. The study was approved by the University of Sydney Human Research Ethics Committee (Ethics Ref: No 13364).

Eligible subjects were randomised, via an equally distributed pre-generated list (www.randomization.com), to one of four intervention groups or a control group as follows:

    i.   High-intensity/low energy expenditure aerobic exercise training (HI:LO)

    ii.   Low-intensity/high energy expenditure aerobic exercise training (LO:HI)

    iii.   Low-intensity/low energy expenditure aerobic exercise training (LO:LO)

    iv.   Progressive resistance training (PRT)

    v.   Stretching, massage and fit-ball training program (control).

Written consent form signed

Baseline testing

- Blood pressure/resting heart rate
- Weight/Height, WC, fitness
- Fasting appetite assessment
- Fasting blood sample for plasma ghrelin and PYY
- 3- day physical activity questionnaire
- 3- day food dairy

Randomized allocation (to 1 of the 4 exercise intervention group or control)

Post- exercise testing

- Blood pressure/resting heart rate
- Weight/Height, WC, fitness
- Fasting appetite assessment
- Fasting blood sample for plasma ghrelin and PYY
- 3- day physical activity questionnaire

- Recruitment
- Telephone screening

GP Screening

-2

-1

0

8

Time (weeks)

Exercise intervention groups

i. High-intensity/low energy expenditure aerobic exercise training (HI:LO)
ii. Low-intensity/high energy expenditure aerobic exercise training (LO:HI)
iii. Low-intensity/low energy expenditure aerobic exercise training (LO:LO)
iv. Progressive resistance training (PRT)

(Abbreviations: WC, waist circumference; PYY, peptide tyrosine tyrosine; GP, general practitioner)

Figure 2-1: Study design and protocol

2.2 Participants

2.2.1 Recruitment and screening

Potential participants were recruited through university noticeboards, electronic bulletins and a departmental clinical trials database. All potential participants who expressed interest in the study were subjected to a telephone screening interview by one of the study coordinators, as shown in Figure 2.1. During the telephone interview a brief overview of the study was given to the potential participant and the importance of consistent commitment to the study was stressed. This was followed by administration of a questionnaire that was designed for preliminary assessment of the eligibility for enrolment in the study (Appendix 1).

Potentially eligible participants were sent an information package (Appendix 2) that they were requested to read and complete before the testing day. This information pack included a screening form to be completed by their general practitioner. The pre-assessment instructions included refraining from ingesting food, alcohol or caffeine, or using tobacco products the night before and morning of testing, fasting at least 12 hours prior to testing, consumption of an adequate meal the night before testing, and ample fluid (water) consumption over the 24-hour period preceding the test to ensure adequate hydration. Participants were also requested to use 3-day physical activity questionnaire (Appendix 3) [147] and a 3-day food diary (Appendix 4) [147] prior to testing and to avoid significant exertion or exercise on the day before and the day of testing. They were also asked to come in light clothing appropriate for exercise and the application of electrodes on their chest wall. Those on regular medications were requested to follow their normal medication regimen.

Immediately prior to commencement of testing, participants were screened for absolute or relative contraindications to exercise according to the American College of Sports Medicine Guidelines for Exercise Testing and Prescription, 2010 [148]

2.2.2 Eligibility criteria

2.2.2.1 Inclusion criteria (eligible)

Potentially eligible participants included those people who were overweight or obese (BMI $\geq$ 25 kg/m^2), aged $\geq 29 \leq 59$ years, were not on lipid lowering medication, had no past history of unstable disease, no hypertension and who were currently undertaking less than 3 structured exercise sessions per week.

2.2.2.2 Exclusion criteria (ineligible)

Participants who were considered ineligible for enrolment included those of BMI (<25 kg/m^2) and/or had terminal/rapidly progressing illness, amputation, currently doing resistance/aerobic exercise $\geq$ 3 sessions/week, were taking prescribed lipid lowering medications, had any musculoskeletal, neuromuscular, or cardiac condition, or any signs or symptoms of unstable disease. In addition, all of those who were unable to sit and exercise on a stationary exercise bike for up to 45 minutes were excluded. Also, pregnant females and those who showed unwillingness to be randomised and to come for training/assessments were excluded. Finally, participants who were on a regular diet or who had lost $\geq$ 5 kg in the last 3 months were also excluded.

2.3 Baseline testing

On arrival at our institute for baseline testing, participants were verbally briefed about the study design and protocols. Any questions were answered and an informed written consent form was then signed by the participant. Blood pressure and resting heart rate were measured. Anthropometric measurements (height and weight) were then taken as shown in Figure 2.1. Height was measured to the nearest 0.1 cm using a wall fixed stadiometer (Tanita, Arlington Heights, Illinois, USA). BMI was then calculated by dividing the body weight (in kg) by height (in m^2). Overweight was defined as a BMI of 25-30 kg/m^2 and obesity as BMI of $>$ 30 kg/m^2 [4]. Waist circumference was measured in the standing position at the smallest circumference between the rib margin and iliac crest [149]. Fasting appetite assessment using the Electronic Appetite Rating Systems was done as described below [144]. Then a fasting blood sample was collected for determination of plasma ghrelin and PYY concentrations. The participants' 3-day physical activity questionnaire [147] (Appendix 3) and 3-day food dairy were also collected. All of these baseline tests were repeated at the end of the 8-week study, as shown in Figure 2.1.

Once all initial baseline testing was completed, participants were given a sealed envelope containing randomized group allocation to one of the four exercise intervention groups or the control group. All of the initial assessments were thus completed with both the participants and the investigators blinded to the group allocation.

2.4 Fasting appetite assessment

Appetite level was assessed in the fasted state using the Electronic Appetite Ratings System, which is a validated electronic version of the visual analogue scale (VAS) [144]. The Electronic Appetite Ratings System is a software program that presents questions about

appetite on the screen and participants are asked to read the question and then use the arrow keys to move a centred vertical cursor along a horizontal line. Once the cursor is in the chosen position, the participants were instructed to press 'continue', and then confirm their response [144]. Questions and anchored responses were: 1) How hungry do you feel now? 2) How full do you feel now? 3) How strong is your desire to eat? 4) How much food could you eat now? 5) How nauseous do you feel now? 6) How satisfied do you feel now? 7) How tired do you feel now?

2.5 Blood sample collection, storage and analysis for plasma ghrelin and PYY

Blood was collected from a suitable antecubital vein into a pre-chilled tube containing K_2EDTA as an anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA). The samples were kept on ice and then centrifuged at 1100g for 10 minutes at 4°C. The plasma supernatant was then aliquot within 30 minutes into two storage tubes (Sarstedt AG & Co, Nümbrecht, Germany) and stored at −80°C until subsequent analysis of plasma total ghrelin and total PYY concentrations by radioimmunoassay using commercially available kits (GHRT-89HK and PYYT-66HK), Merck Millipore, Billerica, MA, USA) in accordance with the manufacturer's instructions. Blood samples were collected at baseline and at week 8, and were always collected at the same time of day.

2.6 Exercise Protocols

2.6.1 Exercise test

Prior to commencement of exercise on the day of baseline testing, cardiorespiratory fitness and work capacity changes in cardiorespiratory fitness were assessed via a graded maximal exercise test to volitional fatigue to assess $VO_{2\ max}$ on the electronically braked cycle ergometer (Figure 2.1.). This test was conducted by an Exercise Physiologist and supervised by a study physician, and $VO_{2\ max}$ was used to design the training programs. $VO_{2\ max}$ was assessed as per previous publications [130-132]. Heart rate, ratings of perceived exertion, blood pressure and ECG were obtained during each stage [147].

2.6.2 Exercise training groups

2.6.2.1 Aerobic groups

2.6.2.1.1 High-intensity/low energy expenditure aerobic exercise training (HI:LO)

Participants in the HI:LO group progressed from cycling for 30 minutes at 60% peak aerobic capacity ($VO_{2\ peak}$) in weeks 1 and 2 to 45 minutes at a high intensity (70% $VO_{2\ peak}$) in weeks

3-8 on a cycle ergometer for two supervised sessions per week. They also undertook one additional session of brisk walking on their own each week, at the same intensity and duration. Participants performed a 3-minute warm up and cool down at 30W.

2.6.2.1.2   Low-intensity/high energy expenditure aerobic exercise training (LO:HI)

In the LO:HI group, participants progressed from cycling for 45 minutes in weeks 1 and 2 to 60 minutes in week 3-8 on a cycle ergometer at a low intensity (50% of peak aerobic capacity (VO_{2peak}), for three supervised sessions per week. They also undertook one additional session of brisk walking on their own each week, at the same intensity and duration. Participants performed a 3-minute warm up and cool down at 30W.

2.6.2.1.3   Low-intensity/low energy expenditure aerobic exercise training (LO:LO)

In the LO:LO group, participants progressed from cycling for 30 minutes in weeks 1 and 2 to 45 minutes in weeks 3-8 on a cycle ergometer at a low intensity (50% of peak aerobic capacity (VO_{2peak}) for two supervised sessions per week. They also undertook one additional session of brisk walking on their own each week at the same intensity and duration. Participants performed a 3-minute warm up and cool down at 30W.

2.6.2.2 Progressive Resistance training group (PRT)

Participants randomized to the PRT group performed 30 to 45 minutes of progressive resistance training three times weekly. All training sessions were supervised and involved 1-3 sets, 8-12 repetitions at 80-85% of one repetition maximum, in accordance with current American College of Sports Medicine guidelines. Exercises were seated leg press, lying chest press, lateral pull-down, triceps push down, lunges, shoulder press, seated row, calf raises bicep curls and abdominal crunches. The total duration of the session was 40 to 55 minutes, which included a 5-minute warm up and cool down at approximately 60% of maximum heart rate on a cycle ergometer.

2.6.3 Control group

Participants in the control group were prescribed a stretch, massage and fitball protocol, designed to elicit no cardiometabolic improvements. Training sessions were home based with one visit per fortnight to receive a program update and cycle at a sub-training load for 5-10 minutes to maintain familiarity with the bike.

2.7   Data Analysis

Data were entered in an Excel file and were exported to Statistical Package for Social Scientists version 20 (IBM, New York, USA) for statistical analysis. Quality control for the entered data was done before analysis. The central tendencies for continuous variables are expressed as mean ± SEM. A test for normality of distribution of the quantitative variables was conducted to decide upon appropriate statistical analyses. As normality was assured, parametric tests were applied on the quantitative variables (age, weight, BMI, waist circumference and circulating appetite regulating hormone concentrations). The baseline anthropometric measurements in the various study groups were analysed according to the type of exercise training intervention using ANOVA. For all other parameters, non parametric tests were adopted i.e. for variables in the Electronic Appetite Ratings System. To test significance of the changes after intervention, Paired Sample t test was used for continuous variables and Wilcoxon signed-rank test was used for non parametric variables. Analysis of covariance (ANCOVA) was carried out to control for the possible influence of waist circumference at base line on its matched measurements after intervention. P values of ≤ 0.05 were accepted as being statistically significant.

3 Results

3.1 Demographic characteristics of participants in the different study groups

Participants (n=28) were assigned to one of four exercise training intervention groups according to the type of exercise training: High-intensity/low energy expenditure aerobic exercise training group (HI:LO), (n=7); Low-intensity/high energy expenditure aerobic exercise training group (LO:HI), (n=8); Low-intensity/low energy expenditure aerobic exercise training group (LO:LO), (n=4); Progressive resistance training group (PRT), (n=4); in addition to a control group (n=5).

The overall age of the studied subjects was 46.9 ± 1.8 years. There were a greater number of female (n=21) than male participants (n=7). However, there was no statistically significant difference in age (48.7 ± 1.8 and 41.7 ± 4.6 years respectively) or BMI (32.5 ± 1.2 and 35.7 ± 2.7 kg/m^2) of females and males at baseline. Similarly, there was no statistically significant difference in age (46.8 ± 4.1 and 47.0 ± 2.1 years respectively), or BMI (33.3 ± 1.1 and 32.5 ± 1.9 kg/m^2) between participants in the exercise training groups and the control group at baseline.

There were no statistically significant differences between different groups at baseline in weight, BMI or waist circumference. However, participants allocated in the HI:LO group had relatively higher weight, BMI and WC measurements than those in other intervention groups.

3.2 Baseline and post-intervention values of anthropometric measurements

There was no change from baseline in body weight or BMI in any exercise group, but there was a decrease in waist circumference with the HI:LO and LO:HI interventions, when comparing pre- and post-intervention values. However, this change was not statistically significant after adjusting for baseline values. Meanwhile, the control group showed slight increments in all anthropometric measurements, but these differences were not statistically significant.

When the data were split by gender, similar results were obtained, and only reduction in WC showed statistical significance in both men and women (data not shown).

For further investigation of the effects of various exercise training intervention program on anthropometric measurements in the four intervention groups, the difference between the baseline and post-intervention values were analysed in each exercise training group separately as well as in the control group. All anthropometric measurements were lower after intervention compared to baseline among all the exercise training intervention groups; however, its magnitude was not similar among groups (Table 3.1.). Notably, overall changes in the WC showed statistical significance among the HI:LO and LO:HI intervention groups but not in the LO:LO and PRT groups. ANCOVA was conducted to control for the possible impact of the differences in baseline readings of waist circumference on the post-intervention values. The regression model revealed that post-intervention waist circumferences were significantly predicted by the baseline values. Moreover, *F test* for significance of different types of exercise revealed that the overall change due to the intervention was not statistically significant after adjusting for the baseline values.

Table 3-1: Anthropometric measurements at baseline and after 8-week exercise training interventions in each of the four study groups and the control group

Study group	Anthropometric measurement	Baseline	Post-intervention	Change	P
(HI:LO)	Weight (kg)	107.0±7.2	105.6±7.4	-1.4±0.6	0.071
	BMI (kg/m^2)	37.1±2.4	36.5±2.4	-0.5±0.2	0.063
	WC (cm)	108.9±5.5	106.3±5.4	-2.5±0.4	**0.001**
(LO:HI)	Weight (kg)	88.1±4.9	87.0±4.3	-1.1±1.1	0.322
	BMI (kg/m^2)	32.0±1.4	32.0±1.2	-0.0±0.6	0.950
	WC (cm)	99.1±4.1	96.7±3.8	-2.4±0.8	**0.022**
(LO:LO)	Weight (kg)	84.9±7.9	84.8±8.3	-0.1±1.1	0.932
	BMI (kg/m^2)	29.5±0.5	29.4±0.8	-0.1±0.4	0.850
	WC (cm)	97.0±4.7	95.4±5.3	-1.6±0.7	0.101
(PRT)	Weight (kg)	89.5±9.9	89.2±9.6	-0.3±0.8	0.732
	BMI (kg/m^2)	33.3±3.3	33.1±3.2	-0.1±0.3	0.692
	WC (cm)	100.1±9.0	98.8±8.9	-1.3±1.0	0.292
Control	Weight (kg)	92.7±7.8	93.9+8.8	1.3±1.4	0.392
	BMI (kg/m^2)	32.5±1.9	32.9±2.1	0.3±0.5	0.519
	WC (cm)	94.6±3.7	95.9±4.7	1.4±1.5	0.424

Data are means±SEM at baseline and after intervention. Abbreviations: WC, waist circumference; BMI, body mass index; (HI:LO), High-intensity/low energy expenditure aerobic exercise training group; (LO:HI), Low-intensity/high energy expenditure aerobic exercise training group; (LO:LO), Low-intensity/low energy expenditure aerobic exercise training group; (PRT), Progressive resistance training group.

3.3 Baseline and post-intervention appetite responses as assessed by the Electronic Appetite Ratings System (EARS)

No statistically significant differences in fasting appetite ratings were apparent among the studied exercise groups or control group, either pre- or post-intervention (Table 3.2).

Table 3-2: Appetite ratings at baseline and after 8-week exercise training interventions in each of the four study groups and the control group

Study group	Appetite responses	Baseline	Post-intervention	P
(HI:LO)	Hunger	36.8±10.6	42.3±8.5	0.726
	Fullness	16.8±8.9	28.0±10.1	0.401
	Desire to eat	40.8±5.5	36.3±10.6	0.696
	Prospective consumption	44.3±12.3	39.8±9.9	0.565
(LO:HI)	Hunger	47.8±16.1	49.0±17.9	0.914
	Fullness	26.5±16.4	24.3±14.1	0.587
	Desire to eat	62.0±5.1	66.8±13.7	0.715
	Prospective consumption	54.3±4.3	54.3±6.4	1.000
(LO:LO)	Hunger	30.3±25.8	38.3±31.1	0.339
	Fullness	37.7±20.8	5.7±5.6	0.192
	Desire to eat	35.7±20.2	47.3±27.9	0.398
	Prospective consumption	28.0±11.4	58.7±21.2	0.119
(PRT)	Hunger	41.8±12.7	35.8±18.3	0.600
	Fullness	16.8±7.4	29.3±17.0	0.303
	Desire to eat	49.0±14.4	42.5±15.6	0.423
	Prospective consumption	46.5±8.8	42.3±15.0	0.599
Control	Hunger	31.3±23.2	65.5±17.7	0.149
	Fullness	25.8±17.5	8.5±3.1	0.374
	Desire to eat	40.3±22.3	67.5±18.6	0.245
	Prospective consumption	41.8±21.1	79.5±7.0	0.076

Data are represented as mean±SEM. Abbreviations: (HI:LO), High-intensity/low energy expenditure aerobic exercise training group; (LO:HI), Low-intensity/high energy expenditure aerobic exercise training group; (LO:LO), Low-intensity/low energy expenditure aerobic exercise training group; (PRT), Progressive resistance training group.

3.4 Plasma concentrations of ghrelin at baseline and after interventions

Table 3.3. demonstrates the values of plasma circulating ghrelin levels before and after the various interventions among the four study groups compared to controls. There was no

statistically significant effect of any type or intensity of exercise on the mean post-intervention concentrations of circulating ghrelin.

Table 3-3: Plasma concentrations of ghrelin (pg/ml) at baseline and after 8-week exercise training interventions in the four study groups and the control group.

Study group	Baseline	Post-intervention	Change	P
(HI:LO)	1306±90	1121±83	-185±118	0.17
(LO:HI)	1624±282	1671±254	47±105	0.67
(LO:LO)	1725±177	1689±281	-36±119	0.78
(PRT)	2286±152	2202±47	-84±164	0.64
Control	1546±223	1457±287	-89±118	0.49

Data are represented as mean±SEM. Abbreviations: (HI:LO), High-intensity/low energy expenditure aerobic exercise training group; (LO:HI), Low-intensity/high energy expenditure aerobic exercise training group; (LO:LO), Low-intensity/low energy expenditure aerobic exercise training group; (PRT), Progressive resistance training group.

3.5 Plasma concentrations of PYY at baseline and after interventions

Table 3.4 demonstrates values of plasma PYY levels before and after the various interventions among the four study groups compared to controls. There was no statistically significant effect of any type or intensity of exercise on the mean post-intervention concentrations of circulating PYY.

Table 3-4: Plasma concentrations of PYY hormone (pg/ml) at baseline and after 8-week exercise training interventions in the four study groups and the control group.

Study group	Baseline	Post-intervention	Change	P
(HI:LO)	403±64	412±58	9±6	0.88
(LO:HI)	312±24	352±32	40±18	0.06
(LO:LO)	419±58	403±67	-16±36	0.68
(PRT)	431±32	391±52	-40±43	0.42
Control	388±42	407±83	19±72	0.80

Data are mean±SEM. Abbreviations: PYY, peptide tyrosine-tyrosine; (HI:LO), High-intensity/low energy expenditure aerobic exercise training group; (LO:HI), Low-intensity/high energy expenditure aerobic exercise training group; (LO:LO), Low-intensity/low energy expenditure aerobic exercise training group; (PRT), Progressive resistance training group.

4 Discussion

The current study indicates that 8-weeks of different doses of supervised aerobic exercise or progressive resistance training did not significantly change subjective appetite ratings or influence the plasma concentrations of the gut-derived appetite regulating hormones ghrelin or PYY in overweight or obese adults that were previously sedentary. Our study did not involve concurrent energy restriction, and the mean weight and BMI of participants in all groups were not significantly affected by the various exercise training interventions. These results are different to what might have been expected based on the results of several acute exercise studies that did not involve concurrent energy restriction [124-126, 128-133], which indicated reductions in appetite and either decreases in circulating concentrations of the appetite-stimulating hormone, ghrelin, and / or increases in circulating concentrations of the appetite-reducing hormone, PYY.

The novelty of the current study is that it is, to our knowledge, the only one that was designed as a randomized controlled trial to investigate the long term effects of various modes of exercise on subjective appetite, plasma concentrations of the appetite regulating hormones ghrelin and PYY, and anthropometric measurements. The majority of the published literature on the benefits of exercise on appetite regulation and its relation to weight management involved acute exercise i.e. exercise for one day and up to one week. In this regard, six randomized controlled trials [124-126, 128, 129, 133] were published since 2007, but they were all limited to acute exercise and were designed as crossover studies (Table 1.1.). In contrast, only very few studies investigated the effects of exercise over a longer period of time, of 12-33 weeks [130-132]. However, these chronic exercise studies were either not randomized [130-132], or did not include a control group [132], or were limited to men [130], or included only lean subjects [131], or were limited to one type of chronic exercise [132], or compared only one mode of aerobic exercise to progressive resistance training [130] (Table 1.1.). The present study aimed to overcome the limitations of previous studies by investigating the chronic effects of various doses of aerobic exercise training and compare it to the effects of a progressive resistance training exercise program on subjective appetite and the plasma concentrations of two appetite regulating hormones: ghrelin and PYY. Additionally, the present randomised controlled trial included overweight or obese men and women.

This study indicated no significant effects of various exercise training programs on fasting appetite measures, including the feeling of fasting hunger or fullness, either at baseline

or after the interventions. The results from our study are different from that of a similar longitudinal uncontrolled study in overweight/obese adolescents [132] that reported increased satiety after 32-weeks of one mode of aerobic exercise [132]. However, the age of participants (adults in our study versus adolescents), as well as the duration of physical activity (8 weeks in our study versus 32 weeks) may have contributed to these discrepant findings. The exercise intensity used in this study is similar to the HI:LO group in our study. In the study on adolescents, the subjective appetite was not assessed similar to our study [132]. Results in the present study are also different from another similar study on the effects of 12-weeks supervised aerobic exercise program on 15 overweight/obese adults [131], which showed an increased feeling of fasting fullness that was accompanied with an increased level of fasting hunger [131]. A more recent longitudinal controlled study investigated the effects of 12-weeks exercise training program and enrolled 33 men of similar age and BMI to our participants. That study indicated an increased feeling of fasting fullness only, but this effect was limited to the aerobic exercise group only and not in those doing progressive resistance training [130]. Of the six studies investigating effects of acute exercise, three [126, 128, 129] reported decreases in the feeling of fasting hunger after acute exercise whereas the remaining three studies [124, 125, 133] did not find a significant effect of acute exercise on the feeling of hunger in the fasted state. These discrepant findings from acute studies may explain the equivocal responses in our present study, as it appears that the effects of aerobic exercise on subjective appetite may be short lived. Taken together, three out of four chronic studies (including the current one) indicate increases in fasting fullness sensations with chronic exercise. However, with such wide variations in study parameters (age of participants, exercise modality and intensity, sex or BMI of participants etcetera), it is not possible to draw definite conclusions about the effects of chronic exercise on appetite without further investigation.

In addition to subjective appetite, other methods were used in previous studies to further investigate effects of exercise on appetite. These methods include measurement of food intake [119, 140], and more recently the assessment of gut-derived appetite regulating hormone concentrations [129, 130]. The latter was selected in the present study to assess appetite control. Our study showed no statistically significant alterations in the circulating total ghrelin concentrations before and after the four exercise interventions compared to controls. However, the changes in mean concentrations of circulating total ghrelin post-intervention varied, and a notable trend to decreased ghrelin was observed in the HI:LO aerobic exercise group. Two previous studies that investigated chronic effects of AE on

ghrelin also indicated no change in the plasma concentrations of acylated or active ghrelin [130, 132]. However one of these studies was limited to adolescents [132] and the other to only men [131] , unlike our study in adults, which included both sexes. In contrast, another study investigating the effects of chronic aerobic exercise demonstrated an increase in the circulating concentrations of total and active ghrelin, accompanied by an increase of the feeling of fasting hunger [131]. The authors attributed the increase in ghrelin concentration to the diet-induced weight loss that was observed in their study, which involved a prescription for dietary restriction [131]. In the current study, participants were not prescribed any sort of dietary restrictions and did not lose a significant amount of weight, and this may explain the lack of change in ghrelin concentrations in response to long term exercise.

In contrast to the current observation of no effect of chronic exercise of different modes and intensities on circulating total ghrelin levels, different findings were reported in response to acute exercise. Half of the reviewed studies (Table 1.1) reported decreased ghrelin levels in response to acute exercise [125, 126, 128], whereas one study reported increased ghrelin levels in females but not in males [123], and two studied reported no change [124, 129]. Again, previous studies used different protocols for assaying ghrelin concentrations, as some measured acylated ghrelin concentrations [126, 130] while other measured total ghrelin [124]. In another study both total and acylated ghrelin were assayed [132].

Similar to plasma ghrelin concentrations, circulating concentrations of total PYY did not show statistically significant alterations after the four different exercise interventions. These findings correlate well with the results for subjective appetite, which also did not alter after any of the exercise interventions. Findings from this study are consistent with that of two previous studies that did not show significant changes in plasma PYY concentrations after long-term exercise [130, 131]. In contrast, one chronic study showed that total PYY concentrations increased in overweight adolescents after 32-weeks of supervised aerobic exercise program [132]. Possible reasons for variations in the results of previous studies are the differences in study designs, exercise protocols and methods used for the determination of plasma PYY concentrations. The total PYY concentration was measured in the present study.

There is a dearth of information in the literature about the link between the intensity, duration and type of exercise and its long-term effects on appetite. In a recent acute exercise trial, 15 young men performed two types of acute exercise: rope skipping and bicycle ergometer to determine the effects of each of these two modes of exercise on appetite. The researchers reported a greater suppression of subjective fasting hunger with rope skipping than with the bicycle ergometer [150]. However, the authors attributed that greater acute

effect of rope skipping on appetite to its weight bearing effects and not to the exercise intensity [150]. In the current study, it appeared that 8-weeks of HI:LO aerobic exercise resulted in a notable, but not significant, decrease in plasma ghrelin concentrations, without any increase in PYY concentrations. In contrast, the LO:LO and the progressive resistance training groups did not demonstrate any such changes in the plasma concentrations of these appetite regulating hormones. Taken together, these findings indicate that high intensity aerobic exercise, but not progressive resistance training, may reduce subjective appetite and / or the appetite-stimulating hormone ghrelin. However, there is still considerable ambiguity about the effect of different doses of acute and chronic exercise on subjective appetite and appetite hormone concentrations in free-living humans, indicating a need for further research in this maturating area of human physiology research.

Results of the current study show that none of the modes or intensities of long-term exercise used in our study had a significant impact on overall body weight or BMI of participants, men or women. It is important to note that participants in our study were not prescribed concurrent energy restriction. Similar previous studies on the effects of various structured chronic exercise training programs on participants who were not on energy intake restrictions [130-132] as one study reported significant reductions in all of anthropometric measures, percentage of body fat and total fat mass after weeks of aerobic exercise program [131]. However, the previous study was limited to lean subjects and may not be compared to results, such as ours, that were conducted on overweight/obese subjects where other researchers indicated a mild decrease in percent of body fat of 2.2% without associated reduction in waist circumference or BMI in a group of 12 adolescents [132]. In the current study, such effects were not seen and even the mild effect on waist circumference in two aerobic exercise groups: HI:LO and LO:HI disappeared after adjusting for the baseline values of waist circumference. Other researchers indicated that the decline in BMI was observed in the aerobic exercise group but not in the progressive resistance training group [130]. Others indicated specifically that aerobic exercise is more effective in reducing the visceral adipose tissue (VAT) content/volume than progressive resistance training that failed to induce VAT reduction [50]. However, progressive resistance training was more related to improving muscle strength and endurance as well as decreasing the metabolic risk factors including type 2 diabetes mellitus and dyslipidaemia [15, 19].

The present study is a pilot study and therefore, one of its limitations is that it included relatively small numbers in each group. This may be avoided in future studies, because the current work has provided preliminary data that can be used in sample size calculations. The

predominance of females participants (75%) may be another limitation of the current study, as some of the previous studies indicated differences in the hormones levels between men and women, particularly in ghrelin concentrations according to the stage of the menstrual cycle [127], albeit in an acute and not in a chronic exercise study. Another limitation is the lack of other indirect methods of subjective appetite assessment such as food intake at an *ad libitum* meal, which assesses energy intake and complements other tools of appetite assessment such as that used in our study. Also, only two appetite-regulating hormones were assessed and perhaps more of these hormones e.g. PP, GLP-1 and CCK could provide greater insight. However, the total ghrelin and PYY hormones were selected in the current study as appetite regulators as they were widely used in a majority of the previous studies and were therefore considered to allow for direct comparisons between findings of the present study and similar studies.

In view of the findings of the current study, larger studies that use more tools of appetite assessment are needed. Such future studies should take in consideration the established role of physical activity in preventing weight regain, and lack of compliance with physical activity prescriptions, as well as unavailability of an effective, safe and licensed weight reducing pharmacological agent [112], understanding the role of various types of chronic exercise in appetite regulation is crucial in weight management programs and subsequent prevention of chronic diseases associated with increasing body weight. Based on the growing evidence of the physiological roles of appetite-regulating gut hormones in energy homeostasis and food intake, such research should also include concurrent investigation of these hormones. A better understanding could lead to more effective exercise prescriptions in weight regain prevention programs in those who have lost weight after long-term dietary restriction. Indeed, these people are at higher risk of obesity relapse as a result of the observed persistent changes in the circulating mediators of appetite including high ghrelin and low PYY concentrations that may ultimately stimulate food intake and weight regain [151]. It will be interesting to determine whether combining long-term exercise with energy intake restrictions will normalise the increased appetite and appetite-stimulating changes in gut-derived hormones that were reported in that study [151]. The current study showed that none of the types and intensities of exercise investigated had any effect on appetite nor on the circulating concentrations of appetite regulating gut hormones. This finding could be taken into consideration in prescribing and recommending exercise to the general public, because it has been shown that some people 'reward' themselves for exercise by eating more [152].

In summary, 8-weeks of different intensities and volumes of regular aerobic exercise and progressive resistance training did not significantly influence subjective appetite or the plasma levels of total ghrelin or PYY of the overweight and obese adults who participated in this randomised controlled trial, in contrary to results that might have been expected from studies on acute exercise that reported an "aerobic exercise-induced anorexia". This "no change" effect of chronic exercise on appetite as reported in the current study is in contrast in some respects to other studies investigating effects of chronic exercise on appetite, albeit in non-randomized or uncontrolled studies in people of differing ages, BMIs and sex to those in the current study. These discrepant findings, combined with the emerging role of gut-derived hormones in the regulation of energy balance, indicate the need for future studies that define the potential roles of different modes and intensities of chronic exercise in weight regain prevention programs, particularly in those subjects on diet-induced weight loss program who have persistent changes in appetite that may ultimately stimulate food intake and obesity relapse.

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Appendix 1

NOTES:

Telephone Screening Form

Date of Telephone call: ____/____/____   **Interviewer:** _____

Source of Participant: _____   Time Commenced: _____

I would like to give you a brief overview of how this exercise study is designed. The research requires about 8 weeks of consistent commitment from you. At the start of the study you will be randomised (like the roll of a dice) into one of five different exercise groups.

The exercise in each group differs in the intensity of exercise you will do as well as the type of the exercise you will be asked to complete. Three of these groups will exercise for 30 - 60 minutes, 2 or 3 times per week for 8 weeks at the Boden Institue of Obesity, Nutrition and Exercise, Camperdown (BIONE), or the University of Sydney, Cumberland Campus, Lidcome, and have an additional home-based session each week. In one group you will perform resistance exercise three times per week at the University of Sydney, Cumberland Campus, Lidcome. In another group, you will be instructed to complete a home-based stretch, fit ball and massage program and attend one supervised session each fortnight.

We will provide you with an initial health screen and exercise test that will be supervised by our study physicians, Professor Ian Caterson**, Professor Stephen Colagiuri and Doctor Namson Lau** at BIONE, Camperdown.

At your initial visit you will be required to undertake a fasting blood test, maximal exercise test and complete a variety of lifestyle questionnaires and forms to help to determine your eligibility to participate and provide a summary of your current health and fitness status. We will also provide you with follow up tests after 8 weeks exercise training to examine the effects of this exercise upon your health and fitness. Each of these assessments will be conducted at BIONE. You will also be required to undergo a MRI/MRS body scan at *Northside Medical Imaging* in Hornsby before and after the 8 weeks of training.

During your training visits you will be supervised by researchers from the University of Sydney.

I will need to ask you some questions to determine whether you are eligible to participate in the study. Are you happy to proceed with this screening?

If no, why not?   YES ☐   NO ☐

Name: _____

2.   Phone Number: h) _____
                           w) _____
                           m) _____
3.   Address: _____
                     _____
                     _____Post Code: _____

4.   What is your date of birth: ____ / ____ / ____   How old are you now? ___ years

        What is your occupation? _____

                                                                    YES   NO
5.   Do you live in: a private home   ☐   ☐
                                  A unit   ☐   ☐
                                  Hostel/retirement home   ☐   ☐
                                  Other (specify)   ☐   ☐

        How would you travel to the Northside Medical Imaging (Hornsby)?
        _____

6.   What is your current: (i) Body weight?   _____ (1 kg = 2.20462262 pounds)
                              (ii) Height?   _____ (1 cm = 0.393700787 inches)
                              (iii) BMI (kg/m^2)? _____ (> 25)

If BMI less than 25, END HERE (If not sure, continue with screening).

Thank you Mr / Mrs …………..……, you are not eligible for this study but if another study
comes up that is more suitable, may we keep your contact information and call you about it?
                                                          YES ☐   NO ☐

THANK YOU FOR YOUR KIND ASSISTANCE

Time Finished: _____

Time Taken to Complete Screening: _____

Are you being followed for: High blood pressure   YES ☐   NO ☐

                                 High cholesterol/TGs   YES ☐   NO ☐

                                 Diabetes   YES ☐   NO ☐

8.   Would you be able to sit and exercise on a stationary exercise bike for up to 45 minutes?

                                           YES ☐   NO ☐

     No, why not? _____

9.   Do you take any medications?   YES ☐   NO ☐

Medication	Dose	Frequency	Reason

Has any of your medication been changed in the last 2 months?   YES ☐   NO ☐

If yes, how?

Do you have any chronic illnesses or conditions?   YES ☐   NO ☐

Disease	Date of Onset/Diagnosis	Currently Stable/Controlled?

11. Do you have angina? YES ☐ NO ☐
   If yes, when was your last attack? Date: ____/____/_____
   How often do the attacks occur? _____
   What usually brings on your angina? _____
   What is your usual treatment? _____

12. Have you ever had a heart attack? YES ☐ NO ☐
   If YES, please specify: Date: ____/____/_____
   Hospital: _____

13. Have you ever had a cardiac stress test? YES ☐ NO ☐
   If YES, please specify: Date: ____/____/_____
   Hospital: _____

14. Do you have a terminal or rapidly progressive disease? YES ☐ NO ☐
   If yes, please specify:
   _____

15. Have you had a blood clot in the lung or leg in the last 6 months?

   YES ☐ NO ☐

   If yes, please give details: _____
   _____

16. Do you have any other chronic disease which is out of control or has changed rapidly
   in the last 6 months? YES ☐ NO ☐
   If yes, please give details: _____
   _____

17. Have you had an amputation? YES ☐ NO ☐
   If yes, please give details: _____

18. Have you had a fracture in the past 6 months? YES ☐ NO ☐
   If yes, please give details: _____
   _____

19. Do you currently participate in any exercise? If yes, please describe?
   YES ☐ NO ☐

Type	Times Per Week	Duration/Session	Intensity (How Hard?)	How long have you been doing this?
Walking/jogging				
Cycling				

Swimming/ Aqua aerobics				
Weight Lifting				
Stretching or Gentle exercise				
Aerobics Classes				
Dancing				
Other (describe)				

Exercise $\geq$ 3/7= ineligible;   Exercise 2-3/7= use discretion;   Exercise 1/7= eligible

Comments: _____

_____

20. Do you smoke / quit smoking in the last 6 months?   YES ☐   NO ☐
If yes, How many cigarettes would you smoke a day?   _____
What type and brand of cigarette do you smoke?   _____

21. Do you drink alcohol and if so, how much? _____   YES ☐   NO ☐

22. Are you pregnant, lactating or have you recently given birth? YES ☐   NO ☐
UNSURE ☐

23. Are you currently participating in any other research studies? YES ☐   NO ☐
If yes, What are they?   _____
Frequency: _____
Time: _____
Type: _____

If accepted for this study, are you willing to be randomised to one of five groups exercising 3-4 days per week for 8 weeks:
- Low intensity/ low energy expenditure aerobic exercise training on an exercise bike
- Low intensity/ high energy expenditure aerobic exercise training on an exercise bike
- High intensity/ low energy expenditure aerobic exercise training on an exercise bike
- Progressive resistance training
- Stretching, fit ball and self-massage at home?

YES ☐   NO ☐
If no, why not?_____

25. Are you willing to:
a) Attend an initial screening and tests before participation at BIONE during weeks 1 and 8 of the study?
b) Attend *Northside Medical Imaging* for MRI/MRS body scans during weeks 1 and 8 of the study?

c) Come to BIONE, or the University of Sydney Cumberland Campus, to undertake training 2-3 times a week for 8 weeks

YES ☐   NO ☐

If no, why?   _____

If yes, would transportation be difficult for you?   YES ☐   NO ☐

Explain transport needs:

_____

Thank you for answering all of these questions. The chief investigator of the study is Dr Nathan Johnson. He will review this questionnaire and we will notify you as whether you are eligible for further screening for this study. Do you have any further questions about the study?

Comments:

_____

_____

Time Finished: _____

Time Taken to Complete Telephone Screening: _____

Appendix 2

THE UNIVERSITY OF SYDNEY

Discipline of Exercise and Sport Science
Faculty of Health Science

ABN 15 211 513 464

Dr. Nathan Johnson

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Participant Information Pack

After reviewing your telephone screening questionnaire, we have decided that you are **potentially eligible** to participate in the research study examining the effects of different volumes, intensities and modes of exercise on liver fat.

This information pack includes the following

1. Information about the study
2. A screening form to be completed by your GP to be returned on the assessment date
3. Information about the upcoming assessment day

Please record the name and address of your regular GP.

General Practitioner

Name: _____

Name of Medical Practice: _____

Address: _____

_____

_____

Phone: _____   Fax: _____

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Ph:

Relationship to you:

The next step in the process is for you to be screened by the study Exercise Physiologist, Shelley Keating. You have indicated that the most appropriate days for this screening are as follows:

1) _____ 2) _____ 3) _____

Do you have any allergies? (If yes, please specify)

_____

_____

_____

Do you have any dietary restrictions or are you on a special diet: (eg low salt, vegetarian, gluten free, etc)

_____

_____

_____

Are you allergic to any known medications?

_____

_____

If you have any questions or concerns about the study before we contact you again, please contact Shelley Keating

> *M: 0405 735 200*

> *E: skea9438@uni.sydney.edu.au*

<u>Please bring this document with you to your initial screening appointment</u>

Initial Assessment/Pre Screening Requirements

The Initial Assessment and pre screening will be conducted at BIONE, Camperdown. The address of BIONE is:

> *Ground Floor, K25*
>
> *Medical Foundation Building (K25) 92-*
>
> *94 Parramatta Road*
>
> *Camperdown NSW 2050*

The closest cross street is Barr Street or Larkin Street, and it is located roughly opposite the BP service station on Parramatta Road.

Prior to commencing exercise testing, you will be asked to complete a Physical Activity Readiness Questionnaire (PAR-Q), a pre screening and risk stratification form and will also be measured for weight, height and waist circumference. Following discussion of the risks associated with exercise testing and training you will be asked to provide written consent to

participate in the exercise study. You will have the opportunity to ask questions throughout the entire screening process.

Please read the "Instructions" and "Exercise Test Information" that has been included in this information pack thoroughly before the testing day. Information regarding the exercise test will also be discussed prior to testing on the assessment day before you are asked to sign an informed consent form.

Assessments include:

> Anthropometry Measures (height, weight, waist circumference)
> BIA (bio-electrical impedance)
> Fasting blood tests
> A graded maximal exercise test
> Provision of lifestyle, exercise and dietary questionnaires

Prior to Assessment- INSTRUCTIONS

- Please refrain from ingesting food, alcohol or caffeine or using tobacco products the night before and morning of testing.

  - o You must have fasted for at least 12 hours prior to testing

  - o Ensure that you consume an adequate meal the night before testing, outside the 12 hour fasting period.

  - o Drink ample fluids (water) over the 24-hour period preceding the test to ensure normal hydration before testing.

- Please ensure that you are rested for the assessment and avoid significant exertion or exercise on the day before and the day of testing

- Clothing should permit freedom of movement and include walking or running shoes.

  - o Women should bring a loose fitting, short sleeved blouse that buttons down the front and avoid restrictive undergarments.

  - o The ECG will require that you wear minimal clothing during the exercise test to allow for the application of electrodes.

- If you take medications, please ensure that you follow your normal medication regimen on your usual schedule on the day of testing. This ensures that the exercise responses are consistent with the responses expected during exercise training.

Following the fasting blood test and graded exercise test, you will be provided with a towel, water, fruit juice, 1-2 cereal/ nut bars, and tea/coffee.

Use of Medical Records

The information that is obtained during exercise testing will be treated as privileged and confidential. It is not to be released or revealed to any person except your referring general practitioner and the research staff without your written consent.

Exercise Test Information

You will perform an exercise test of a cycle ergometer. The exercise intensity will begin at a low level and will be advanced in stages depending on your fitness levels. We may stop the test at any time because of signs of fatigue or changes in your heart rate, ECG, or blood pressure, or symptoms you may experience. It is important for you to realize that you may stop the test when you wish because of feelings of fatigue or any other discomfort.

Attendant Risks and Discomforts

There exists the possibility of certain changes occurring during the test. These include abnormal blood pressure, fainting, irregular, fast or slow heart rhythm, and in rare instance, heart attack, stroke, or death. Every effort will be made to minimise these risks by evaluation of preliminary information relating to your health and fitness and by careful observations during testing. Emergency equipment and trained personnel are available to deal with unusual situations that may arise.

Responsibilities of the Participant

Information you possess about your health status or previous experiences of heart-related symptoms (e.g. shortness of breath with low-level activity, pain, pressure, tightness, heaviness in the chest, neck, jaw, back and/or arms) with physical effort may affect the safety of your exercise test. Your prompt reporting of these and any other unusual feelings with effort during the exercise test itself is very important. You are responsible for fully disclosing your medical history, as well as symptoms that may occur during the test. You are also expected to report all medications (including non prescription) to the testing staff.

Benefits to be Expected

The results obtained from the exercise tests may assist in evaluating your current level of fitness. They also help to ensure that it is safe for you to participate in exercise.

Inquiries

Any questions about the procedures used in this exercise test or the results of your test are encouraged. If you have any concerns or questions, please ask us for further explanations.

THE UNIVERSITY OF SYDNEY
Faculty of Health Sciences

Dr. Nathan Johnson, Discipline of Exercise and Sport Science,
East Street (P.O. Box 170), Lidcombe, NSW, Australia 2141
Tel: 9351-9137, Fax: 9351-9204, Email: nathan.johnson@sydney.edu.au

Dr

Address line 1

Address line 2

Date

Re: NAME   **D.O.B:**

Mr./Ms._____ has agreed to participate in a research study entitled: *'Exercise Stretegies for Liver Fat Reduction'.* **Ethics Ref: No 13364 03-2011**

Dear Doctor,

We are currently conducting a research project investigating the effects of exercise intensity, energy expenditure and exercise modality in reducing liver fat in overweight or obese individuals with pre-diabetes. The aim of the study is to compare the effects of different exercise intensities, volumes and modalities in overweight and obese (BMI > 25) adults (29-59 years) with pre-diabetes.

Initial screening involves a combination of blood tests, MRI/MRS scans and a maximal exercise test which will be supervised by a physician from the Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders (University of Sydney) or the Westmead Millennium Institute. These tests will be repeated following an 8 week intervention period. Should any clinical issues arise during these investigations, the participant will be informed and the results will be forwarded to your surgery immediately.

Subjects will be randomised to an 8 week training intervention involving either of i) low-intensity/low energy expenditure aerobic exercise training ii) low-intensity/high energy expenditure aerobic exercise iii) high-intensity/low energy expenditure aerobic exercise training (HI-LO) iv) progressive resistance or v) stretching and fit-ball training program.

The training groups will be required to attend supervised one hour training sessions two- three days per week at the Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, the Westmead

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Millennium institute or The University of Sydney Cumberland Campus, and complete one additional session at home each week. They will also be monitored for dietary intake throughout the study.

The primary outcome measure of this research is the changes in hepatic and visceral fat. Secondary outcome measures are blood parameters including glucose, insulin and liver enzyme concentrations. A detailed explanation of these procedures can be found in the attached Participant Information Statement.

In order to participate in this research we request that volunteers obtain written consent from their Medical Practitioner. For your convenience we have attached a standardized letter which you may wish to use. We thank you for your consideration of this matter.

If you have any further queries or have other patients who may be interested in participating in the study, please contact the undersigned.

Kind Regards

Nathan Johnson, PhD
Discipline of Exercise and Sport Science
University of Sydney
Ph: (02) 9351 9137

Email: nathan.johnson@sydney.edu.au

Point of contact: Miss Shelley Keating
                           Ph: 0405 735 200
Email: skea9438@uni.sydney.edu.au

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MEDICAL PRACTITIONER CONSENT FORM

Re: Patient NAME   **D.O.B: _____**

Dr: _____

Provider Number: _____

Address of Practice: _____

                             _____

                             _____

Practice Phone Number: _____

Absolute Contraindications to Exercise Testing:

Tick any of the following conditions that apply to your patient:

☐ A recent significant change in the resting ECG suggesting significant ischemia, recent myocardial infarction (within 2 days),m or other acute cardiac event.

☐ Unstable angina

☐ Uncontrolled cardiac dysrythmias causing symptoms or hemodynamic compromise

☐ Symptomatic severe aortic stenosis

☐ Uncontrolled symptomatic heart failure

☐ Acute pulmonary embolus or pulmonary infarction

☐ Acute myocarditis or pericarditis

☐ Suspected or known dissecting aneurysm

☐ Acute systemic infection, accompanied by fever, body aches, or swollen lymph glands

☐ None of the above

Relative Contraindications to Exercise Testing:

Tick any of the following conditions that apply to your patient:

- ☐ Left main coronary stenosis
- ☐ Moderate stenotic valvular heart disease
- ☐ Electrolyte abnormalities (e.g. hypokalemia, hypomagnesia)
- ☐ Severe atrial hypertension (i.e. systolic BP of >200mm Hg and/or a diastolic BP of >110mm Hg) at rest
- ☐ Tachydysrythmia or bradydysrythmia
- ☐ Hypertrophic cardiomyopathy and other forms of outflow tract disruption
- ☐ Neuromuscular, musculoskeletal, or rheumatoid disorders that are exacerbated by exercise
- ☐ High-degree atrioventricular block
- ☐ Ventricular aneurysm
- ☐ Uncontrolled metabolic disease (e.g. diabetes, thyrotoxicosis, or mycedema)
- ☐ Chronic infectious disease (e.g. mononucleosis, hepatitis, AIDS)
- ☐ Mental or physical impairment leading to inability to exercise adequately
- ☐ Other (eg other acute illness or unstable chronic disease) (specify)

  _____

- ☐ None of the above

(Adapted from the American College of Sports Medicine Guidelines for Exercise Testing and Prescription, 2010)

Mr./Ms._____is eligible to participate in a research study entitled: *'Exercise Strategies for the Reduction of Liver Fat'*. **Ethics Ref No: 13364 03-2011**

I have reviewed the participant information sheet provided and consent to NAME being included as a volunteer in the above named study. They are a suitable candidate to undertake exercise testing and training. Please **do / do not** provide me with copies of all medically relevant results collected throughout the duration of the study for my patient.

Signed: _____

Date: _____

Name:

Appendix 3

Patient Code Number:
Dates Completed:

BOUCHARD QUESTIONNIARE

This questionnaire is designed to assess the level of physical activity you do in order to determine the amount of energy you use each day.

The questionnaire needs to be completed for three days. Two of these days can be any day of the week, however the third day needs to be a Saturday or Sunday.

For each 15-minute period during these days, you need to enter a categorical value ranging from 1 to 9, which corresponds to the dominant activity you have done for that period. A table of these categorical values and examples of the activities they correspond to is shown on the questionnaire. If you do an activity that is not listed, you should apply the closest categorical value for an activity of comparable frequency.

At the end of the day, you will need to add up the frequency of each category and record it down the bottom of the questionnaire.

An example of a completed diary record for one day is given on the following page.

Reference: C. Bouchard, A. Tremblay, C. Leblanc, G. Lortie, R. Savard and G. Theriault 1983

DAY 1

A) Details:

Date: 1/4/05
Day of the week (please circle): Mon/Tue/Wed/Thu/Fri/Sat/Sun

B)Categorical Values Table:

Categorical Value	Example of Activities
1	Sleeping; resting in bed
2	Sitting: eating, listening, writing etc
3	Light activity standing: washing, shaving, combing, cooking etc
4	Slow walk (<4 km/h), driving, to dress, to shower etc
5	Light manual work: floor sweeping, window washing, driving a truck, painting, waiting on tables, nursing chores, several house chores, electrician, barman, walking at 4 to 6 km/h
6	Leisure activities and sports in recreational environment: baseball, golf, volleyball, canoeing or rowing, archery, bowling, cycling (<10 km/h), table tennis etc
7	Manual work at moderate pace: mining, carpentry, house building, lumbering and wood cutting, snow shovelling, loading and unloading goods etc
8	Leisure and sport activities of higher intensity (not competitive): canoeing (5 to 8 km/h), bicycling (>15 km/h), dancing, skiing, badminton, gymnastic, swimming, tennis, horse riding, walking (>6 km/h) etc
9	Intense manual work, high intensity sport activities or sport competition: tree cutting, carrying heavy loads, jogging and running (>9 km/hr), racquetball, badminton, swimming, tennis, cross country skiing (>8 km/h), hiking and mountain climbing etc

C)Physical Activity Record Card:

Minute	Hour																							
	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23
0-15	2	1	1	1	1	1	1	1	1	3	5	5	5	2	5	5	2	2	2	2	2	8	4	2
16-30	2	1	1	1	1	1	1	1	1	4	5	5	5	2	5	5	2	2	2	2	2	8	4	2
31-45	2	1	1	1	1	1	1	1	1	3	5	5	5	5	5	2	2	4	4	2	2	8	2	2
46-60	1	1	1	1	1	1	1	1	1	4	5	5	2	5	5	2	2	2	4	2	2	4	2	1

D)Category Frequency Summary:

Categorical Value	Daily Frequency
1	34
2	30
3	2
4	8
5	19
6	0
7	0

DAY 2

A) Details:

Date:
Day of the week (please circle): Mon/Tue/Wed/Thu/Fri/Sat/Sun

B)Categorical Values Table:

Categorical Value	Example of Activities
1	Sleeping; resting in bed
2	Sitting: eating, listening, writing etc
3	Light activity standing: washing, shaving, combing, cooking etc
4	Slow walk (<4 km/h), driving, to dress, to shower etc
5	Light manual work: floor sweeping, window washing, driving a truck, painting, waiting on tables, nursing chores, several house chores, electrician, barman, walking at 4 to 6 km/h
6	Leisure activities and sports in recreational environment: baseball, golf, volleyball, canoeing or rowing, archery, bowling, cycling (<10 km/h), table tennis etc
7	Manual work at moderate pace: mining, carpentry, house building, lumbering and wood cutting, snow shovelling, loading and unloading goods etc
8	Leisure and sport activities of higher intensity (not competitive): canoeing (5 to 8 km/h), bicycling (>15 km/h), dancing, skiing, badminton, gymnastic, swimming, tennis, horse riding, walking (>6 km/h) etc
9	Intense manual work, high intensity sport activities or sport competition: tree cutting, carrying heavy loads, jogging and running (>9 km/hr), racquetball, badminton, swimming, tennis, cross country skiing (>8 km/h), hiking and mountain climbing etc

C) Physical Activity Record Card:

Minute	Hour																							
	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23
0-15																								
16-30																								
31-45																								
46-60																								

D)Category Frequency Summary:

Categorical Value	Daily Frequency
1	
2	
3	
4	
5	
6	
7	
8	
9	

DAY 3

A) Details:

Date:

B)Categorical Values Table:

Categorical Value	Example of Activities
1	Sleeping; resting in bed
2	Sitting: eating, listening, writing etc
3	Light activity standing: washing, shaving, combing, cooking etc
4	Slow walk (<4 km/h), driving, to dress, to shower etc
5	Light manual work: floor sweeping, window washing, driving a truck, painting, waiting on tables, nursing chores, several house chores, electrician, barman, walking at 4 to 6 km/h
6	Leisure activities and sports in recreational environment: baseball, golf, volleyball, canoeing or rowing, archery, bowling, cycling (<10 km/h), table tennis etc
7	Manual work at moderate pace: mining, carpentry, house building, lumbering and wood cutting, snow shovelling, loading and unloading goods etc
8	Leisure and sport activities of higher intensity (not competitive): canoeing (5 to 8 km/h), bicycling (>15 km/h), dancing, skiing, badminton, gymnastic, swimming, tennis, horse riding, walking (>6 km/h) etc
9	Intense manual work, high intensity sport activities or sport competition: tree cutting, carrying heavy loads, jogging and running (>9 km/hr), racquetball, badminton, swimming, tennis, cross country skiing (>8 km/h), hiking and mountain climbing etc

C)Physical Activity Record Card:

Minute	Hour																							
	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23
0-15																								
16-30																								
31-45																								
46-60																								

D)Category Frequency Summary:

Categorical Value	Daily Frequency
1	
2	
3	
4	
5	
6	
7	
8	
9	

Appendix 4

Food Record

Name_____ Participant Code: ___ ___ ___ ___

Date of Birth: _____

Height_____ cm

Weight at start of diary_____ kg & at end of diary_____ kg

Contact telephone: _____

Email: _____

Started Diary on __/__/_____ and Finished on __/__/_____

GUIDELINES FOR KEEPING YOUR FOOD DIARY

HOW LONG DO I NEED TO RECORD?

- Record **all** the food and drinks that you consume for three (3) days (two "work" days and one "rest/weekend" day)

You will need to do this

- Baseline: in the time between initial screening and the start of testing (record 3 days/ week)
- Week 8 during training (record 3 days/ week)
- Week 12 for 3 days before final testing (record 3 days/ week)

Please bring the completed diary with you to your next appointment. If you are in doubt about how to record an item, describe it in a way that you will remember and ask at your next appointment. If you have any problems please contact the study researcher on 0405735200.

Try to carry the diary with you and record the food or drink as you consume it, so that nothing is forgotten. Start a new page for each day.

HOW DO I RECORD MY FOOD IN THE DIARY?

- Record all food and drink on the diary sheets provided. Try to carry the diary with you always and record food as you eat it, so that nothing is forgotten.
- Record all food and drink no matter how small or large
- Start a new page for each day.
- Follow the example diary page so you can see how much detail is required.

WHAT SHOULD I RECORD? (See sample sheet provided)

Please include:

1) **MEAL TYPE**

   e.g. breakfast, lunch, dinner, snack.

2) **TIME OF FOOD CONSUMPTION**

3) **TYPE OF FOOD AND DRINKS**

   a) Name of the food

   b) Brand name, if applicable

   c) Any special feature of the food e.g. low fat yoghurt, polyunsaturated margarine

   d) For mixed dishes, estimate the ingredients separately (e.g. salad with 1 lettuce leaf, ½ tomato and three slices of cucumber dressed with 1 teaspoon of French dressing) OR attach the recipe to the food diary and then record the amount you had (e.g. 1 cup of vegetable stew)

   e) Don't forget to include accompaniments such as butter, gravy, salt, milk and sugar in coffee etc

   f) Don't forget to include all fluids including water and alcohol

- Give as much detail as possible, indicating the cut of meat, kind of bread eg
  - **BBQ Lean beef sirloin, large serve (approximately the size of 1 hand) with fat removed before eating**
  - White sliced bread (TipTop) – 3 slices
  - Custard cream biscuits (Arnotts) – 4 biscuits

1) COOKING METHOD

Note if the food is grilled, deep fried, stir fried, boiled, baked etc.

If fat is added during cooking write what kind e.g. 1 tablespoon of olive oil.

Don't forget accompaniments such as; butter, gravies, salt, butter added to vegetables, milk and sugar in coffee

2) QUANTITY OF FOOD AND DRINKS

An approximate amount of food eaten in grams or household measures such as cups, tablespoons or teaspoons e.g. 1 cup cooked pasta or 1 tablespoon of gravy. Check the food label for a weight when appropriate e.g. half a 185 g can of salmon, 40 g Mars bar, 150 g steak.

Include the number of portions eaten e.g. 3 slices of white bread, ½ apple.

- Record the type of exercise, the time it took place, and the duration.
- Record all fluids even water and estimate the volume consumed in cups* or millilitres

  * Indicate the size of the cup (eg a middi glass, a paper cup, coffee mug)

Note: If you are in any doubt about how to record any item, describe it in a way that you will remember and we shall check it at the end of the week.

DAY One Date: ___/___/_____, Day of the Week: _____, Weight (morning):_____kg

Meal	Time	Food Eaten		Drinks	
		Description eg. Weetbix	**Quantity** eg. 3 biscuits	**Description** eg. Coke Cola	**Quantity** eg. 600mls
Breakfast		_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____
Snacks		_____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____

DAY One Date: ___/___/_____, Day of the Week: _____, Weight (morning):_____kg

Meal	Time	Food Eaten		Drinks	
		Description eg. Weetbix	**Quantity** eg. 3 biscuits	**Description** eg. Coke Cola	**Quantity** eg. 600mls
Lunch		_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____

DAY One Date: ___/___/_____, Day of the Week: _____, Weight (morning):_____kg

Meal	Time	Food Eaten		Drinks	
		Description eg. Weetbix	**Quantity** eg. 3 biscuits	**Description** eg. Coke Cola	**Quantity** eg. 600mls
Snacks		_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____	_____ _____ _____ _____
Dinner		_____ _____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____
Supper/ dessert		_____ _____ _____ __	_____ _____ _____ __	_____ _____ _____ _____	_____ _____ _____

Physical Activity: - TYPE: _____   Intensity   (1-10)_____

Duration_____min

Did you drink any alcohol today?   Yes   No

If yes how much? (eg 3 schooners of beer) _____

Please name any supplements taken and the amount (eg, 1 multivitamin)

_____

Is today different to your usual routine? If so please briefly describe

_____

_____

_____