INVESTIGATION OF THE ROLE OF EXERCISE AND RESTRAINED EATING BEHAVIOUR ON APPETITE CONTROL

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To my parents for being the best family I could have wished for, for always believing in my dreams and supporting me throughout a journey that was unknown to themselves.
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Abstract

Obesity is a global epidemic; physical inactivity and increased consumption of highly palatable energy-dense food are likely to be the main drivers. However, little is known about the impact of exercise and dietary restraint on appetite control. The role of a six-week moderate intensity exercise intervention on short-term appetite regulation was investigated. Energy intake at a buffet test-meal was measured 60 minutes following two covertly manipulated preloads (607 and 246kcal) in healthy sedentary normal-weight volunteers at baseline and after the exercise intervention. Exercise was shown to improve short-term appetite control, leading to a more sensitive eating behaviour in response to previous EI, both acutely at a test-meal, and for the next 24h.

To try to elucidate the mechanisms behind the beneficial role of exercise on appetite control, the effects of 1h of moderate intensity exercise, performed in the fed state, on the plasma levels of appetite-related hormones/metabolites were investigated in normal-weight individuals. Acute exercise significantly increased postprandial levels of polypeptide YY (PYY), glucagon-like peptide-1 (GLP-1) and pancreatic polypeptide (PP) but had no impact on ghrelin, suggesting that exercise can trigger physiological changes in hormone secretion which could help in appetite control and weight maintenance.

The role of dietary restraint on the plasma levels of appetite-related hormones/metabolites and subjective/objective measures of appetite was also investigated in normal-weight volunteers. Restraint did not impact on PYY or triacylglycerol (TAG) plasma levels, but was associated with lower fasting insulin plasma levels and a lower release of both insulin and glucose in the postprandial state. Moreover, restrained eaters showed better insulin sensitivity, both fasting and postprandially, and higher fullness ratings in the fed state.

Finally, the predictive value of three different questionnaires used to measure restrained eating behaviour (Revised Restrained Scale (RRS), 18-items revised Three Factor Eating Questionnaire (TFEQ-18R) and Dutch Eating Behaviour Questionnaire (DEBQ)) was investigated, in their ability to predict disinhibition in the laboratory in a sample of normal-weight women, using preloads of different energy content and a buffet as the test-meal. None of the questionnaires was able to predict disinhibition; however, the loss of compensation observed with increased levels of restraint was best forecasted by the RRS.
Taken together these findings provide strong evidence for a positive role of exercise on appetite regulation and weight maintenance, reinforcing the need to meet current national physical activity targets. The role of dietary restraint in predicting disinhibition seems to be minor; however, this eating behaviour was found to be associated with altered carbohydrate metabolism.
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<td>ARC</td>
<td>Arcuate nucleus</td>
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<td>AgRP</td>
<td>Agouti-related peptide</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>BIA</td>
<td>Bioimpedance</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BMR</td>
<td>Basic metabolic rate</td>
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<tr>
<td>bpm</td>
<td>Beats per minute</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>CHO</td>
<td>Carbohydrate</td>
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<tr>
<td>CIU</td>
<td>Clinical Investigation Unit</td>
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<tr>
<td>CRH</td>
<td>Corticotrophin releasing hormone</td>
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<tr>
<td>CSS</td>
<td>Charcoal stripped serum</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<td>DEBQ</td>
<td>Dutch eating behaviour questionnaire</td>
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<td>EB</td>
<td>Energy balance</td>
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<tr>
<td>EE</td>
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<td>Energy intake</td>
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<td>DQ</td>
<td>Food Quotient</td>
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<td>FFM</td>
<td>Fat-free mass</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>LEP</td>
<td>Low-energy preload</td>
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<td>MET</td>
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<td>NSB</td>
<td>Non-specific binding</td>
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<td>Physical activity level</td>
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<td>Post-buffet food diary</td>
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<td>PP</td>
<td>Pancreatic polypeptide</td>
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<td>Physical activity</td>
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<td>Propiomelanocortin</td>
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<td>Polypeptide YY</td>
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<td>QC</td>
<td>Quality control</td>
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<td>REI</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>Standard deviation</td>
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<td>Triacylglycerol</td>
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<td>Maximal oxygen uptake</td>
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<td>α-melanocyte-stimulating hormone</td>
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Chapter One
Chapter 1. Literature review

1.1 Introduction

Obesity is a global epidemic with large public health implications (World Health Organization, 2006). Despite its complex aetiology, it is widely accepted that obesity results from a state of long-term positive energy balance (EB), with energy intake (EI) exceeding energy expenditure (EE) (Prentice & Jebb, 1995; British Nutrition Foundation, 1999; WHO, 2003). This is likely to result from a breakdown in the normal mechanisms that regulate appetite, a very complex phenomenon resulting from constant biological and environmental inputs (Blundell, 1991).

It is undeniable that profound changes in lifestyle have lead to a reduction in physical activity (PA) levels and that the omnipresence of highly palatable food in the surrounding environment creates the need, in an increased number of people in Western countries, to actively restrain food intake, in order to maintain or lose weight. In face of this, it is extremely important to understand how exercise and dietary restraint impact on appetite and eating behaviour in order to achieve a better knowledge of the aetiology and/or potential treatments of obesity.

The following review of the literature will summarize the incidence and aetiology of obesity, describe the concepts of hunger, appetite and satiety and experimental approaches used in appetite research and discuss the mechanisms involved in the control of appetite. In addition, the effects of restrained eating behaviour on appetite and the impact of both acute and chronic exercise on EB and appetite will also be reviewed.

1.2 Obesity

Obesity has become a global epidemic especially in (though not restricted to) developed countries, with more than 1.6 billion adults overweight (body mass index (BMI) > 25 kg/m²) and at least 400 million clinically obese (BMI > 30 kg/m²) worldwide (World Health Organization, 2006). In England, between 1980 and 2004, the prevalence of obesity increased almost threefold, with present numbers indicating that over 60% of the population is overweight and around 24% obese (HSE, 2005). If this
rate of growth continues, it is expected that by 2010 between 28% and 33% of the population in England will be obese, approaching the levels of obesity currently seen in the United States (Department of Health, 2006).

The public health consequences of this phenomenon are huge. Obesity increases all-cause mortality and is an independent risk factor for diabetes (increasing the risk five-fold in men and almost 13-fold in women), cardiovascular disease, hypertension and osteoarthritis (National Audit Office, 2001; IOTF and EASO, 2002). It appears that obesity, especially intra-abdominal obesity, increases disease risk and shortens life expectancy by having an adverse effect on metabolism, which leads to hypertension, dyslipidemia and insulin resistance (British Nutrition Foundation, 1999; IOTF and EASO, 2002; World Health Organization, 2003). The socio-economic costs of obesity and overweight are substantial (Thompson and Wolf, 2001). In Europe, it has been estimated that up to 8% of health budgets has been spent on obesity, either directly (diagnosis and treatment of obesity and its co-morbidities) or indirectly (lower productivity, absenteeism) (IOTF and EASO, 2002). The cost of obesity, in England, in 2002, was estimated to be between £3.3 and £3.7 billion (House of Commons, 2004).

In the face of this, new approaches are urgently needed to address the challenge of preventing and treating obesity. To achieve this, the aetiology of obesity needs to be understood, a difficult task due to its complexity and multifactorial origins. Although genetic factors can be important in determining individual susceptibility to weight gain, with the discovery of the first obesity susceptibility gene been recently published (Frayling et al., 2007), the dramatic rise in obesity in the past few decades cannot be explained by genetic factors alone, since our genetic pool has remained almost unchanged. The dominant factor in the aetiology of obesity is a long-term positive EB, (Prentice & Jebb, 1995; British Nutrition Foundation, 1999; WHO, 2003).

At an individual level, however, it is rather hard to ascertain whether the positive EB is caused by excessive EI, inactivity or a combination of both (House of Commons 2004). Although the majority of the studies report a lower EI in obese, compared with lean subjects (British Nutrition Foundation, 1999), obesity continues to be associated with gluttony (Weststrate, 1995). Underreporting food intake, a classic feature found in the obese population (Lichtman et al., 1992), probably accounts for the lower EI observed in some studies. Moreover, even if obese subjects do not have, currently, a
high EI that does not mean that there have not been periods of hyperphagia in the past, with consequent positive EB, which contributed to weight gain (King and Tribble, 1991; Pearcey and De Castro, 2002).

It is undeniable that PA has decreased over the past few decades, driven by dramatic changes in lifestyle (Prentice & Jebb, 1995). The current recommendations for adults, shared by the English Department of Health and the American College of Sports Medicine, are to accumulate at least 30 minutes of moderate PA on most, preferably all, days of the week (Pate et al., 1995; American College of Sports and Medicine, 1998; Department of Health, 2004). However, in England, only 25% of women and 37% of men were achieving that target in 1998 (Department of Health, 2004). The picture in other European countries is no better, with the prevalence of a sedentary lifestyle, defined as expending less than 10% of leisure time expenditure in activities involving ≥4 metabolic equivalents (the ratio of energy expended during a PA to the metabolic rate of sitting quietly), ranging from 43.3% in Sweden to 87.8% in Portugal. This is especially worrying among obese individuals, where a significantly higher prevalence of inactivity is observed regardless of the gender, country or criteria used to define inactivity (Varo et al., 2003).

Whether inactivity is a cause or a consequence of obesity is a rather complex question. It is a paradox that obesity is increasing while overall EI has been falling over the past 20 years in the United Kingdom. It has been proposed that a greater reduction in PA levels and an inability of the organism to down-regulate EI to a similar extent in order to match the decreased EE may be the dominant factor driving the increasing prevalence of obesity (Moore, 2000). However, this does not mean that physical inactivity alone explains this epidemic, or that diet has no role in the aetiology of obesity (British Nutrition Foundation, 1999; IOTF and EASO, 2002; House of Commons, 2004). It seems that the primary cause for the increasing prevalence of obesity is an increased consumption of highly palatable, energy-dense food (which deregulates normal satiety mechanisms, inducing a state of “passive over-consumption”) combined with reduced PA levels (IOTF and EASO, 2002; World Health Organization, 2003; Prentice and Jebb, 2004). Prentice & Jebb (1995), using epidemiological data from different population-based studies, showed that the increase in the prevalence of obesity in the past 50 years was closely related to measures of PA.
and practically unrelated to diet (Prentice and Jebb, 1995). However, at the individual level, little evidence is available that obese subjects are significantly less active than lean subjects and, therefore, that inactivity is a predictor of obesity, until obesity reaches a severity threshold where performing any type of movement becomes highly limited (Prentice et al., 1996).

The role of PA on obesity prevention was recently reviewed by Wareham and colleagues in a metanalysis published in 2005. Although cohort studies tended to show that individuals with high levels of PA were less likely to gain weight, the evidence regarding the predictive effect of baseline PA on subsequent weight gain was rather inconsistent, probably due to confounding factors, reverse causality and measurement error. Most of the observational studies showed a negative association between PA levels and body weight, but they do not help to ascertain the direction of causality. Intervention trials, on the other hand, were scarce and inconsistent in their conclusions regarding the effectiveness of increasing PA levels in obesity prevention (Wareham et al., 2005). Although a final conclusion has not been reached as to whether inactivity is a cause or a consequence of obesity, PA levels are likely to provide valuable information for a better understanding of the obesity epidemic.

1.3 Hunger, appetite and satiety – general overview

Eating behaviour is a complex phenomenon that can be conceptualised by the size and frequency of eating episodes and our everyday food choices, which together determine total energy and macronutrient intake. This phenomenon is the result of constant inputs, not only biological (or physiological) but also environmental (Blundell, 1991; Blundell and Halford, 1994). In fact, it is accepted that the physiological mechanisms that control food intake can be easily overridden by strong social and environmental factors (British Nutrition Foundation, 1999) (Figure 1.1). As emphasized by Blundell & Fischer (2004), while behaviour, in the form of PA, only accounts for 40 to 60% of total EE, EI is 100% behaviour (Blundell and Fischer, 2004).
Figure 1.1. Interactions between biological and environmental factors in determining eating behaviour. Adapted from Blundell & Halford, 1994.

Appetite and satiety are two subjective concepts used to explain the control of food intake. In a very simplified way, it can be said that these phenomena have in their foundation both objective (unconditional or physiological) and subjective (conditioned or learned) components (Stubbs et al., 2000).

Appetite can be defined as a range of parameters associated with food consumption that predict normal eating behaviour; such as sensations of hunger, drive for energy, selection of specific nutrients and tastes and craving for certain foods (Blundell, 1991; King et al., 1997b). The biological drive to eat which causes subjects to start looking for food is known as hunger. This aspect of human feeding behaviour determines not only how much to eat, but also what and when. In a simplistic way, it can be said that while appetite is a psychological desire to consume certain foods, aroused by environmental cues such as the sight and smell of a freshly-baked cake, hunger is a more basic physiological sensation, an urgent drive to eat food.

After food consumption, hunger feelings start to decrease and different physiological mechanisms are activated that inhibit further eating. This process by
which an eating episode reaches its end is known as satiation. On the other hand, the inhibition of hunger and eating as a result of previous food intake is known as satiety: the process that delays the onset and restricts food intake at the next eating episode. These two processes are also described as intra- and inter-meal satiety, respectively (Blundell, 1991; Gerstein et al., 2004).

Blundell & Halford (1994) have proposed a model to describe the various mechanisms involved in the control of satiety and the amount of food consumed at an eating episode. The sensations involved in the regulation of feeding include hunger, appetite, satiety and satiation. Eating behaviour is determined by the combined action of satiety and satiation (Blundell, 1991). There is a clear link between appetite, as a drive for energy, and the ‘satiety power’ of food. Satiety power can be described as the capacity of a specific food to subsequently suppress hunger and delay the onset of a new eating episode and, therefore, produce a longer inter-meal interval. This outcome is the result of the interaction between food’s characteristics; nature, quantity and sensoral properties, and the psychobiological responses to its consumption. This can be conceptualised in the form of a cascade - “satiety cascade”- with different processes taking place during and following food consumption, designated as sensory, cognitive, post-ingestive (or pre-absorptive) and post-absorptive, which will ultimately determine satiety and satiation (Blundell, 1991; Blundell and Halford, 1994) (Figure 1.2).

Figure 1.2. Components of the satiety cascade. Taken from Green et al, 1997.
According to this cascade, food is sensed and processed by the biological system, and as a result, different neural and humoral signals are generated that control appetite. According to Blundell (1991), the biopsychological system involved in the expression of appetite operates at three different levels. The bottom level includes psychological (such as subjective hunger, cravings and hedonic feelings) and behavioural events (such as meals, snacks, energy and macronutrient intake), the intermediary level includes peripheral physiology and metabolic events and the top level includes neurotransmitters and interactions within the brain (Blundell, 1991). The expression of appetite results, therefore, from complex interactions between these three levels.

1.4 Experimental approaches in appetite research

In the area of appetite research different aspects need to be taken into account including, not only food intake and motivation to eat, but also food preferences and macronutrient selection.

1.4.1 The preload paradigm

Food intake in the laboratory environment can be measured during an eating episode or as a result of manipulating a previous meal, assessing respectively the effect of a food attribute on satiation and satiety. These procedures are known as concurrent evaluation and the preload paradigm, respectively (Kissileff, 1985).

The preload paradigm, also known as “preload-test-meal” paradigm, is the most commonly used, involving the consumption of a vehicle food containing the attribute under investigation (energy and/or nutrients) – the “preload” and the measurement of EI at a subsequent meal, usually known as the “test-meal”. This paradigm has been widely used to assess the short-term effects of dietary manipulations on subjective motivation to eat and subsequent food intake using a within-subject repeated measures design (Kissileff, 1985). The preload paradigm has been consistently used in the area of appetite research to study homeostatic feedback control, a process that if missing or not tightly regulated can lead to energy imbalances and weight gain (Hill et al., 1987). The term “energy or caloric compensation” refers to the adjustment of EI at a test-meal as a result of changes in preload energy content.
Energy compensation is a complex phenomenon and multiple factors have been shown to impact on it, namely subject characteristics (e.g. age, gender, body weight); the physical properties of the preload (energy density, macronutrient composition, volume, physical state, texture, flavour and taste/palatability); the time-interval between preload and test-meal and psychological, cognitive and social factors (Blundell and Halford, 1994; Almiron-Roig et al., 2003; Hill et al., 1987; Rolls et al., 1994). It has also been shown that any potential effect of the preload manipulation on subsequent food intake at the test-meal will decay as the time between preload and test-meal increases (Rolls et al., 1991; Stubbs et al., 1998). Despite being highly variable and dependent on the nature and size of the food attribute under investigation, most of the studies tend to present the test-meal one hour after the preload (Green et al., 1997).

1.4.2 Assessment of ad libitum food intake

*Ad libitum* food intake at a test-meal is usually used in association with the preload paradigm to investigate the impact of different preloads on subsequent EI. In practice, subjects are presented with a pre-weighed amount of food that is re-weighed after they had finished eating, allowing energy and macronutrient intake to be calculated (Hill et al., 1995b).

Growing interest in the area of feeding behaviour and food choices has led to a slightly different approach – the “buffet test-meal”, in which different food items of varied macronutrient composition are presented on a tray or table within a single eating episode (Hill et al., 1995b). The use of buffet test-meals, as a measure of spontaneous energy and macronutrient intake, was shown to have a high reproducibility and, therefore, to be a reliable method in the laboratory environment (Arvaniti et al., 2000). Although this method allows the collection of both quantitative and qualitative data, it is very laborious and expensive, and the artificial laboratory environment can distort typical eating behaviour. However, this last limitation can be greatly minimized if this approach is used in a within-subject design (Stubbs et al., 1998). It can also be argued that test-meals where a multitude of foods are presented do not represent everyday life and usually result in overconsumption, therefore lacking external validity (Hill et al., 1995b). To try to overcome this drawback, subjects are not presented with their favourite foods, although it needs to be ensured that familiar food-items are provided.
(Stubbs et al., 1998). Additionally, because a repeated measurement design is usually used, it is important to keep constant the type and amount of foods presented.

Several factors have been shown to impact on eating behaviour, including the immediate environment (location, weather conditions, noises); temporal stimuli (hour of the day, day of the week, season of the year); social stimuli (presence of other people); psychological stimuli (subjective state of anger and anxiety) and finally the properties of the foods presented (palatability, energy content, etc) (De Castro, 1997; Kissileff, 1985). One way of minimizing the influence of those factors is to standardize test-meal conditions, by using always the same room, with constant light, free of odours, sounds and other disturbing factors, as well as to keep subjects in separate cubicles during test-meals, in order to avoid social interaction.

Although the amount of food eaten at a specific eating episode, and within a specific context may be used as a measure of appetite, the degree to which actual intake reflects appetite is controversial. This inconsistency is probably the result of the influence of different factors in the pathway between appetite and food intake, namely cognitive factors, the hedonic properties of food and the environmental circumstances. However, if measured in standardised conditions, actual food intake can be used as an indicator of appetite (de Graaf et al., 2004).

1.4.3 The disposition to eat

1.4.3.1 Subjective ratings of motivation to eat

One of the most common systems of assessing motivation to eat (hunger, desire to eat, fullness, etc) is the use of visual analogue scales (VAS). VAS were initially used to assess pain, but became particularly popular in appetite research in the eighties (Blundell and Rogers, 1980; Hill and Blundell, 1982). They take the form of a straight line, usually 10cm long, with words anchored at each end describing extreme sensations. Subjects are asked to express their motivation to eat by putting a vertical mark across the line and this is usually repeated at different time points to generate a temporal profile. In the area of appetite regulation, a cluster of six questions is usually used: "How hungry do you feel?"; "How full do you feel?"; "How strong is your desire to eat?"; "How much do you think you could eat now?"; "Urge to eat" and
"Preoccupation with thoughts of food" (Stubbs et al., 2000). VAS can also be used to assess the palatability of different foods or meals, which is of great importance since perceived palatability may affect food intake (Stubbs et al., 1998).

The use of VAS to assess subjective motivation to eat presents several advantages: it is easy and quick to perform, simple to analyse and its standardized format allows comparisons among different studies (Stubbs et al., 2000). However, VAS are sometimes regarded as not very reliable. According to Stubbs and collaborators (2000) different questions need to be addressed to evaluate the reliability and validity of VAS in assessing motivation to eat and, therefore, its usefulness in the area of appetite research: 1) Do the ratings of motivation to eat accurately predict food intake?; 2) Are the ratings sensitive to experimental manipulations? 3) Are they reproducible?

Positive correlations have been reported between subjective hunger ratings and subsequent EI, in free-living conditions using self-recorded food intake over a seven-day period (de Castro and Elmore, 1988). However, in another study, with a similar design, hunger ratings and reported EI in the following hour were positively correlated during week but not weekend days, and no association was observed when a within-subject analysis was performed (Mattes, 1990). Another study, with a well controlled environment, showed a small but significant correlation between pre-meal perception of hunger and fullness and EI at a test-meal in old subjects (65-85 years old), while in young subjects (18-35 years old) that association was only significant for hunger ratings (Parker et al., 2004). Hunger ratings do not necessarily predict food intake, which is not completely unexpected since hunger and eating are not always coupled (Blundell and Rogers, 1980; Hill et al., 1995a). We do not eat only when we are hungry and we do not always stop eating when we are full, many other factors are involved in the relationship between motivation to eat and feeding behaviour. Even though subjective ratings of appetite are usually correlated with subsequent food intake and, therefore, can be used under some, but not all circumstances, as a valid indicator of eating behaviour in relation to meal size and frequency, they should not be seen as an accurate proxy of EI (Hill et al., 1987; Mattes, 1990; Flint et al., 2000; Stubbs et al., 2000).

There is evidence that VAS are sensitive to various treatments (Blundell and Rogers, 1980) and dietary manipulations, including changes in macronutrient
composition and EI (Stubbs et al., 2000; Flint et al., 2000). Subjective ratings of motivation to eat are, however, not easily reproduced when tested after similar meals, in the same subject, on different days. This low reproducibility is probably the result of both true biological day-to-day variation, as well as methodological variation (how people interpret and use the scale) (Flint et al., 2000; Raben et al., 1995). Another limitation of VAS relates to the fact that subjects tend to mark their ratings very close to the upper or lower ends of the scale despite being told that they would represent the most extreme sensations they had ever had (Hill et al., 1995b). Although this may bring problems, there are some statistical transformations that can be applied to minimize this effect (Long, 2000). All these drawbacks and limitations do not necessarily mean that VAS are not useful and valid, but instead that they should be carefully interpreted and used in combination with other measurements of eating behaviour. Because inter-individual variation may account for as much as 50% of the variance in VAS observed in a group of subjects, motivation to eat is better measured in the context of a within-subject repeated-measurement design (Stubbs et al., 2000).

The measurement of subjective motivation to eat has been criticised as a very subjective and even superfluous analysis based on psychometric data, since the primary outcome in most of the studies is food intake. However, although the assessment of “actual food intake” may seem, at the first glance, a very straightforward measurement, it also suffers from some drawbacks, especially when subjects have a very narrow selection of foods available (Stubbs et al., 2000). Gathering information regarding the motivation to eat may overcome some of those limitations. A person may feel hungrier in condition A compared with B, but end up eating the same on both conditions due to cognitive, social or environmental confounders.

1.4.3.2 Food preference lists

The preference for specific foods is an important aspect to consider in the complex pathway underlying eating behaviour and food intake (Hill and Blundell, 1982). Food preference lists are usually used in parallel with VAS, as a measure of disposition to eat. Here subjects are asked to select any or all the food items listed according to what they would like to eat at that moment in time. The checklists comprise foods with different energy and macronutrient composition allowing the assessment of, not only total energy but also, macronutrient preference (Hill et al.,
One of the most commonly used food checklists was developed by Hill and collaborators (1987) and consists of different food-items divided into: high-fat, high-carbohydrate, high-protein, low-energy and mixed items (Hill et al., 1987). A significant positive correlation has been described between total items selected and subsequent total food intake, as well as, between subjects’ preference for protein and carbohydrate (CHO) and prospective consumption of these macronutrients (Blundell and Rogers, 1980; Hill and Blundell, 1986).

1.5 Central and peripheral control of appetite

Appetite and food intake are under the control of both the brain and multiple hormones, produced mainly by the gastrointestinal (GI) tract, but also the pancreas, the adrenal glands and the adipose tissue (Hellstrom et al., 2004). These two types of regulation are known, respectively, as central and peripheral.

It has been known for a long time that the hypothalamus plays a key role in the central regulation of eating behaviour in humans. The hypothalamic region of the brain is constantly receiving and processing neural, metabolic and endocrine signals from the periphery, enabling it to adjust not only EI but also EE. The area within the hypothalamus most actively involved in the regulation of feeding is the arcuate nucleus (ARC) which express receptors for many of the hormones and peptides known to be involved in eating behaviour (Neary et al., 2004).

Two distinct populations of neurons involved in the regulation of food intake can be found on the ARC: one population co-expresses the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) and the other the anorexigenic cocaine and amphetamine-regulated transcript (CART) and propiomelanocortin (POMC). POMC is the precursor of the α-melanocyte-stimulating hormone (α-MSH), an anorectic peptide agonist of the melanocortin 3 and 4 receptors (MC3 and MC4) (Neary et al., 2004; Murphy and Bloom, 2004). NPY exerts its potent orexigenic effects probably via Y1, Y2 and Y5 receptors (Murphy and Bloom, 2004; Batterham and Bloom, 2003) and its secretion seems to be sensitive to signals reflecting body fat stores (Kokot and Ficek, 1999) (Figure 1.3).
Figure 1.3. Peripheral signals and neuronal circuits involved in energy homeostasis. Continuous lines indicate stimulatory effects and dashed lines indicate inhibitory effects. PYY – peptide YY, GLP-1 – glucagon-like peptide 1, PP – pancreatic polypeptide, NPY – neuropeptide Y, AgRP – agouti gene-related peptide, POMC – pro-opiomelanocortin, αMSH – α-melanocyte stimulating hormone, MC3 and MC4 – melanocortin 3 and 4. Adapted from Murphy & Bloom, 2004.

Much recent research in the area of appetite and feeding behaviour has focused on peripheral signals from the GI and adipose tissue (such as gut-derived peptides and hormones, leptin and different cytokines) and how they modulate hypothalamic function (British Nutrition Foundation, 1999; Neary et al., 2003).
1.5.1 Short-term regulation of appetite

Shortly after foods have been ingested, the brain starts to receive information from the GI tract needed to regulate nutrient and energy balance (EB). That information can be originated from distinct levels: luminal or absorbed nutrients, GI distension and GI hormones (Gutzwiller et al., 2004).

1.5.1.1 Nutrient signals

The short-term regulation of food intake and appetite was initially thought to be driven by single nutrients, probably because it was described that different nutrients had distinct satiety powers (Blundell, 1991) and that specific areas within the hypothalamus were sensitive to specific nutrients (Wilding, 2002). In the fifties, three different theories were proposed as to how the circulating levels of glucose, lipids (free fatty acids, glycerol, etc) and amino-acids (tryptophan, L-arginine, etc) could modulate hunger and fullness and regulate food intake in the short-term (British Nutrition Foundation 1999). These theories have become known as glucostatic, lipostatic and aminostatic, but despite some evidence supporting their role in food intake and appetite regulation, it is unlikely that they fully represent the complex mechanisms involved in eating behaviour.

In the late eighties, a new theory for day-to-day regulation of food intake was proposed, whereby CHO stores in the form of glycogen would exert a negative feedback on subsequent food intake - the glycogenostatic model (Flatt, 1987b). According to this model a constant storage of CHO is maintained over time through selective food intake. Therefore, if CHO are limited or removed from the diet, hyperphagia is inevitably promoted as a regulatory mechanism to provide the extra CHO needed to achieve a new balance in glycogen stores. However, the available evidence regarding this theory remains controversial (British Nutrition Foundation, 1999; Stubbs, 1995).

Another theory - the energostatic model - started to develop in the nineties. It states that appetite is controlled by the oxidation rate of metabolites in the liver and other peripheral tissues as a direct result of energy levels (mainly but not restricted to body fat) (Friedman et al., 1990). More recently, Stubbs (1995) updated this theory by proposing that it is the position of macronutrients in the “oxidative hierarchy” that
controls satiation (Stubbs, 1995). Apart from knowing which of these systems regulates appetite control, Goldberg and colleagues (1998) raised the fundamental question as to whether any of them can override the strong social and environmental factors that modulate eating behaviour in our modern societies (Goldberg et al., 1998).

1.5.1.2 Peripheral hormonal signals

Nutrients in the GI tract stimulate the coordinated production and release of several satiety hormones such as cholecystokinin (CCK), glucagon like-peptide-1 (GLP-1) and peptide YY (PYY). Some of these hormones are directly involved in gastric emptying, while others have longer-lasting postprandial effects that will affect not only satiation but also satiety (de Graaf et al., 2004; Cummings et al., 2001).

CCK was the first gut hormone to be implicated in appetite regulation. More than 20 years ago Kissileff and colleagues (1981) showed that CCK infusion decreases food intake in men (Kissileff et al., 1981; Murphy and Bloom, 2004). This hormone is rapidly released into the bloodstream in response to the presence of digestive products, mainly fat, in the intestinal lumen, increases gradually over the first 10-30 minutes after a meal, and despite slowly decreasing after that, may remain elevated for three to five hours postprandially (de Graaf et al., 2004; Moran and Kinzig, 2004). CCK acts both within the GI tract, maximizing nutrient absorption (by stimulating pancreatic and gallbladder secretions, delaying gastric emptying and modifying intestinal motility) and through vagal stimulation, providing negative feedback signals to the brain. CCK has been shown to quickly inhibit food intake by reducing meal size and duration, therefore contributing to meal termination and postprandial satiety. However, these effects are usually brief (de Graaf et al., 2004; Moran, 2000; Geary, 2004; Moran and Kinzig, 2004). It has been suggested that the role of CCK on feeding behaviour is not restricted to the short term and that it may also be involved in the long-term regulation of food intake and body weight by synergizing the actions of leptin and insulin (Moran, 2000; Ukkola, 2004; McMinn et al., 2000).

GLP-1 is secreted from the distal ileum in response to a mixed meal. Its concentration starts to increase 10-20 minutes after a meal and peaks after 60 minutes. For this reason GLP-1 is unlikely to be the major signal involved in meal termination, since most eating episodes last, on average, only 20 minutes, but is probably more
involved in satiety by suppressing food intake and reducing hunger feelings in the postprandial state (Blundell and Naslund, 1999; Gutzwiller et al., 1999). This gut hormone is thought to be involved in the "ileal brake" mechanism, which inhibits acid secretion, gastric emptying and gut motility in response to feeding, allowing for a gradual release of nutrients to the small intestine (de Graaf et al., 2004; Blundell and King, 1999). The mechanism by which GLP-1 inhibits food intake is not clearly understood. Although delayed gastric emptying has been proposed as a potential mechanism (Blundell and Naslund, 1999), GLP-1 has been shown to be able to cross the blood-brain barrier (Kastin et al., 2002), therefore suggesting that both central and peripheral mechanisms are involved in the impact of GLP-1 on satiety. Additionally, GLP-1 also acts as an incretin, enhancing insulin and suppressing glucagon secretion after a meal (Kreymann et al., 1987).

PYY is co-secreted with GLP-1 from endocrine L-cells of the distal ileum and colon in response to feeding, in proportion to the energy content of the meal (Bottcher et al., 1984) and is as effective as GLP-1 in delaying gastric emptying and transit time (Allen et al., 1984; Savage et al., 1987). Two forms of PYY are released: the full length \( \text{PYY}_{1-36} \) and the truncated biologically active form \( \text{PYY}_{3-36} \), created by cleavage of the N-terminal residues by dipeptidyl peptidase IV. \( \text{PYY}_{3-36} \) acts as an agonist of the Y2 receptors in the hypothalamus, thereby regulating subsequent food intake by modulating the activity of NPY and POMC neurons in the ARC (Batterham and Bloom, 2003). PYY probably exerts its effects on the complex cascade of feeding behaviour through the stimulation of anorexigenic neurons such as POMC and/or inhibition of orexigenic neurons such as NPY (Neary et al., 2003). Evidence for these direct effects of PYY on feeding comes from studies showing that \( \text{PYY}_{3-36} \) is able to cross the blood-brain barrier bidirectionally by non-saturable mechanisms, at least in mice (Nonaka et al., 2003).

Pancreatic polypeptide (PP) is a member of the PP peptide family, which also includes PYY and NPY. It is produced by endocrine cells of the pancreatic islets in response to food consumption and its levels may remain elevated in the blood for as long as six hours postprandially (Adrian et al., 1976). PP release is known to be sensitive to plasma glucose levels (Adrian et al., 1977) and although the exact mechanisms by which PP reduces food intake are not completely understood, it is thought that its anorectic effects may be mediated via the neuropeptide Y4 receptor.
(Batterham et al., 2003b). Although controversial, it has also been suggested that the effects of PP on food intake may be mediated through a decrease in gastric emptying (Katsuura et al., 2002).

Ghrelin, a peptide with growth hormone-releasing properties, is produced mainly by the stomach (Ariyasu et al., 2001). Ghrelin concentrations increase with fasting and quickly decline after a meal, suggesting that ghrelin can signal pre-meal hunger and meal initiation and, therefore, can be used as a biomarker of satiety (Cummings et al., 2001; Korbonits et al., 2004). In fact, ghrelin is the only peripherally-active peptide known to have orexigenic properties. Its stimulatory effects on appetite and subsequent food intake are achieved through stimulation of orexigenic neurons (NPY and AgRP) and inhibition of gastric vagal afferent nerves (Korbonits et al., 2004). Interestingly, ghrelin seems to be involved not only in short-term regulation of appetite (by controlling meal initiation), but also in the long-term regulation of EB after weight loss (by reducing fat oxidation) (de Graaf et al., 2004), making it one of the more important scientific discoveries in the area of appetite in recent years. Ghrelin responds both to acute and chronic changes in nutritional state; its levels are significantly reduced in obese subjects (Tschop et al., 2001) and markedly elevated in patients with anorexia nervosa (Ariyasu et al., 2001). The low plasma ghrelin levels observed in obese subjects are probably the result of elevated insulin and/or leptin levels, which exert a negative feedback control over ghrelin (Ravussin et al., 2001).

1.5.2 Long-term regulation of appetite and body weight

It has been proposed that adipostatic factors generated by the adipose tissue can auto-regulate body fat levels by negative feedback pathways. Leptin is one of those factors. It is synthesised mainly by the adipose tissue in proportion to body fat levels, providing information to the hypothalamus about the state of energy stores. Leptin has been shown to reduce food intake and body weight and increase EE (de Graaf et al., 2004). It is, therefore, an apparent paradox that despite leptin being an anorexigenic signal, obese subjects present with high levels of the hormone. In a similar way to insulin, obese subjects seem also to suffer from leptin resistance (British Nutrition Foundation, 1999; Considine et al., 1996). Murphy & Bloom (2004) have suggested that leptin may play a critical role in periods of starvation, but be of little importance in
normal-everyday life where food is freely available (Murphy and Bloom, 2004). When fat stores are reduced, leptin production is down-regulated leading to the stimulation of orexigenic NPY/AgRP neurons and inhibition of anorectic POMC neurons (Wilding, 2002). However, leptin is also expressed in non-adipose tissue sites, in particular the stomach. Gastric leptin has been shown to be sensitive to acute nutritional state, with increased secretion after feeding. Leptin is, therefore, likely to be involved in both acute and chronic regulation of food intake by acting as a messenger to the brain of the availability of external (food) and internal (adipose tissue) energy supplies (Pico et al., 2003).

Although insulin is not released from adipocytes, it may also act as an adiposity signal. Its basal plasma levels correlate with body fat levels and obesity is usually associated with insulin resistance. Insulin is thought to exert a direct anorectic effect by inhibiting the expression of NPY/AgRP neurons in the ARC (Murphy and Bloom, 2004). An interesting model for the regulation of appetite and EB has been proposed according to which, the concentration of adiposity-related long-term signals such as leptin and insulin would modulate the sensitivity of short-term meal-related signals, such as CCK and GLP-1 (Schwartz et al., 1999).

In summary, three types of mechanisms seem to operate in the complex system that regulates appetite and EI: long-term including leptin and insulin; intermediate including post-absorptive signals associated with macronutrient oxidation such as glucose and free fatty acids plasma levels and, finally, short-term mechanisms involving immediate post-ingestive, but pre-absorptive, signals arising from the GI tract, such as CCK, GLP-1 and PYY (Blundell, 1991; King et al., 1997b). Probably because feeding is a basic human need, there is considerable overlap and redundancy between the different pathways involved in appetite control (British Nutrition Foundation, 1999).

Apart from this complex homeostatic system, where hunger and satiety signals are tightly balanced in order to maintain EB, food intake is also under hedonic control (Saper et al., 2002). While the homeostatic system is activated by energy deficits (hunger), the hedonic system is activated by the availability of palatable food (reward) (Lowe and Levine, 2005). In fact, if food intake was solely under homeostatic control, obesity would not be one of the biggest epidemics of the 21st century. Eating is a
pleasure for most of us and few experiences in life bring more satisfaction than a well prepared meal with rich flavours, textures and smells.

In the same way that the homeostatic system includes a multitude of hormones, peptides and neurotransmitters (previously discussed), the hedonic system is also well characterized and includes opioids, endocannabinoids and dopamine pathways, among others (Yeomans et al., 2004). Despite the independence of these systems, interactions between them have been described (Saper et al., 2002; Blundell and Fischer, 2004). Although these interactions are complex, an asymmetry seems to exist favouring positive EB via passive over-consumption (Yeomans et al., 2004). Blundell & Fisher (2004) have suggested that a high susceptibility to hedonic signals may be a risk factor for weigh gain (Blundell and Fischer, 2004).

1.6 Restrained eating behaviour and appetite

Apart from physiological processes; including both central and peripheral mechanisms, already described, appetite is also regulated by external stimulus arising from food and the surrounding environment. Environmental, psychological, social, and cultural stimuli have been shown to exert powerful effects on food intake (De Castro, 1996). The increased availability of highly palatable food in the present society may lead to the chronic activation of the hedonic appetite system and explain why an increased number of people in Western societies need to restrain their food intake, in order to maintain their body weight within a social acceptable range. Dietary restraint has, therefore, become an important behavioural concept.

Dietary restraint refers to the extent to which individuals are concerned with their body weight and attempt to control it by dieting (Herman and Mack, 1975). It is characterized by a self-imposed resistance to the internal and external cues that regulate eating behaviour motivated by the desire to maintain or suppress body weight (Herman and Mack, 1975). The concept was first developed in the context of obesity aetiology, to try to explain differences in eating behaviour between obese and normal-weight subjects. In the early seventies, Nisbett (1972) proposed a set-point model, according to which everyone has a tightly regulated body weight, determined by the overall number of adipocytes. Obese individuals would have a higher set-point and, at least some of them, due to social pressure would suppress their weight below their physiological set
point by actively restricting food intake (Nisbett, 1972). Nisbett’s model was later expanded by Herman & Mack (1975) by showing that concerns with body weight and dieting, what they collectively called “restraint” is independent of body weight and can be found also in normal weight individuals (Herman and Mack, 1975). Moreover, they were able to show that differences in restraint, rather than obesity in itself, can predict individual differences in eating behaviour (Herman and Mack, 1975; Herman and Polivy, 1980).

Herman & Mack (1975) were able to describe an astonishing phenomenon using the “preload-test-meal” paradigm: while unrestrained subjects eat less after a preload, restrained subjects tend to eat more, two opposite behaviours that became known as “regulatory” and “counter-regulatory eating”. Although females tend to present with higher levels of restraint compared with males, this pattern was replicated in both genders (Polivy, 1976; Woody et al., 1981; Rotenberg and Flood, 2000) and independently of body weight (Herman and Polivy, 1980; Herman and Mack, 1975).

Two hypotheses were developed to try to explain this abnormal eating behaviour of restrained eaters in response to preloading. The “disinhibition theory” proposes that certain events, collectively known as “disinhibitors”, such as preloading, can act cognitively by temporarily disrupting the commitment for dieting (Herman and Polivy, 1980). It is possible that this disruption of self-control, observed in restrained eaters, may be mediated by the emotional distress caused by the consumption of a forbidden food or the perception of having overcame a pre-established energy threshold (Ruderman, 1986; Mela and Rogers, 1998). Once the perceived EI overcame a critical individual threshold, restrained eaters would abandon their self-imposed restrictive behaviour, becoming susceptible to underlying hunger signals and therefore more prone to overeating (Herman and Polivy, 1980). In addition to preloading, other types of disinhibitors, such as alcohol consumption and negative emotional states (e.g. anxiety and depression), had already been found to disinhibit food consumption in restrained and inhibit food consumption in unrestrained eaters (Herman and Polivy, 1980). According to Ruderman (1986) restrained individuals tend to see dieting in “a rigid all-or-nothing fashion” and, as such, a violation of the diet is seen as an excuse to abandon it, at least, temporarily.
The “disinhibitory theory” was further expanded by the introduction of a “boundary model” (Ruderman, 1986) (Figure 1.4.).

![Boundary model diagram](image)

Figure 1.4. Boundary model. Adapted from Ruderman, 1986

According to this model, food intake is regulated within a specific range, called “biological indifference” through normal hunger and satiety physiological signals. In restrained eaters, the hunger boundary would be much lower and the satiety boundary much higher than in unrestrained eaters, resulting in a wider zone of biological indifference. This would mean that restrained eaters would feel hungry only when very deprived and would reach fullness much later. Moreover, restrained eaters would also have a cognitively self-imposed “diet boundary” below their physiological fullness boundary which determines the allowed amount of calories they can eat. In the presence of a small preload or no preload, restrained individuals tend to eat only a small amount during the test-meal in order to maintain their intake below the diet boundary. However, if the preload is big enough to overcome their diet boundary, restraint is abolished with consequent overeating until the fullness boundary is reached (Ruderman, 1986).

The response to preloading was also shown to be modulated by social factors (Herman and Polivy, 1980; Ruderman, 1986). The presence or absence of the experimenter was shown to impact in the response to preloading. While unrestrained subjects were unaffected by the presence of the experimenter, restrained eaters reversed their abnormal counter-regulatory response and showed a sensible eating behaviour (i.e. compensated) similar to unrestrained eaters (Herman and Polivy, 1979). Interestingly,
when individuals were exposed to a “preload-test-meal” paradigm along with another person who restricted her/his food intake or overate, therefore identifying her/himself, directly or indirectly, as a dieter or non dieter, restrained and unrestrained subjects responded in the same direction, reducing or increasing their *ad libitum* food intake in order to match the dieting model. Unexpectedly, when left to engage with someone who identified herself as a dieter and then overate, restrained and unrestrained subjects showed a counter-regulatory response later on when left alone at a test-meal, suggesting that restraint may be seen as a state and not necessarily a trait (Polivy et al., 1979). This and other studies (Tomarken and Kirschenbaum, 1984) suggest that restraint is not a dichotomous, but instead a continuous construct, with both restrained and unrestrained individuals being able to show regulatory and counter-regulatory eating. Only its relative prevalence and the power of the conditions required to produce those behaviours is different. Unrestrained eaters may be seen as “less restrained eaters” who present a higher threshold for counter-regulation compared with their restrained counterparts (Herman and Polivy, 1980; Tomarken and Kirschenbaum, 1984).

Although “dieting” and “restraint” are sometimes used interchangeably, they reflect different constructs. Restrained eaters may be seen as in a vicious cycle, with chronic dieting undermining lapses in restraint (disinhibition) with consequent episodes of overeating and this creating the need for further dieting (Heatherton et al., 1988). Dietary restraint was initially measured using the restraint scale (Herman and Mack, 1975), which has since been developed and refined into a 10-items questionnaire focusing on concerns with dieting and weight fluctuation – the revised restrained scale (RRS) (Herman and Polivy, 1980). However, this scale seems to suffer from psychometric and conceptual drawbacks, since it confounds restraint with disinhibition and is inadequate in the obese population (Heatherton *et al.*, 1988). The problem is that the restraint scale was developed to identify dieters and unfortunately most of the dieters display both restraint and disinhibition. In an attempt to overcome this limitation and isolate the true restraint construct new questionnaires were developed in the eighties: the Three Factor Eating Questionnaire (TFEQ) that measures three dimensions of eating behaviour: restraint; disinhibition and hunger (Stunkard and Messick, 1985) and the Dutch Eating Behaviour Questionnaire (DEBQ), which allows the measurement of three different eating behaviours: restrained, emotional and external eating (van Strien *et al.*, 1986). Emotional eating can be described as excessive eating resulting
from a state of confusion between internal signals, such as anger, fear and anxiety, for which the normal response is loss of appetite, and hunger. External eating is the over response to food related stimuli such as look and smell, regardless of the internal status of hunger and fulness (van Strien et al., 1986). Although all these questionnaires are conceptually related, they reflect different dimensions of dietary restraint. In opposition to the RRS, the restraint subscale of the TFEQ and the DEBQ have few items on overeating and weight fluctuations and more on cognitive and behavioural strategies for restricting EI (Mela and Rogers, 1998; Laessle et al., 1989a).

It has become a common practice to exclude restrained eaters from appetite studies based on the assumption that they exhibit an atypical eating behaviour in response to preloading (King and Blundell, 1995; Lluch et al., 1998; Westerterp-Plantenga et al., 1997; Mattes, 1996; Foltin et al., 1992). However, this exclusion has been carried out arbitrarily and no consensus exists on either the questionnaire or cut point to be used. Rather surprisingly, a close analysis of the literature reveals that only a few studies have been able to show a true “counter-regulatory eating behaviour” (with a significant preload x restraint interaction) in response to preloading in restrained eaters (Herman and Mack, 1975; Rotenberg and Flood, 2000; Woody et al., 1981; Polivy et al., 1988; Spencer and Fremouw, 1979; Hibbscher and Herman, 1977) (see Table 1.1).

Interestingly, in all the studies showing counter-regulation in restrained eaters, a single highly palatable and “diet-breaking” food – ice cream, was used as the test-meal and restraint was always measured by the RRS. It seems, therefore, that the response to preloading, in terms of eating behaviour, is dependent on both, the questionnaire used to assess restraint and the type of test-meal presented. Moreover, a variation of the “preload-test-meal” paradigm was used in all these studies – the “preload taste-test” paradigm using ice cream as the taste-test. Here, participants are asked to taste and rate different types of ice-cream. They can taste as much or as little ice-cream as they want and they are invited to help themselves at the end with the remaining ice-cream.
Table 1.1 Studies assessing the effects of restraint on food intake after preloading

<table>
<thead>
<tr>
<th>Study</th>
<th>Tool</th>
<th>Cut-off point</th>
<th>Design</th>
<th>Preload</th>
<th>Test-meal</th>
<th>Results</th>
</tr>
</thead>
</table>
| Herman & Mack, 1975    | RRS  | Median split (8.5) | Parallel study        | Milkshake   | Taste-test Ice cream | Significant Preload x Restraint interaction  
|                        |      |               |                         | - no preload | Ice cream       | Restrained counter-regulated Unrestrained compensated                  |
| Polivy, 1976          | RRS  | Median split (11.5) | Parallel study        | High and low energy chocolate pudding | Taste-test Sandwiches | Tendency to a significant Preload x Restraint interaction (P=0.07)  
|                        |      |               |                         | - ↑ energy told ↑ energy chocolate pudding |               | Restrained eaters counter-regulated but only when perceiving the preload as ↑ energy  
|                        |      |               |                         | - ↑ energy told ↓ energy chocolate pudding |               | Unrestrained ate approximately the same regardless of the condition (did not compensate) |
| Hibscher & Herman, 1977| RRS  | Median split (14) | Parallel study        | Milkshake   | Taste-test Ice cream | Significant Preload x Restraint interaction  
|                        |      |               |                         | - no preload - preload | Ice cream       | Restrained counter-regulated Unrestrained compensated                  |
| Spencer & Fremouw     | RRS  | Median split (?) | Parallel study         | Milkshake   | Taste-test Ice cream | Significant Preload x Restraint interaction  
|                        |      |               |                         | - told ↑ energy | Ice cream       | Restrained counter-regulated Unrestrained compensated                  |
| Woody et al, 1981     | RRS  | Median split (16.5) | Parallel study         | Good or bad tasting milkshake | Taste-test Ice cream | Counter-regulation only found when preload was perceived as ↑ energy and test-meal was good tasting |
|                        |      |               |                         | - no preload - good or bad tasting preload (presented as ↑ or ↓ energy) | Ice cream       |                                                                 |
| Ruderman & Christensen, 1983 | RRS  | Median split (13) | Parallel study         | Milkshake   | Taste-test Ice cream | No significant Preload x Restraint interaction  
<p>|                        |      |               |                         | - no preload - preload | Ice cream       | Restrained ate more after preloading compared with no preload but not significant. |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Tool</th>
<th>Cut-off point</th>
<th>Design</th>
<th>Preload</th>
<th>Test-meal</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wardle &amp; Beales, 1987</td>
<td>DEBQ</td>
<td>Median split (?)</td>
<td>Parallel study</td>
<td>Milkshake</td>
<td>Taste-test</td>
<td>No significant Preload x Restraint interaction</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ice cream</td>
<td></td>
</tr>
<tr>
<td>Jansen et al., 1988</td>
<td>RRS</td>
<td>Median split (12)</td>
<td>Parallel study</td>
<td>Milkshake</td>
<td>Taste-test</td>
<td>No significant Preload x Restraint interaction</td>
</tr>
<tr>
<td></td>
<td>DEBQ</td>
<td>Median split (2.9)</td>
<td></td>
<td></td>
<td>Ice cream</td>
<td></td>
</tr>
<tr>
<td>Polivy et al., 1988</td>
<td>RRS</td>
<td>Median split (15)</td>
<td>Parallel study</td>
<td>Milkshake</td>
<td>Taste-test</td>
<td>Significant Preload x Restraint interaction</td>
</tr>
<tr>
<td>Lowe &amp; Kleifield, 1988</td>
<td>TFEQ</td>
<td>Median split (10)</td>
<td>Parallel study</td>
<td>Milkshake</td>
<td>Taste-test</td>
<td>Restrained counter-regulated</td>
</tr>
<tr>
<td>Ogden &amp; Watt, 1990</td>
<td>DEBQ</td>
<td>Median split (?)</td>
<td>Cross-over</td>
<td>High and low</td>
<td>Sandwiches</td>
<td>No significant Preload x Restraint interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Energy drinks</td>
<td></td>
<td>and cookies</td>
<td></td>
</tr>
<tr>
<td>Ogden &amp; Wardle, 1991</td>
<td>DEBQ</td>
<td>Median split (?)</td>
<td>Parallel study</td>
<td>Mars bar vs</td>
<td>Taste-test</td>
<td>No significant Preload x Restraint interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>plain cracker</td>
<td>Biscuits</td>
<td></td>
</tr>
<tr>
<td>McCann et al, 1992</td>
<td>RRS</td>
<td>Median split (24)</td>
<td>Parallel study</td>
<td>Milkshake</td>
<td>Taste-test</td>
<td>Both restrained and unrestrained counterregulated (not surprisingly since even</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(obese participants)</td>
<td></td>
<td></td>
<td>Ice cream</td>
<td>unrestrained eaters had relatively high restraint scores</td>
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<td></td>
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<td></td>
<td></td>
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</tbody>
</table>
Table 1.1 Studies assessing the effects of restraint on food intake after preloading (cont.)

<table>
<thead>
<tr>
<th>Study</th>
<th>Tool</th>
<th>Cut-off point</th>
<th>Design</th>
<th>Preload</th>
<th>Test-meal</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dritschel et al., 1993</td>
<td>RRS</td>
<td>Median split (?)</td>
<td><em>Parallel study</em></td>
<td>Milkshake</td>
<td>Taste-test Biscuits</td>
<td>No significant Preload x Restraint interaction (regardless of the questionnaire used to measure restraint)</td>
</tr>
<tr>
<td></td>
<td>TFEQ</td>
<td>Median split (?)</td>
<td></td>
<td>- water</td>
<td>- preload</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DEBQ</td>
<td>Unrest &lt;2.75 Rest &gt;mean+1SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Strien et al, 2000</td>
<td>RRS</td>
<td>Restraint was treated as a continuous variable</td>
<td><em>Parallel study</em></td>
<td>Milkshake</td>
<td>Taste-test Ice cream</td>
<td>Ice cream consumption was related to the RRS and restraint subscale of the DEBQ</td>
</tr>
<tr>
<td></td>
<td>TFEQ</td>
<td></td>
<td></td>
<td>- water</td>
<td>- preload</td>
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</tr>
<tr>
<td></td>
<td>DEBQ</td>
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</tr>
<tr>
<td>Rotenberg &amp; Flood, 2000</td>
<td>RRS</td>
<td>Restraint was treated as a continuous variable</td>
<td><em>Parallel study</em></td>
<td>Milkshake</td>
<td>Taste-test Cookies</td>
<td>The amount of cookies eaten significantly increased as a function of restraint in the preload condition</td>
</tr>
<tr>
<td></td>
<td>TFEQ</td>
<td></td>
<td></td>
<td>- water</td>
<td>- preload</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DEBQ</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ouwens et al, 2003</td>
<td>RRS</td>
<td>Restraint was treated as a continuous variable</td>
<td><em>Parallel study</em></td>
<td>Milkshake</td>
<td>Taste-test Cookies</td>
<td>Cookies consumption was not associated with restraint (regardless of the questionnaire)</td>
</tr>
<tr>
<td></td>
<td>TFEQ</td>
<td></td>
<td></td>
<td>- no preload</td>
<td>- preload</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DEBQ</td>
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<td></td>
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</tbody>
</table>

These results are not completely unexpected since the RRS tends to identify individuals susceptible to disinhibition in opposition to the restraint subscales of the TFEQ and DEBQ (Laessle et al., 1989a). In fact, it has been previously reported that only those who simultaneously present with high levels of restraint and disinhibition show counter-regulatory behaviour in response to preloading (Westenhoefer et al., 1994). Dietary restraint has been shown not to be an homogeneous construct and two dimensions have been identified: “rigid control of eating” and “flexible control of eating” (Westenhoefer, 1991). Those exhibiting a “pure” rigid control are more likely to show counter-regulatory eating behaviour, since they tend to set their cognitive diet boundary as a rigid point, which if passed means that further attempts to diet are hopeless with consequent disinhibition and overeating.

It is interesting to note that counter-regulatory eating behaviour in restrained eaters can also be observed when the subjects’ perception of the energy content of the preload, instead of its real content, is manipulated (Spencer and Fremouw, 1979; Polivy, 1976). When normal-weight males were given a high or low energy pudding and either correctly or incorrectly informed of its energy content, restrained eaters, classified according to the RRS, counter-regulated, by eating more sandwiches after the true high calorie pudding and even more after what they believed to be a HEP, regardless of its actual energy content (Polivy, 1976). Similar results were obtained in underweight, normal-weight and overweight females using an ice-cream “taste test” (Spencer and Fremouw, 1979).

As previously discussed, restrained individuals may, under certain circumstances, present an atypical eating behaviour in response to preloading in the laboratory environment. Moreover, in a free-living environment, restrained eaters have been shown to consume less energy, take fewer meals and exhibit a higher preference for low caloric and healthy food (Laessle et al., 1989b), including fruits and vegetables (Beiseigel and Nickols-Richardson, 2004) compared with unrestrained eaters. Interestingly, an increasing number of studies has been reporting a potential link between restrained eating and altered metabolic (Laessle et al., 1989b; Hibscher and Herman, 1977) and endocrine functions (Keim and Horn, 2004; Pirke et al., 1990; Burton-Freeman, 2005). These physiological abnormalities could put restrained eaters at
an increased risk for weight gain and explain why they need to cognitively restrict their food intake in order to maintain or loose weight (Pirke et al., 1990).

1.7 Energy balance

EB describes the relationship between EI and EE. Traditionally EE has been divided in three major components: basal metabolic rate (BMR), thermogenesis and PA in itself. BMR is the minimum energy needed for the organism to maintain its basic functions and represents around 65% of total EE in a sedentary person. Thermogenesis accounts for around 10% of total EE and includes; thermoregulatory thermogenesis - the energy needed to maintain body temperature, thought to be of minor importance in humans, diet induced thermogenesis - the obligatory heat loss associated with food absorption, transport and metabolism and finally and despite being controversial in humans, adaptive thermogenesis - the regulated production of heat in response to environmental changes in temperature and diet (British Nutrition Foundation, 1999). PA is the most complex and variable component of EE and can account from 30 to 80% of total EE, in sedentary individuals and athletes, respectively. It can be defined as the sum of all voluntary and involuntary movements performed by the body that results in EE. Although PA and exercise are sometimes used interchangeably, exercise is a subtype of PA that is planned, structured, repetitive and aimed at improving or at least maintaining fitness and/or health (British Nutrition Foundation, 1999; American College of Sports Medicine, 2000). It is important to emphasize, in the context of EB regulation, that while only the last component of EE - PA is under voluntary control, EI is fully voluntary, with the exception of clinical conditions.

As previously discussed, it is accepted that the steady increase in the prevalence of obesity, in the last decades, has in its foundation a state of chronic positive EB. This state presupposes an uncoupling between EI and EE, most likely due to a failure in down-regulating EI to match the widespread levels of inactivity. However, the contribution of both factors, EI and EE, to the final steady state remains controversial (Moore 2000), and will be dependent on the ability of diet and exercise to compensate for disruptions in EB.

One of the biggest problems in assessing the relationship between EE and EI involves the difficulty in accurately measuring these variables under free living
conditions. Activity diaries, pedometers, heart-rate monitors and accelerometers have been adopted to estimate EE, and food diaries to estimate EI, despite the limitations associated with these devices. In short-term interventions, EE and EI can be accurately measured in the laboratory using indirect calorimetry and foods of known nutritional composition and weight. However, this is not sustainable for long-term interventions and do not reflect normal free-living behaviour.

The homeostatic mechanisms involved in the control of appetite and EB are not symmetrical and the coupling between EI and EE seem to be different depending on energy status (deficit or surplus) (Moore, 2000) (Figure 1.5).

![Energy Balance Diagram](image)

Figure 1.5. Asymmetry in energy balance regulation. Energy deficits created by exercise and/or diet are difficult to attain due to strong homeostatic mechanisms that are activated to restore energy balance. In opposition, it is relatively easy to attain a state of energy surplus by the adoption of a sedentary lifestyle and/or a high-fat diet, since the homeostatic mechanisms activated are rather weak. Adapted from Moore, 2000.

A negative EB, achieved either through diet or exercise, is difficult to attain due to strong feedback mechanisms that operate to normalize EB and protect against weight loss. In contrast, it is relatively easy to reach a state of positive EB through a lifestyle characterized by inactivity and high-fat diets, which leads to passive over-consumption of energy, since the homeostatic mechanisms involved in restoring EB seem to be rather
weak in this situation (Moore, 2000). It has already been shown that the adoption of a sedentary lifestyle is not followed, at least in the short- to medium-term, by a compensatory decrease in EI, with consequent attainment of a positive EB (Murgatroyd et al., 1999; Stubbs et al., 2004a). This pattern clearly suggests that humans can tolerate energy surplus much better than energy deficit (Blundell, 1991; Moore, 2000; Stubbs et al., 2004b), which is probably an evolutionary mechanism needed to ensure the survival of the species (Goldberg et al., 1998).

Despite this asymmetry in the regulation of EB, with stronger homeostatic mechanisms being activated in situations of energy deficit compared with energy surplus (Moore, 2000), acute energy deficits induced by diet or exercise seem to activate distinct pathways and have different effects on appetite control, compensatory responses and EB. Hubert and colleagues (1998) studied the effects of acute energy deficits created by dietary manipulation or exercise on appetite responses and subsequent EI in unrestrained women. They found that hunger ratings, ad libitum EI at a buffet lunch and food cravings during the day were significantly increased in the low-energy compared with the high-energy breakfast, while exercise did not induce a significant effect on any of these variables, resulting in a negative EB (Hubert, King et al. 1998). It has been hypothesized that this slow compensatory response to exercise may arise from the fact that a higher priority is given to the maintenance of fluid balance than EB, being possible that, at least in acute studies, fluids can substitute for food in an attempt to tightly regulate water balance (Blundell et al., 2003; Durrant et al., 1982; Stubbs et al., 2004b). Exercise may, therefore, be more effective in creating an acute negative EB compared with energy restriction.

Additional evidence for the relative ineffectiveness of energy restriction in creating a negative EB in the short-term was provided by Hill and colleagues (1987) and Goldberg and colleagues (1998). Hill and colleagues (1987) measured short-term energy compensation in normal-weight subjects using, high- and low-energy lunch meals (HE and LE), differing mainly in their fat content. The acute energy deficit created by the LE lunch meal was quickly compensated by a significant increase in EI during the next two hours, despite lower afterwards. When EI throughout the day was added up until eight hours after the meal, a 43% energy compensation was obtained after the LE lunch (Hill et al., 1987). Using whole body calorimetry, Goldberg and colleagues (1998) studied
the short-term appetite response of lean subjects to dietary energy deficits, induced by a 15% reduction on either fat or CHO content relative to a control diet. They found that on day two, when participants were allowed to eat freely from an \textit{ad libitum} buffet, complete compensation occurred, independently of the macronutrient used to induce the energy deficit. In fact, a slight over-consumption occurred, since EE decreased significantly (-4%) during both energy deficits. EI seems to be auto regulated in the short-term and a specific pattern has been described, by which food intake at day one exerts a negative feedback control over EI on the following days, with the maximum effect being observed two to three days later. Both meal size and frequency seem to be targeted (De Castro, 1997).

\subsection*{1.8 Effects of exercise on appetite and EB}

The impact of exercise on appetite and EB is controversial. There is a belief that exercise drives up hunger and increases food intake to compensate for the energy deficit incurred, thereby making it futile as a method of weight control (Mayer et al., 1956). However, this concept is probably an oversimplification of the complex mechanisms involved in EB regulation, since it has been consistently shown that exercise can induce weight loss and/or prevent weight gain (Racette et al., 1995; Blundell et al., 2003). It is likely that this view of exercise as a fruitless way of inducing an energy deficit, based on the hypothesis that EB is tightly regulated, results from a poor monitoring of EI in intervention studies (King et al., 1997b).

There is a wide range of possibilities regarding the impact of exercise on EI and EB. An increase in EE induced by exercise may increase EI, although the extent of this compensation could vary; suppress EI through either metabolic or psychological mechanisms or, alternatively, EI may remain constant, but changes in food choices and nutrient selection may occur (King et al., 1994; King et al., 1997b). According to Blundell and collaborators (2003) different mechanisms may impact on the effects of exercise on appetite regulation, either by improving the sensitivity of the satiety cascade system, by regulating macronutrient preferences and food choices or, finally, by altering the hedonic response (pleasure) to food (Blundell et al., 2003).

Apart from physiological and metabolic mechanisms, exercise may also impact on EB through its effects on behavioural/cognitive and psychological variables. The
impact of exercise on EI may be modulated through changes in cognitive factors including eating behaviour; namely restrained and disinhibited eating; attitudes and beliefs associated with exercise (the belief that exercise increases appetite) and individual goals of exercising (exercise to lose weight, to increase fitness, to tone up muscle) (King, 1999). Regular exercise has also been show to have positive psychological effects (King and Tribble, 1991), improving for instance the sense of well-being, self-confidence, self-image and anxiety (Durrant et al., 1982), which can affect EI. People usually tend to overestimate exercise-induced EE and underestimate the energy value of foods, especially high-energy dense foods, as well as perceive food as a reward for exercise. These misjudgements probably explain why some individuals fail to appropriately adjust their eating behaviour in response to exercise and, therefore, why exercise alone is not always a successful approach to lose weight (King, 1999).

A review paper published in 1999 showed that in short- to medium-term intervention studies, around 19% reported an increase in EI in response to exercise, 16% showed a decrease and the remainder 65% showed no change. Moreover, when observational studies were analysed, only half showed a relationship between EI and EE (Blundell and King, 1999), therefore suggesting a rather weak coupling between EI and EE at least in the short- to medium-term. In a more recent review, about PA and appetite control, from the same research group, it was concluded that in the short (1-2 days) to medium term (7-16 days), exercise can produce a negative EB, with no substantial compensatory responses in EI being observed. In the long-term (more than 16 days), EI starts to increase, although the observed compensation is usually partial and incomplete accounting for around 30% of the energy cost associated with exercise (Blundell et al., 2003).

### 1.8.1 Effects of acute exercise on appetite and EB

In the short-term, exercise does not seem to drive up hunger or appetite, nor increase EI (King and Blundell, 1995; King et al., 1996; King et al., 1997a; Lluch et al., 1998; Westerterp-Plantenga et al., 1997; Blundell and King, 1999; Thompson et al., 1988). In fact, vigorous exercise has been described to acutely suppress hunger, a phenomenon known as “exercise-induced anorexia” (King et al., 1994; King and Blundell, 1995), despite short-lived and unlikely to have any significant effect on EI.
(King et al., 1997b; Bellisle, 1999; Thompson et al., 1988; King et al., 1994). However, a few studies show an increase in appetite sensations (Maraki et al., 2005), an increase in subsequent EI (Verger et al., 1992; Verger et al., 1994; Pomerleau et al., 2004) or even a decrease in EI (Westerterp-Plantenga et al., 1997) in response to acute exercise. This lack of consistency is probably due to differences in methodology, namely the intensity of exercise (Thompson et al., 1988), nutritional state (Durrant et al., 1982), gender (Imbeault et al., 1997), macronutrient composition of the test-meal (Tremblay et al., 1994) and the time-lag between exercise and eating (Verger et al., 1992). King and colleagues (1997a) have suggested that the inability of some studies to show a beneficial effect of acute exercise on EB derives from the fact that they do not account for the energy cost associated with it (King et al., 1997a). When the acute effects of exercise on appetite, EI and EB are under investigation, it is extremely important to measure not only absolute EI, but also relative energy intake (REI), that meaning the EI after accounting for the EE induced by exercise (King et al., 1994; King et al., 1997b).

Motivation to eat and subsequent EI in response to acute exercise seem to be modulated by gender, body weight and dieting behaviour, among other factors, as stated previously. In general, acute exercise has no effect on subsequent EI in men, while in women an increase in usually observed, either decreasing or abolishing the effects of exercise on EB (Imbeault et al., 1997; Thompson et al., 1988; Pomerleau et al., 2004). Unrestrained normal-weight women, unlike men, report an increased palatability of foods with exercise and do not experience the transient suppression of hunger – observed immediately after exercise (King and Blundell, 1995; King et al., 1996). However, “exercise induced anorexia” has been reported in restrained and obese females (Lluch et al., 1998; Tsouliou et al., 2003). Regarding body weight, it has been shown that while normal-weight individuals increase their EI in response to a three-day exercise intervention, compared with a similar period of rest, obese subjects fail to show such a compensatory response (Durrant et al., 1982). Similar results were reported after a moderate or intense bout of exercise, with only normal-weight women showing a compensatory increase in EI, regardless of the exercise intensity (Kissileff et al., 1990; Durrant et al., 1982). However, in another study a temporary reduction in hunger and a decrease in EI was reported after moderate exercise in both obese and non-obese men (Westerterp-Plantenga et al., 1997). Because in Durrant and colleagues’ study (1982)
the majority of the subjects were females (14 vs 2), it is possible that the effects of body weight on EI on the post-exercise period are modulated by gender.

Hill and colleagues (1995a) proposed a model according to which body weight and eating behaviour would modulate the coupling between EI and EE. According to this model, individuals with distinct eating behaviours: unrestrained, restrained and disinhibited, would be more or less responsive to physiologic cues of hunger and fullness and, therefore, present different patterns of energy compensation in response to increased PA (Hill et al., 1995a). According to them, unrestrained lean individuals would respond to increased PA by increasing their EI, while unrestrained overweight individuals would only partially compensate in terms of EI, therefore resulting in a negative EB. In restrained eaters, EI in response to exercise would be dependent on their levels of disinhibition. Restrained eaters with low levels of disinhibition ("successful dieters") would self-impose a tight cognitive control over their EI and, therefore, not to increase their EI in response to increased PA, independently of their body weight. In restrained eaters with high levels of disinhibition ("unsuccessful dieters"), their response to exercise would be dependent on their current dieting status. During a period of restrained eating, physiological appetite cues would be ignored and EI maintained or even decreased, while in a period of uncontrolled eating brought on by disinhibition, an increase in EI would be observed (Hill et al., 1995a). In fact, exercise has been shown to be more effective in creating a negative EB in restrained compared with unrestrained eaters (unrestrained eaters increased EI while restrained eaters tended to decrease EI) (Lluch et al., 2000), suggesting according to King (1999) that exercise may act as a controlling mechanism over eating in restrained individuals, and not as a disinhibitor as initially thought (King, 1999).

Even though EI and EE do not seem to be tightly coupled in the short-term (De Castro, 1997; Edholm et al., 1970; Edholm, 1977; Blundell et al., 2003), that does not invalidate the hypothesis that gross regulatory mechanisms operate in the medium- to long-term to guarantee that EB is maintained (King et al., 1997b). Short-term studies involving only two or three days are likely to provide a very narrow picture of EB and, longer periods of time may be needed to accurately estimate the coupling between EI and EE (King et al., 1997b). Unfortunately, the majority of the studies looking at the impact of acute exercise on EB assess only immediate EI at the subsequent meal (King
et al., 1996; Maraki et al., 2005), during the rest of the day (King and Blundell, 1995), the following day (Murgatroyd et al., 1999; King et al., 1997a; King et al., 1997a) or exceptionally on the next two (King et al., 1994) or three days (Pomerleau et al., 2004). Pomerleau and colleagues (2004) were able to show that although a bout of high intensity, but not low intensity, exercise, inducing a similar energy cost, significantly increases EI at a subsequent meal in normal-weight women, no significant differences were observed in cumulative EI throughout the day or during the following three days. Acute exercise is, therefore, able to create a negative EB that can be sustained for at least three days. It can be suggested that if acute bouts of exercise are repeated over time that this will result in a longstanding negative EB and progressive weight loss.

Even if acute exercise does lead to a compensatory increase in EI, it is the extent of that response that will determine its final impact on EB. Two hours of exercise in lean men were reported to increase EI, at a test-meal 30 minutes later, by around 25% (440 kcal) compared with a similar period of rest. This upregulation in EI, however, was not big enough to fully compensate for the increase in EE (800 kcal) and a negative EB was achieved (Verger et al., 1994). It is important to emphasize that the time interval between exercise and eating is extremely important in determining the net EB in response to acute exercise, and there is evidence showing that the later a meal is presented after exercise (from 0 to 120 min after a two-hour exercise), the higher the hunger ratings and the larger the amount of food consumed and total EI (Verger et al., 1992). Although it has been suggested that, in the short-term, an increase in EE induced by exercise will not be compensated for by an increased EI unless the volume of exercise is big enough to cause a substantial disruption on EB, no changes in hunger or EI were reported in response to two bouts of vigorous exercise, inducing an average of 1191 kcal deficit, both during that day or the following 24 hours (King et al., 1997a).

It has been proposed that the compensatory increase in EI in response to exercise may be abolished, or at least reduced, due to its stimulatory effects on the plasma levels of cytokines (Hubert et al., 1998), such as IL-1β (Cannon et al., 1989) and free fatty acids (FFA) (Larue-Achagiotis and Louis-Sylvestre, 1987), known to have anorectic properties. However, the exact mechanisms involved remain unknown. It is possible that the effects of acute exercise on motivation to eat and subsequent EI may be
modulated by its impact on post-ingestive, but pre-absorptive, satiety signals released by the GI tract, known to control appetite and food intake.

1.8.2 Effects of chronic exercise on EI, EB and body weight

The general view regarding the impact of long-term exercise on EB is that, in the absence of energy restriction, only very modest results are observed in terms of weight loss (Bensimhon et al., 2006). This does not mean, however, that exercise should not be present in all interventions intended to tackle the problem of obesity. Although diet alone may seem at first glance a more effective way of losing weight than exercise, its efficacy in the long-term is questionable. In a recent comprehensive analysis of 31 long-term studies on the effect of energy restriction on weight loss, Mann and colleagues (2007) found that despite an initial 5-10% weight loss in the first six months of dieting, more than two thirds of the subjects regained more weight than they had originally lost within four to five years (Mann et al., 2007). Moreover, exercise is now seen as crucial in preventing weight gain or regain in the long-term (Bensimhon et al., 2006).

In an exhaustive review paper published 10 years ago, Wilmore (1996) was able to show that over a one year period of an exercise training program designed to induce weight loss, a typical subject could expected to lose, on average, 3.2kg of body weight and 5.2kg of fat mass and gain 2.0kg of fat-free mass (FFM) (Wilmore, 1996). Although the net weight loss may look rather disappointing, it should be noted that the degree of weight loss in response to exercise is greatly underestimated due to the progressive substitution of fat by FFM (Prentice and Jebb, 2004). However, a great variability was found among studies, with some reporting weight loss, maintenance or even a small gain after a PA intervention (Wilmore, 1996). It is possible that methodological differences among studies explain some of these differences, namely the macronutrient composition of the diet and the characteristics of the exercise intervention (type, duration, frequency and intensity) (Wilmore, 1996).

It has been suggested that the large inter-individual variation in the response of body weight to exercise is due to differences in the coupling between EI and EE (Moore, 2000). Unfortunately, most of the studies looking at the impact of chronic exercise on EB are limited by the fact that neither EI nor EE are controlled or accurately
measured. Free-living subjects tend to upregulate their EI in response to exercise, even if told otherwise and not to comply with the exercise prescription (Miller et al., 1997; Garrow and Summerbell, 1995; Ballor and Poehlman, 1994). Moreover, the increase in total EE induced by exercise, presupposes, that normal activity throughout the rest of the day remains unchanged or increases (Wilmore, 1996), which has been reported in some (Meijer et al., 1991), but not all studies (Goran and Poehlman, 1992). It should be noted, however, that this latter study was performed in the elderly and included a vigorous endurance training program.

In fact, there is evidence that substantial weight loss can be achieved with exercise alone, if EI remains unchanged over time and compliance with exercise is good. Ross and colleagues (2000) randomly assigned obese men to a diet or exercise induced weight loss programme, where all the exercise sessions were supervised and food intake was maintained constant throughout the study period. After three months, they were able to show a similar weight loss in both groups (8%) and a significantly higher fat mass loss in the exercise group (Ross et al., 2000). A similar weight loss was reported after a three-month period in men who were kept in a residential facility, where exercise was supervised and EI was kept constant (Bouchard et al., 1990). It should be emphasized, however, that the amount of exercise needed to attain this weight loss (between one to two hours per day in six to seven days of the week) although achievable, represents probably a great commitment, particularly in the obese population. Interestingly, significant reductions in body weight can also be achieved under ad libitum food intake if the compliance with the exercise programme is good, as shown in a military environment (Lim and Lee, 1994).

The effect of long-term exercise on EI, EB and body weight has been a matter of extensive research over the last century. Mild to moderate exercise programmes (from 18 to 57-days long) were shown not to induce any compensatory increase in EI in obese women, with weight loss being achieved, while in normal-weight men and women a compensatory increase in EI has been observed (Woo et al., 1982a; Woo et al., 1982b; Woo and Pi-Sunyer, 1985; Keim et al., 1989; Blair et al., 1981), despite a large inter-individual variability in the response to exercise. It is possible that compensatory responses to exercise are not initiated until fat reserves are decreased below a specific level, which would support the differences in EI in response to exercise between lean
and obese subjects. A few studies show a decrease in EI in response to chronic exercise (Leon et al., 1979; Watt et al., 1976). In Leon and colleagues’ (1979) study, after a transitory increase in EI in the first four weeks of a 16 week vigorous walking program, in healthy sedentary obese men, a progressive decrease in EI was observed ending below baseline levels. Overall, a 61% energy compensation was found allowing a 5.7kg weight loss (Leon et al., 1979). In Watt and colleagues’ study (1976), however, no significant change in body weight was reported, suggesting food misreporting or, alternatively, a compensatory decrease in non-exercise EE.

As for acute exercise, EB in response to long-term exercise also seems to be modulated by gender, with men much more responsive than women (Garrow and Summerbell, 1995; Donnelly et al., 2003; Saris et al., 2003). This is likely to be due to differences in energy compensation. A larger weight loss and a more pronounced change in body composition, after a 40-week endurance exercise intervention, was reported in males compared with females (19.4<BMI<26.4 kg/m²), probably related to the fact that while females increased their EI, males had an opposite response, especially on the latter stages of the program (Westerterp et al., 1992; Tremblay et al., 1984). Similar results were obtained in normal-weight men and women after a 19 to 20-week exercise intervention (Woo and Pi-Sunyer, 1985; Tremblay et al., 1984).
The disruption created by PA, on both energy and macronutrient balance, cannot be sustained indefinitely and a new steady state needs to be achieved over a certain period of time. The point of reversion is probably modulated by both physiological, as well as behavioural mechanisms (Figure 1.6).

![Diagram](image)

**Figure 1.6.** Physiological and behavioural mechanisms operating when energy balance is disturbed by exercise (transition period), before a new steady-state is achieved. VO\textsubscript{2} max - maximal oxygen uptake, Ecost of Ex - energy cost of exercise, BMR - basic metabolic rate, EI - energy intake, EE - energy expenditure, ↑ - increase, ↓ - reduction. Adapted from King, 1999.

During the uncoupling between EI and EE, known as the "transition period", several physiological mechanisms are activated in order to achieve a new steady state. Weight loss is associated with a compensatory reduction in total BMR, probably driven by changes in body composition in favour of decreased FFM (Ross et al., 2000). Moreover, as maximal oxygen uptake (VO\textsubscript{2} max) increases with exercise training, a lower EE is achieved for the same volume of exercise. Finally, as body weight decreases, the net exercise-induced EE is also reduced (King, 1999). However, it is unlikely that these physiological/metabolic mechanisms are responsible, on their own, for the attainment of a new steady state by reversing the uncoupling between EI and EE. Behavioural mechanisms are probably also involved (Blundell and King, 1999; Blundell and King, 2000). There are two major behavioural mechanisms that can reverse the
energy imbalance created by exercise: a compensatory increase in EI, a reduction in EE, likely through down-regulation of spontaneous PA (non-exercise EE), or both (Hill et al., 1995a; Hill and Peters, 1995). The increase in EI may be the expression of raised hunger or result from a more relaxed dietary regime, especially towards more energy-dense foods, due to the widespread belief that the energy cost of exercise can offset dietary indulgences (Hubert et al., 1998). EI can be up-regulated by increasing the amount of food consumed within an eating episode, increasing the frequency of eating episodes, increasing the energy density of foods or even by increasing the consumption of energy-rich drinks (King, 1999; Blundell and King, 1999). If EI does not increase, or the increase is insufficient, and EE is maintained constant, a new steady state may still be achieved, although it would take longer, through changes in metabolism, as explained before (Hill and Peters, 1995). It has been suggested that a new state will be achieved when weight loss is enough to reduce BMR below a specific level at which total EE equals EI (Hubert et al., 1998).

1.8.3 Cross-sectional studies

The first study in humans examining the relationship between EI and EE in groups of individuals according to their habitual PA levels was carried out as early as 1956 by Mayer and collaborators (1956), in a male population of mill workers in West Bengal. They compared EI in different groups according to their levels of PA in their jobs and found a good correlation between EI and energy requirements. However, the rise in EI for increasing activity levels was only observed within a specific range of PA that the authors designated as “normal activity range”. If PA was below that range, as happens in sedentary individuals, a decrease in EE was not followed by a proportional decrease in EI, but instead by an increase, therefore leading to a positive EB and weight gain. This justifies the increased body weight observed in this group when compared with more active ones (Mayer et al., 1956). It seems, therefore, that the coupling between EI and EE, and appetite control in general, is disrupted at low levels of PA.

Prentice and Jebb (2004) proposed that this asymmetry in appetite control between sedentary and active people has in its foundation an imbalance between the effectiveness of hunger and satiety signals. According to them, active people are likely to have energy requirements that are above the “cultural norm” and their energy
homeostasis rely on efficient hunger signals. Sedentary people, on the other hand, have energy requirements that are below the "cultural norm" and their energy homeostasis rely on inefficient satiety signals (Prentice and Jebb, 2004). They go further and suggest that it is for this reason that many people have to adopt a cognitive behaviour of dietary restraint in order to maintain a normal body weight in face of the current levels of inactivity.

In their review of the effects of exercise on EB, King and colleagues (1997) presented additional evidence that individuals with high levels of PA usually have greater EI, compared with individuals with a sedentary lifestyle, but paradoxically lower BMI, suggesting a tight coupling between EI and EE at high levels of PA. This raised the hypothesis that exercise might sensitize the physiological mechanisms involved in appetite control, thereby improving the coupling between EI and EE (King et al., 1997b). Further evidence for the role of exercise on appetite control was provided by the same research group in 1999, when they showed that active males were able to detect differences in the energy content of covertly manipulated drinks, after an exercise challenge (50 minutes at 70% of VO$_2$ max), and to adjust for that at a subsequent meal. After a high-energy drink active males significantly suppressed their subsequent EI, compared with after a low-energy drink, with a near almost perfect energy compensation being achieved (King et al., 1999).

Further support for the hypothesis that exercise may sensitize the physiological mechanisms involved in appetite control comes from a study by Long and colleagues (2002) showing a better compensatory response to covert preload energy manipulation in habitual exercisers compared with sedentary individuals (Long et al., 2002). Using a cross-over design, healthy lean men were randomized into a low- (LEP) or high-energy preload (HEP) in two different occasions, followed 60 minutes after that by an ad libitum buffet lunch. Although buffet EI following the LEP and HEP was not significantly different in the sedentary group, an almost perfect energy compensation (90%) was observed in the exercise group, achieved by significantly down-regulating buffet EI after the HEP. However, a cross-sectional design does not prove causality and it is possible that the better energy compensation observed in active men may be due to other factors and not exercise in itself. It would be interesting to investigate whether exercise can, indeed, improve the sensitivity of compensation for previous EI in
previously sedentary individuals, using a longitudinal design, and the potential mechanisms involved. If it is proved that exercise can lead to a more sensitive eating behaviour in response to previous EI, its role on EB will extend far beyond its direct effects on EE.

1.8.4 Effects of exercise on the neuro-endocrine-appetite system

Very little research has been carried out exploring the effect of exercise on the complex neuro-endocrine system that regulates appetite and feeding behaviour (King et al., 1997b), both in the short- and long-term.

Fasting leptin levels do not seem to change in response to acute (hours or days) (Perusse et al., 1997; Tsofliou et al., 2003; Dirlewanger et al., 1999) or chronic exercise (months to years) (Perusse et al., 1997; Kohrt et al., 1996; Keim et al., 1998; Hasbun et al., 2006), independent of reductions in fat mass. Other studies, however, have reported a reduction in leptin levels after intense prolonged exercise (25 km swimming race) in highly trained subjects (Karamouzis et al., 2002), as well as, after a one year exercise intervention, even after adjusting for BMI and fat mass (Reseland et al., 2001). However, in Karamouzis and colleagues’ (2002) study a huge negative EB was imposed and the subjects were not in a fasting state, therefore allowing the release of insulin, which is known to exert a negative feedback over leptin secretion. It has been proposed that an exercise intervention that allows improvements in insulin sensitivity would have the ability of altering leptin levels independently of changes in fat mass, due to changes in insulin and cortisol levels, which are known to modulate leptin synthesis (Considine, 1997). Support for this hypothesis comes from another study showing that physical activity EE and fasting leptin levels are negatively correlated in both genders, independently of body composition, suggesting, according to the authors, that exercise may be important in modulating leptin levels probably due to changes in catecholamine levels (Franks et al., 2003). This would help to explain, at least partially, the great inter-individual variability observed in the relationship between body fat and plasma leptin levels, despite its high correlation at the population level (Considine et al., 1996).

The effects of exercise on the plasma levels of GI hormones/metabolites known to be involved in appetite regulation remains largely unknown and the few existing
studies have been performed in areas where appetite was not the primary outcome. Significant increases in fasting ghrelin levels were observed after weight loss induced by diet and/or exercise in normal-weight and overweight women (Leidy et al., 2004; Foster-Schubert et al., 2005). However, no significant effects were found in the absence of weight loss, suggesting that exercise in itself has little or no impact on fasting ghrelin levels in the long-term. Moreover, acute exercise is not associated, at any intensity, with increased fasting ghrelin concentrations in the recovery period in normal weight and overweight subjects (Schmidt et al., 2004; Dall et al., 2002). Acute exercise was shown to increase fasting plasma levels of CCK (Bailey et al., 2001) and GLP-1 (O'Connor et al., 1995; O'Connor et al., 2006), although the last two studies were performed in athletes. Acute exercise has also been shown to increase PP plasma levels, not only in fasting (Sullivan et al., 1984; Hilsted et al., 1980; O'Connor et al., 1995) but also postprandially (Greenberg et al., 1986), despite dependent on the intensity of exercise (Holmqvist et al., 1986). Chronic exercise, on the other hand, was shown not to increase fasting CCK levels in active men (Bailey et al., 2001), but to induce a slight increase in fasting PP plasma levels, as well as on its postprandial peak (Hurley et al., 1991), in previously sedentary males. Interestingly, the magnitude of the increase observed in PP plasma levels with acute exercise, seems to decrease with training (Gingerich et al., 1979). However, most of the above studies looked only at the impact of exercise on the fasting levels of these gut peptides. CCK, GLP-1 and PP are satiety hormones released in the postprandial state and, therefore, changes in fasting levels provide very little information. What is missing at this point are studies that relate changes in the plasma levels of these appetite-related hormones, in response to both acute and chronic exercise, with alterations in subjective and objective measures of appetite and net EB.
In 1995, Richard (1995) proposed that the potential role of exercise on modulating EI and EB is probably related with its effects on corticotrophin releasing hormone (CRH) - NPY balance (Richard, 1995) (Figure 1.7).

Exercise has been shown to stimulate adrenocorticotropic hormone (ACTH) secretion in response to CRH, as well as cortisol in humans, proportionally to its intensity, although an adaptation to regular exercise is likely to occur over time (Luger et al., 1987). CRH, a potent anorectic neuropeptide, is known to decrease EI and increase sympathetic nervous system-mediated thermogenesis, although the heat generated by muscular activity may inhibit this last effect (Richard, 1995). In fact, animal studies have shown that CRH is involved in the acute anorectic effects of exercise (Rivest and Richard, 1990). If sustained, exercise would, therefore, allow a reduction in body weight. However, in a similar way to food restriction, exercise can also stimulates NPY activity within the brain, at least in animal studies (Lewis et al., 1993), which, according to Richard (1995) may contribute to the resistance to lose...
weight seen in some obese people who regularly perform exercise (Richard, 1995). The net result of the simultaneous activation of these two neuronal systems (CRH and NPY), with opposite effects on EB, is not known, but it is possible that one may overcome the effects of the other, depending on the duration and intensity of exercise. It should be emphasized that although several studies have shown that exercise, especially of high intensity, has the ability of increasing NPY plasma levels (Karamouzis et al., 2002; Jensen et al., 1994; Lacroix et al., 1997; Pernow et al., 1986), this does not imply that the activity of NPY within the ACR of the hypothalamus also increases and, therefore, that the effects of exercise on EI and appetite are directly mediated through NPY.

1.8.5 Interactions between exercise and diet composition on EI and EB

The macronutrient composition of the foods available after exercise has been shown to modulate EI and EB and to determine, in the longer term, the net results in terms of weight control (Tremblay et al., 1994; King and Blundell, 1995; King et al., 1997b; Murgatroyd et al., 1999; Lissner et al., 1997; Hubert et al., 1998). It has been shown both in males (Tremblay et al., 1994; King and Blundell, 1995) and females (King et al., 1996) that, if high-fat foods are available in the post-exercise period, a significant increase in EI is observed, with consequent compensation for the energy cost of exercise. In contrast, if low-fat "healthy" foods are presented, a short-term negative EB is usually achieved. This has important implications in the light of the actual obesogenic environment characterized by inactivity and high-fat diets, since the benefits of exercise on EB are directly dependent on a strong self-discipline, concerning both compliance with the exercise programme and compliance with a "healthy diet" to minimize the risk of passive over-consumption and food indulgence. Further evidence for this hypothesis comes from a longitudinal study in sedentary women, where weight changes in response to exercise were reported to be directly dependent on the fat content of the foods available (Lissner et al., 1997). Taken together, these studies clearly suggest that the energy density of the foods available after exercise may be a stronger predictor of overall EB than the amount of EE induced by exercise (Bellisle, 1999). High-fat diets have, therefore, the power of minimizing, or even completely abolishing,
the negative EB induced by exercise, in both restrained and unrestrained individuals (Lluch et al., 1998; King and Blundell, 1995; King et al., 1996).

The significantly higher EI observed when individuals are presented with high-fat foods, occurs despite a smaller amount of food consumed, a phenomenon that has been termed as “fat paradox” (Blundell et al., 1995). There is now consistent evidence available showing that high-fat diets can induce a state of “passive over-consumption” by deregulating basic mechanisms involved in appetite control (Blundell et al., 1995; King et al., 1997a). Two main factors seem to contribute to a delay or weakness of fat-induced satiety signals: first, high-fat foods have a high energy density and second, fat produces strong orosensory stimulation, which results in positive feedback and facilitates further intake (King et al., 1996). Therefore, a larger amount (and therefore energy) of high-fat foods can be consumed before satiety signals became fully operative (Weststrate, 1995; Blundell and Halford, 1994).

1.8.6 Effect of exercise on macronutrient selection, food choices and sensory responses

The effects of exercise on food choices and macronutrient selection, both in the short- and long-term, are controversial and the hypothesis that exercise can lead to healthier food choices remains unproven (Tremblay and Drapeau 1999). In the same way that it is believed that EB is only achieved when EI equals EE, it is also accepted that a state of equilibrium is needed between the proportion of macronutrients oxidized (Respiratory Quotient – RQ) and the proportion of macronutrients ingested (Food Quotient – FQ) (Flatt, 1987a). According to Flatt’s hypothesis, a negative EB can only be obtained if the average RQ is lower than the average FQ (Flatt, 1987a).

Different mechanisms may be activated in response to imbalances in body energy stores to ensure weight maintenance over time: changes in food intake and macronutrient selection, in the composition of the substrate mix used for generating energy and/or in the overall rate of EE (Flatt, 1987a). Fat and CHO are the macronutrients thought to be more involved in EB, due to the small relative contribution of proteins to both, EI and EE, and to the tight regulation of protein stores (Tremblay and Buemann, 1995). It has been suggested that EI is more sensitive to changes in the small reserves of CHO compared with fluctuations on the large fat depots (Flatt, 1987a).
Exercise may modulate the relative contribution of fat and CHO to spontaneous EI, since it has the capacity, depending on its type and duration, of altering the oxidation status and storage of these macronutrients (Hill et al., 1995a; King et al., 1997b). It has been hypothesized that in the post-exercise period, individuals would choose foods rich in the depleted macronutrient, in order to replace its reserves, suggesting, therefore, a relationship between substrate oxidation and post-exercise macronutrient selection (King et al., 1997b).

However, in the short-term, most of the studies have failed to show any effect of exercise on macronutrient selection (King et al., 1994; Tremblay and Buemann, 1995; Tremblay et al., 1994; Imbeault et al., 1997). Some studies, nevertheless, show an increased preference for CHO acutely after exercise, independent of its intensity (Westerterp-Plantenga et al., 1997; Thompson et al., 1988). Verger and colleagues (1992) reported, in young athletes, an effect on macronutrient selection. When a meal was presented shortly after a two-hour exercise bout, the later the meal (in the first two hours post-exercise), the greater the contribution from CHO for total EI (Verger et al., 1992). However, this finding (which did not achieve statistical significance) was not reproduced in a later study from the same research group, where instead an increase in protein intake was reported (Verger et al., 1994). In the long-term, again, most of the studies show no effect of exercise training on macronutrient selection (Tremblay and Drapeau, 1999; Westerterp et al., 1992), even after a 16-month intervention (Donnelly et al., 2003), although an increase in CHO intake was reported in one study (King et al., 1997b). This lack of evidence for an association between exercise and macronutrient preference reinforces the need for controlling fat intake in order to favour a more negative EB (Tremblay and Drapeau, 1999).

A significant correlation seems to exist between changes in substrate oxidation (RQ) induced by exercise and post-exercise EI. Almeras and colleagues (1995) presented evidence for a role of fat oxidation in modulating the impact of exercise on EB. They were able to show that individuals with a low RQ (high-fat oxidizers) had a lower post-exercise EI, compared with those with a high RQ (high-CHO oxidizers) (Almeras et al., 1995). According to them, because high fat oxidizers spare CHO stores, they are less prone to increase their EI after exercise as an attempt to restore CHO balance and are, therefore, more likely to achieve a negative EB (Almeras et al., 1995).
However, no relationship between the composition of the substrate mix oxidized during exercise and the food consumed in the post-exercise period has been reported in more recent studies (Tremblay and Drapeau, 1999; Imbeault et al., 1997; King, 1999).

Although food choices and macronutrient selection in response to exercise is under metabolic and hormonal influences, other factors are likely to be involved (King et al., 1997b), namely psychological (dietary restraint, health and food related attitudes and long-term food habits and preferences) and social variables (traditions, food availability, normal times and places of eating) (Bellisle, 1999; Verger et al., 1994). Although it is possible that the increase in CHO intake with exercise, observed in some studies, may be secondary to an increased consumption of CHO-rich drinks (King, 1999), an increased preference for sweets in response to exercise has also been described in one study (Westerterp-Plantenga et al., 1997).

Even though few studies have addressed the potential effect of exercise on sensory responses (Bellisle, 1999), an increase in the pleasantness and the palatability of some foods after acute exercise was already reported in restrained and unrestrained normal-weight women, respectively (Lluch et al., 1998; King et al., 1996) as well as in man (King and Blundell, 1995).

### 1.9 Aims of the current work

Obesity has become a global epidemic and reduced PA levels and increased consumption of highly palatable energy-dense food are likely to be the main drivers. The current work aims to elucidate further the role of exercise, both in the short- and long-term, and restrained eating behaviour on appetite control in man. Moreover, because restrained eaters have been repeatedly excluded from appetite studies on the basis of their abnormal eating behaviour in the laboratory, another aim of the current research will be to identify the best tool in predicting disinhibition in restrained eaters.

To achieve these aims the following objectives have been proposed:

A. To examine whether increasing habitual PA levels in sedentary normal-weight volunteers, can improve the sensitivity of short-term appetite regulation in response to two covertly manipulated preloads, using a longitudinal design. Short-
term appetite regulation will be assessed at baseline and after a six-week moderate intensity exercise intervention, using subjective ratings of motivation to eat and objective measures of food intake at a buffet meal and over a 24-hour period, following two preloads of different energy content.

B. To investigate the effects of acute exercise, when performed in the fed-state, on the postprandial levels of appetite-related hormones and metabolites, subjective ratings of motivation to eat and prospective food intake at a test-meal. These variables will be assessed simultaneously to elucidate the degree to which changes in subjective and objective measures of appetite following acute exercise can be attributable to differences in the release of appetite-related hormones and metabolites.

C. To investigate the effect of dietary restraint on fasting and postprandial plasma levels of appetite-related hormones and metabolites. Subjective ratings of motivation to eat and prospective food intake at a test-meal, and for the next 24 hours, will also be measured and correlated with potential abnormalities in the plasma levels of the hormones and metabolites measured in restrained eaters.

D. To investigate the predictive validity of three scales that measure dietary restraint: the Revised Restrained Scale (RRS), a shortened version of the Three Factor Eating Questionnaire-18R (TFEQ-18R) and the Dutch Eating Behaviour Questionnaire (DEBQ) in their ability to predict disinhibited eating behaviour in the laboratory. This will be achieved by measuring ad libitum food intake at a buffet test-meal, one hour after a HEP and LEP clearly labelled with their exact energy content. Moreover, EI outside the laboratory for the rest of the day will also be assessed. Correlations between the extent of energy compensation and different constructs of eating behaviour will be established.
Chapter Two
Chapter 2. Materials and methods

The following chapter describes the materials and methods used in carrying out the different experiments. Individual protocols will be described at the beginning of each chapter, together with any alteration to the overall methods described here.

2.1 Materials

2.1.1 Apparatus and equipment

- Co-oximeter (Instrumentation Laboratory, Warrington, UK)
- Gamma counter - Wizard 1470 with Multicalc level 4.M software (Wallac International, Turku, Finland)
- ILAB 650 (Instrumentation Laboratory, Milan, Italy)
- Microhematocrit centrifuge - Haematospin 1400 (Hawksley, Lancing, UK)
- Microplate Luminometer LB 960 Centro (Berthold Technologies, Wildbad, Germany)
- Microplate Washer AW1 (Anthos Labtec Instruments, Wals, Austria)
- Pedometer YAMAX SW-200 (Digi –Walker, Tokyo, Japan)
- Randox Space Alfa WasserMann Analyser (Randox Co., Antrim, UK)
- YSI 2300 Stat Plus Glucose & Lactate Analyzer (Yellowsprings, Ohio, USA)
- Heart rate monitor - Polar Elecro Oy (Polar, Kempele, Finland)
- Cateye Fitness EC-C400 Upright exercise bike (Cateye, Dallas, USA)
- Stationary exercise bikes
- Bodystat - 1500MMD bioimpedance machine (Bodystat Limited, Isle of Man, British Islands)
- Seca 888 digital scale (Seca, Cheshire, UK)
- Harpenden stadiometer
- Flexible measuring tape
- Butterfly needles, 0.8 x 22.2 mm (Venissystems, Sligo, Ireland)
- Canunulae Y-can 19G, 1.1 x 30 mm (Beldigo SA, Marche, Belgium)
- Fluoride oxalate tubes, 2.5 ml (L.I.P. Ltd, Shipley, UK)
- Lithium heparin tubes, 10 ml (L.I.P. Ltd, Shipley, UK)
- Potassium EDTA tubes, 10 ml (L.I.P. Ltd, Shipley, UK)
- Apex microtubes, 2 ml (Alpha Laboratories, Hampshire, UK)
- Test tubes, LP3 (L.I.P. Ltd, Shipley, UK)
- Syringes 1 ml, 5 ml, 10 ml, 20 ml and 30 ml (BD Plastipak, Devon, UK)

### 2.1.2 Chemicals, reagents and antisera

All chemicals were of AnalaR grade unless otherwise stated.

- Disodium hydrogen orthophosphate anhydrous (Na$_2$HPO$_4$) (Fisher, Leicestershire, UK)
- Sodium dihydrogen orthophosphate (NaH$_2$PO$_4$H$_2$O) (Fisher, Leicestershire, UK)
- Human biosynthetic insulin (Eli Lilly, Hants, UK)
- Albumin, Bovine Serum (BSA) Fraction V powder (Sigma Chemical Company Ltd, Poole)
- Anti-guinea pig Sac-Cel (IDS, Tyne and Wear, UK).
- Charcoal stripped serum (gift from Prof. Linda Morgan, University of Surrey)
- Guinea pig anti-insulin antisera (gift from Dr. Shelagh Hampton, University of Surrey)
- Aprotinin (Sigma Chemical Company Ltd, Poole, UK)
- Immuno-chemiluminometric insulin assay kit (Invitron, Monmouth, UK)
- Activated charcoal (Sigma Chemical Company Ltd, Poole, UK)

### 2.1.3 Miscellaneous

- Double cream (Tesco)
- Maltodextrin - Maxijul Super Soluble 2.5 kg (SHS International, Liverpool, UK)
- Nesquik (Nestle, Vevey, Switzerland)
- Cadbury's drinking chocolate (Cadbury's, Birmingham, UK)
- Dried milk (Marvel)
- Olive oil (Tesco)
- Skimmed milk (Tesco)
2.2  Methods

2.2.1  Measurement of eating behaviour

Different questionnaires were used to measure dietary restraint, as well as other eating behaviours.

2.2.1.1  Revised Restrained Scale

The restraint scale was first developed in 1975 (Herman and Mack, 1975) and further developed and refined into a 10-items questionnaire focusing on concerns with dieting and weight fluctuation – the Revised Restrained Scale (RRS) (Herman and Polivy, 1980). Each individual question is scored between 0 and 3 or 0 and 4 depending on the number of possible answers, with 0 being given if the first answer is chosen, 1 if the second is chosen and so on (Appendix I). The total score derived from the RRS can range from a minimum of 0 to a maximum of 35.

2.2.1.2  Three Factor Eating Questionnaire

The Three Factor Eating Questionnaire (TFEQ) was developed in the eighties to try to address problems with both the predictive and construct validity of the RRS and isolate the “restraint” construct (Stunkard and Messick, 1985). The TFEQ consists of 51 items and measures three dimensions of eating behaviour: restraint; disinhibition (loss of control over eating) and hunger (subjective feeling of hunger and food cravings) (Stunkard and Messick, 1985).

A shortened version of the TFEQ, with only 18 items (TFEQ-18R) was developed in 2000 (Karlsson et al., 2000) and its use validated in the general population (Lauzon et al., 2004). The TFEQ-R18 measures three dimensions of eating behaviour: cognitive restraint; uncontrolled eating, made up from most of the disinhibition and hunger items of the TFEQ and reflecting the inability to restrict food intake and a high sensitivity to external stimuli and a new dimension - emotional eating (Karlsson et al., 2000) (Appendix II). The dimension of cognitive restraint consists of six questions, with a total score ranging from 6 to 24; uncontrolled eating consists of nine questions, with a
total score raging from 9 to 36 and emotional eating of three questions, with a total score raging from 3 to 12 (Appendix II).

2.2.1.3 Dutch Eating Behaviour Questionnaire

The Dutch Eating Behaviour Questionnaire (DEBQ) was developed also in the eighties with the aim of improving the understanding of the complex eating behaviour patterns exhibited by obese individuals (van Strien et al., 1986). In a similar way to the TFEQ, one of its main purposes was to isolate the restraint construct. Moreover, van Strien and colleagues (1986) were also interested in measuring emotional and external eating behaviour. The DEBQ consists of 33 items, all with the same response format — never, seldom, sometimes, often and very often, together with a non relevant category in items that are presented in a conditional format (Appendix III). The 33 items are distributed by three behavioural dimensions: restrained eating — 10 items, emotional eating — 13 items and external eating — 10 items. Each item has a five-point response format (1-5): never (1), seldom (2), sometimes (3), often (4) and very often (5), respectively, with the exception of item 26, where a reverse scoring is applied. Scores are then added up for each dimension (restrained, emotional and external eating behaviour) and the final score calculated individually as the total score divided by the number of items (see example below).

\[
\text{e.g. Restrained score} = \frac{\text{total score from "restrained" items}}{\text{n° restrained items}}
\]

\[
\text{e.g. Restrained score} = \frac{28}{10} = 2.8
\]

If at a specific item, the "non relevant" option is chosen, that item is given a score of 0 and the number of items used as the division factor in the above described equation is reduced by 1 for that particular dimension.

2.2.2 Measurement of appetite and food intake

2.2.2.1 Visual analogue scales

Subjective feelings of hunger, desire to eat, fulness and thirsty were assessed using self-rated visual analogue scales (VAS) (Blundell and Rogers, 1980; Hill and
Blundell, 1982). The reliability and validity of VAS in assessing motivation to eat has been discussed in Chapter 1 (1.3.3.1).

Throughout each study period, participants were asked to rate their subjective feelings of motivation to eat at regular intervals specified in each experimental protocol to generate a temporal profile. Each scale consists of a 10 cm line with words anchored at each end describing extreme sensations (Figure 2.1).

![Figure 2.1. Example of visual analogue scales used to assess motivation to eat](image)

Participants were instructed to rate themselves by placing a vertical mark through the line for each question to describe how they were feeling at that moment in time. They were also asked to regard both ends of the lines as indicating the most extreme sensations they have ever felt. In order to analyse the data statistically, the ratings were then converted into a score in centimetres.

VAS were also used to measure the palatability of preloads and/or test-meals, as well as cognitive and emotional state. VAS were presented as booklets, with each question presented on a separate page, and participants instructed not to look at their previous responses.
2.2.2.2 Food preference lists

Food preference lists were used in parallel with VAS, as a measure of appetite and were already described in Chapter 1 (1.3.3.2). Participants were asked to select any or all the food items listed according to what they would like to eat at that moment in time (Hill et al., 1995b). The food checklist used was developed by Hill and collaborators (1987) and consists of different food items divided into: high-fat, high-CHO, high-protein, low-energy and mixed items (Hill et al., 1987) (Appendix IV). Participants were instructed to consider each item in turn and independently from the other items rather than constructing a menu. This data was analysed in terms of the number of items selected in each category and the energy provided by them.

2.2.2.3 Preloads

Two liquid preloads differing in their energy content: high-energy (HEP) and low-energy preloads (LEP), were used in chapter 3 and 6 of this thesis. The preloads were presented as 450 ml flavoured milkshakes (chocolate, strawberry or banana according to participant preference) differing in their energy content by around 360 kcal through the addition of a CHO energy supplement of maltodextrin (Table 2.1). Milkshakes were designed to have similar sensory properties and were prepared approximately 30 minutes before participants’ arrival, stored in the fridge until required and re-whisked immediately before presented. The ingredients of the milkshakes were specifically chosen to achieve a certain nutritional composition based on previous experiments (Long, 2000).
Table 2.1. Ingredients and nutritional composition of the low- and high-energy preloads

<table>
<thead>
<tr>
<th>Ingredients (g):</th>
<th>Low-energy preload</th>
<th>High-energy preload</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double cream</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Maltodextrin <em>(Maxijul)</em></td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Nesquik *</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutritional composition:</th>
<th>Low-energy preload</th>
<th>High-energy preload</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>246</td>
<td>607</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>10.5</td>
<td>100.7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>22.2</td>
<td>22.2</td>
</tr>
</tbody>
</table>

Amounts and nutritional composition per 450 ml serving, made up to this volume with water. * chocolate, strawberry or banana flavoured

In chapter 4 and 5, a hot chocolate drink was used as preload. This chocolate drink was designed with the specific aim of stimulating the release of gut peptides (Robertson et al., 2003) and contains 15% of protein, 38% of fat and 47% of CHO (Table 2.2). This proportion of macronutrients was based on the average UK macronutrient intake (Henderson et al., 2003).

Table 2.2. Ingredients and nutritional composition of the chocolate drink (per serving)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g)/serving</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Carbohydrates (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadbury’s drinking chocolate</td>
<td>18</td>
<td>1.1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Skimmed milk</td>
<td>200</td>
<td>6.6</td>
<td>0.2</td>
<td>10</td>
</tr>
<tr>
<td>Marvel (dried milk)</td>
<td>31</td>
<td>11.2</td>
<td>0.2</td>
<td>16.4</td>
</tr>
<tr>
<td>Olive oil</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Maltodextrins</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>TOTAL ± 250 ml</td>
<td>± 250 ml</td>
<td>18.9</td>
<td>21.4</td>
<td>59.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Energy</th>
<th>kcal</th>
<th>505.8</th>
<th>75.6</th>
<th>192.6</th>
<th>237.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td>15%</td>
<td>38%</td>
<td>47%</td>
<td></td>
</tr>
</tbody>
</table>

A serving = 250 ml
2.2.2.4 *Ad libitum* test meals

*Ad libitum* food intake at a test-meal was used in association with the previously described preloads in the context of the “preload-test-meal” paradigm to investigate the impact of different preloads on subsequent EI. Foods were weighed and/or counted before participants sat down to the meal and re-weighed/re-counted after they had finished eating, allowing energy and macronutrient intake to be calculated (Hill et al., 1995b). Participants were instructed to eat as much or as little as they wanted until comfortably full, with no time limit regarding the duration of the meal. In order to minimise social interaction, known to impact on food intake (De Castro, 1997), participants were separated from one another in individual booths during the consumption of the test-meals. Moreover, all efforts were done to standardize test-meal conditions, by using always the same room, with constant light, free of odours, sounds and other disturbing factors.
2.2.2.4.1 Buffet test-meal

In chapters 3, 4 and 6 of this thesis a standardised *ad libitum* "buffet test-meal", using foods appropriate to the time of day, and in excess of expected consumption, was used. In an attempt to avoid over or under-consumption due to the presence of highly desirable foods or, conversely, non-palatable foods, participants were asked before the start of the study to rank different food items by order of preference and cross out those that they did not like (Rogers, 1993) (Appendix V). Based on this questionnaire, the buffet consisted of the second food preference within each category and second and third preference of sandwich fillings. Foods were presented in small bite portions of fixed size (1/4 of sandwich, sliced fruit, ½ chocolate mini roll, ¼ jam tart) in order to deprive participants from familiar cognitive and visual clues used to self-monitor food consumption (Stubbs *et al.*, 1998), avoid partial consumption and simplify nutritional calculation.

The buffet test-meal consisted of a variety of lunch-type foods, with different macronutrient composition (sandwiches, salad, fruit, cake, biscuits, crisps, yogurt, mayonnaise and mustard) (see Table 2.3). The average energy (kcal) and macronutrients (g) available at the buffet test-meal, calculated from the mean values from each food group, was of 2331 kcal, 69.5 g of protein, 332 g of CHO and 79.6 g of fat (or 4100 kcal, 85 g of protein, 373 g of CHO and 250 g of fat, if sauces were included: mayonnaise and mustard).

Foods were purchased from a local supermarket and were not modified in any way. They were prepared in the Clinical Investigation Unit (CIU) kitchen in the morning of each study day and kept in the refrigerator until served. Each food group was placed in separate plates covered by plastic film, except the mayonnaise and mustard that were given in their own jars, and presented on two trays. A glass of water (250 ml) was also provided and more was given if requested. Participants were presented with exactly the same type and amount of food throughout the study. To avoid opportunistic over-consumption due to the availability of free food, participants were told that they could take with them any food left after the buffet lunch.
Table 2.3. Food items, amounts presented and macronutrient composition (per unit) of the buffet test-meal

<table>
<thead>
<tr>
<th>Food item</th>
<th>Unit</th>
<th>N° of units</th>
<th>Energy (Kcal)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandwiches(^1) (2 slices of Hovis Farmhouse wholemeal bread (88g) + 10g flora margarine) and:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40g Tesco tune chunks in brine, drained</td>
<td></td>
<td>12 in total (6 of each filling selected)</td>
<td>72</td>
<td>5</td>
<td>8.2</td>
<td>2.2</td>
</tr>
<tr>
<td>20g Tesco freshly grated HealthyLing mild cheese</td>
<td>(\frac{1}{4})</td>
<td></td>
<td>75</td>
<td>4</td>
<td>8.2</td>
<td>2.9</td>
</tr>
<tr>
<td>35g Tesco British honey roast sliced ham</td>
<td></td>
<td></td>
<td>71</td>
<td>4.1</td>
<td>8.4</td>
<td>2.4</td>
</tr>
<tr>
<td>35g Bernard Matthews premium chicken roast</td>
<td></td>
<td></td>
<td>72</td>
<td>4.2</td>
<td>8.2</td>
<td>2.4</td>
</tr>
<tr>
<td>35g Scrambled egg</td>
<td></td>
<td></td>
<td>75</td>
<td>3.6</td>
<td>8.2</td>
<td>3.1</td>
</tr>
<tr>
<td>40g Quorn Dely (chicken, ham or turkey style)</td>
<td></td>
<td></td>
<td>73</td>
<td>4</td>
<td>8.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Salad:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>50g</td>
<td>1</td>
<td>7</td>
<td>0.4</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Tomato</td>
<td>90g (3 pieces)</td>
<td>1</td>
<td>15</td>
<td>0.6</td>
<td>2.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Cucumber</td>
<td>65g (sliced)</td>
<td>1</td>
<td>7</td>
<td>0.5</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Walter crisps (ready salted or flavoured)</td>
<td>50g</td>
<td>1</td>
<td>265</td>
<td>3.3</td>
<td>24.5</td>
<td>17</td>
</tr>
<tr>
<td>Tesco low fat yoghurt (strawberry, apricot or cherry)</td>
<td>200g</td>
<td>1</td>
<td>180</td>
<td>8.4</td>
<td>28.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Cakes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Kippling almond slice</td>
<td>(\frac{1}{2}) slice</td>
<td>8</td>
<td>68</td>
<td>1.2</td>
<td>10.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Mr Kippling lemon slice</td>
<td>(\frac{1}{2}) slice</td>
<td>8</td>
<td>60</td>
<td>0.6</td>
<td>9.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Mr Kippling country slices</td>
<td>(\frac{1}{2}) slice</td>
<td>8</td>
<td>61</td>
<td>0.7</td>
<td>9.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Tesco chocolate mini rolls</td>
<td>(\frac{1}{2}) roll</td>
<td>8</td>
<td>55</td>
<td>0.7</td>
<td>7.1</td>
<td>2.7</td>
</tr>
<tr>
<td>Tesco jam tarts assorted</td>
<td>(\frac{1}{2}) tart</td>
<td>8</td>
<td>65</td>
<td>0.6</td>
<td>10.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Biscuits:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tesco all butter (round)</td>
<td></td>
<td>8</td>
<td>41</td>
<td>0.5</td>
<td>5.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Tesco fruit shortcake</td>
<td>1 biscuit</td>
<td>16</td>
<td>18</td>
<td>0.2</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Macvities Lincoln</td>
<td>8</td>
<td></td>
<td>43</td>
<td>0.5</td>
<td>5.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Tesco value jaffa cakes</td>
<td>8</td>
<td></td>
<td>43</td>
<td>0.5</td>
<td>7.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Fruit:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple (peeled)</td>
<td>260g (in (\frac{1}{4}))</td>
<td>1</td>
<td>117</td>
<td>1.0</td>
<td>29.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Banana</td>
<td>130g (sliced)</td>
<td>1</td>
<td>124</td>
<td>1.6</td>
<td>30.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Satsumas</td>
<td>330g (in (\frac{1}{4}))</td>
<td>1</td>
<td>121</td>
<td>3.0</td>
<td>28.4</td>
<td>0.3</td>
</tr>
<tr>
<td>White grapes</td>
<td>200g</td>
<td>1</td>
<td>120</td>
<td>0.8</td>
<td>30.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Hellmans mayonnaise</td>
<td>200g jar</td>
<td>1</td>
<td>1444</td>
<td>2.2</td>
<td>2.6</td>
<td>158.2</td>
</tr>
<tr>
<td>Tesco English mustard</td>
<td>190g jar</td>
<td>1</td>
<td>325</td>
<td>13.3</td>
<td>38.2</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Macronutrient composition calculated according to manufacturers' information. CHO – carbohydrates.
2.2.2.4.2 Pasta based test-meal

In chapter 5, because changes in macronutrient selection were not under investigation and buffet test-meals are very expensive and laborious to prepare, a pasta based test-meal was used. The pasta meal consisted of ad libitum pasta and tomato sauce plus ad libitum cheddar cheese (Mild Tesco cheddar) in excess of expected consumption. 300g of raw pasta was cooked (Tesco macaroni pasta - standard macaroni, not egg, wholemeal, or specifically quick cook) and mixed with one jar (500g) of Ragu original tomato sauce and 30g of olive oil. The nutritional composition of the pasta based test-meal can be seen in Table 2.4.

Table 2.4. Energy and macronutrient composition of the pasta based test-meal

<table>
<thead>
<tr>
<th></th>
<th>Energy (kcal)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato based pasta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100g (cooked)</td>
<td>128.1</td>
<td>3.6</td>
<td>21.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Total (±1252g)</td>
<td>1604.4</td>
<td>44.5</td>
<td>268.9</td>
<td>69.5</td>
</tr>
<tr>
<td>Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100g</td>
<td>410.0</td>
<td>25.0</td>
<td>0.1</td>
<td>34.4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1956.4</td>
<td>71.1</td>
<td>252.6</td>
<td>105.4</td>
</tr>
</tbody>
</table>

The tomato-based pasta was presented inside a plastic box on a tray together with a bowl with 100 g of grated cheese and an empty soup plate with cutlery. A glass of water (250 ml) was also provided and more given if requested. Pasta and cheese were weighed separately before participants sat down and re-weighed after each subject had finished eating, and energy and macronutrient intake calculated.

2.2.3 Sub-maximal fitness test

Maximal oxygen uptake (VO\textsubscript{2} max) as a measure of fitness was estimated using a sub-maximal exercise test on a cycle ergometer. The YMCA protocol (American College of Sports Medicine, 2000) was chosen, which involves two to four, three-minute stages of continuous exercise. It is designed to raise the steady-state heart rate (HR) of the subject to between 110 beats per minute (bpm) and 85% of the age-predicted maximal HR (MHR) for at least 2 consecutive stages. MHR was calculated as 220 - age (years) (American College of Sports Medicine, 2000). Following the
guidelines of the American Colleague of Sport and Medicine (2000), participants were instructed, in advance to the fitness test, to:

- Avoid food, tobacco, alcohol and caffeine for at least 3 hours before testing;
- Avoid exercise or strenuous physical activity the day of the test;
- Wear comfortable, loose-fitting clothing;
- Drink plenty of water/fluids over the 24h period preceding the test to ensure normal hydration prior to the testing.

The YMCA sub-maximal fitness test was performed in a Cateye Fitness EC-C400 Upright exercise bike (Cateye, Dallas, USA). The general procedure is described below:

1. The exercise test started with a two to three minutes warm-up in order to acquaint the participant with the cycle ergometer and prepare him/her for the exercise intensity in the first stage of the test.

2. Participant were instructed to position themselves correctly on the cycle ergometer (i.e., upright posture, 5 degree bend in the knee at maximal leg extension, hands in the proper position on the handlebars)

3. The protocol consisted of three-minute stages with appropriate increments in workload (see Table 2.5).

4. HR was monitored twice during each stage, on the last 15 to 30 seconds of the second and third minutes of each stage. If HR was higher than 110 beats/min, a steady state HR (i.e., two HRs within ± 6 beats/min) had to be reached before the workload was increased.

5. Participants were monitored regularly for appearance and onset of any adverse symptoms.

6. The test ended when the participant either reached 85% of his/her age-predicted MHR, failed to conform to the exercise test protocol, experienced signs of excessive discomfort, or if an emergency situation arose.
7. A cool-down was performed consisting of either continued pedalling at a work rate equivalent to that of the first stage of the exercise test protocol or lower; or a passive cool-down if the subject experienced signs of discomfort or in the case of an emergency situation (American College of Sports Medicine, 2000).

Table 2.5. YMCA cycle ergometer protocol

<table>
<thead>
<tr>
<th></th>
<th>1st Stage</th>
<th>150 kgm/min (0.5 kg – 25 W)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR&lt;80</td>
<td>HR 80-89</td>
</tr>
<tr>
<td>2nd Stage</td>
<td>750 kgm/min (2.5kg – 125W)</td>
<td>600 kgm/min (2.0kg – 100W)</td>
</tr>
<tr>
<td>3rd Stage</td>
<td>900 kgm/min (3.0kg – 150W)</td>
<td>750 kgm/min (2.5kg – 125W)</td>
</tr>
<tr>
<td>4th Stage</td>
<td>1050 kgm/min (3.5kg – 175W)</td>
<td>900 kgm/min (3.0kg – 150W)</td>
</tr>
</tbody>
</table>

All the workloads were performed at 50 rpm. Taken from ACSM, 2000
The HR measured during the last minute of each stage (when a steady state was achieved) was then plotted against workload in a graph (Figure 2.2). The line generated from the plotted points was then extrapolated to the age-predicted MHR and a perpendicular line dropped to the x-axis to estimate maximal workload and VO\textsubscript{2} max (Figure 2.2). VO\textsubscript{2} max in L/min was then converted to ml/kg/min by multiplying the L/min value by 1000 and then dividing it by body weight in kg.

<table>
<thead>
<tr>
<th>Example:</th>
<th>Data:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, age = 28 years</td>
<td>Workload (kgm/min)</td>
</tr>
<tr>
<td>Estimated MHR = 188 bpm</td>
<td>150</td>
</tr>
<tr>
<td>85% MHR = 160 bpm</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>450</td>
</tr>
</tbody>
</table>

Figure 2.2. How to determine VO\textsubscript{2} max from sub-maximal heart rates obtained during the YMCA sub-maximal fitness test. Adapted from ACSM, 2000.

### 2.2.4 Anthropometry and body composition

Anthropometry and body composition were performed in the morning after a 12 hour overnight fast. All the participants were instructed not to exercise on the day, not to consume alcohol in the previous 48 hours and asked to urinate immediately before the measurements were performed (Heyward, 1998).
Body weight was measured with the participants dressed in light clothes and without shoes on a Seca 888 digital scale (Seca, Cheshire, UK) and recorded to the nearest 0.1 kg. Height was measured with a Harpenden stadiometer without shoes, with the person standing erect, looking straight in a horizontal plane with the feet together and the knees straight and recorded to the nearest 0.5 cm. The heels, buttocks, shoulder blades and the back of the head had to touch against the wall. Waist and hips were measured with a flexible measuring tape with the person standing erect and the arms relaxed along the body. The waist was measured at the narrowest point, below the rib-cage but above the belly button, while the hips were measure at the widest point around the buttocks. Both measures were recorded to the nearest 0.1 cm.

Percentage of body fat was measured by bioimpedance (BIA) using Bodystat 1500MM bioimpedance machine (Bodystat Limited, Isle of Man, British Islands), following standardised procedures fully described in the literature (Heyward, 1998). Each participant was asked to lie down in the bed, making sure that his/her legs and arms were abducted approximately 45° (so that there would be no contact between the thighs or between the arms and the trunk). Four electrodes were then placed on the right side of the body, two in the hand (dorsal surface of the hand proximal to the metacarpal phalangeal joint and mid point of the wrist) and two in the feet (dorsal surface of the feet proximal to the metatarsal phalangeal joint and between the medial and lateral malleoli of the ankle) (Heyward, 1998) and measurements performed.

2.2.5 Laboratory analyses

2.2.5.1 Blood sample collection

Blood samples were taken at regular intervals during the investigation period (see study protocols for further details on specific blood sampling times). Venous blood was collected into 2.5 ml fluoride oxalate tubes for analysis of glucose, 5 ml lithium heparin tubes for triacylglycerol (TAG), non-esterified fatty acids (NEFA), total cholesterol, HDL-cholesterol and insulin and 5 ml potassium EDTA-coated tubes, containing 200 kIU aprotinin/ml of whole blood, for the measurement of PYY, GLP-1, PP and ghrelin. Following collection tubes were kept in an ice bath to reduce degradation. Whole blood was then centrifuged at 1750 g (3000 rpm) for 10 minutes. After centrifugation the plasma was separated from the red cells using a plastic Pasteur
pipette and stored in 2 ml microtubes at -20°C for subsequent analysis. All samples were batch analysed at the end of each study to minimise inter-assay variability.

2.2.5.2 Analysis of plasma glucose

Two different analysers were used to measure glucose.

A. The YSI 2300 Stat Plus Glucose & Lactate Analyzer (YSI Incorporated, Yellow Springs, Ohio, USA) measures glucose using an immobilised enzyme biosensor. In this method, an enzyme specific for glucose is immobilized between two membrane layers; one of polycarbonate and the other of cellulose acetate. As it enters the enzyme layer, glucose is oxidized with the production of hydrogen peroxide.

\[ \text{D-glucose} + \text{O}_2 \xrightarrow{\text{Glucose oxidase}} \text{D-glucono-}\sigma\text{-lactone} + \text{H}_2\text{O}_2 \]

Hydrogen peroxide then passes through the membrane of cellulose acetate into a platinum electrode where the hydrogen peroxide is oxidized. The current produced is proportional to the concentration of the substrate; in this case glucose.

\[ \text{H}_2\text{O}_2 \xrightarrow{\text{Platinum Anode}} 2\text{H}^+ + \text{O}_2 + 2\text{e}^- \]

Quality controls (QCs) were included at the beginning and end of each assay. Intra- and inter-assay coefficients of variation (CVs) were at 6.3 mmol/L: 1.6% and 2.9% and at 14.5 mmol/L: 2.0% and 2.9%, respectively.

B. The ILAB 650 (Instrumentation laboratory, Milan, Italy), measures glucose using an enzymatic colorimetric method. In this method, glucose reacts with oxygen and water in the presence of glucose oxidase, with the production of gluconic acid and hydrogen peroxide.

\[ \text{B-D-glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + \text{H}_2\text{O}_2 \]

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

\[ 2\text{H}_2\text{O}_2 + \text{phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{red quinoneimine} + 4\text{H}_2\text{O} \]
The concentration of the red dye is directly proportional to the amount of glucose in the sample, which is determined by measuring the absorbance of red quinoneimine at 510 nm.

QCs were included at the beginning and end of each assay. Intra- and inter-assay CVs were at 5.31 mmol/L: 1.8% and 1.5% and at 14.0 mmol/L: 0.9% and 0.7%, respectively.

2.2.5.3 Analysis of plasma TAG

Plasma TAG concentrations were measured by an enzymatic colorimetric method on the ILAB 650 (Instrumentation laboratory, Milan, Italy) and Randox Space AlfaWasserMann Analyser (Randox Co., Antrim UK). After two intermediate steps, it ultimately measures the oxidative coupling of 4-chlorophenol and 4-aminophenazone to a red coloured quinoneimine compound in the presence of hydrogen peroxide. Hydrogen peroxide is proportional to the concentration of TAG in the sample, as well as, to the amount of the quinoneimine dye, which is produced by the reactions shown below.

\[
\begin{align*}
\text{TAG} & \xrightarrow{\text{Lipoprotein lipase}} \text{glycerol + fatty acids} \\
\text{Glycerol + ATP} & \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-P + ADP} \\
\text{Glycerol-3-P + O}_2 & \xrightarrow{\text{Glycerol-3-phosphate oxidase}} \text{dihydroxyacetone phosphate + H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + 4\text{-chlorophenol and 4-aminophenazone} & \xrightarrow{\text{Peroxidase}} \text{quinoneimine dye + H}_2\text{O}_2
\end{align*}
\]

The concentration of quinoneimine generated in the reaction is also directly proportional to the concentration of TAG in the sample, which is determined by measuring absorbance at 510 nm.

QCs were included at the beginning and end of each assay. For the ILAB 650, intra- and inter-assay CVs were at 1.41 mmol/L: 1.09 and 1.17% and at 2.51 mmol/L: 1.51% and 7.78%, respectively. For the Randox Space AlfaWasserMann Analyser, intra- and inter-assay CVs were at 1.13 mmol/L: 2.80% and 2.50% and at 2.55 mmol/L: 3.41% and 3.14%, respectively.
2.2.5.4 Analysis of plasma total cholesterol and HDL-cholesterol

Total cholesterol was measured on the Randox Space AfaWasserMann Analyser (Randox Co., Antrim UK), using an enzymatic three-step colorimetric method. In this method, cholesterol esters in the plasma are completely hydrolysed to free cholesterol and free fatty acids by cholesterol esterase. The liberated cholesterol, together with any free cholesterol originally present in the plasma, is then oxidized by cholesterol oxidase. Finally, in a reaction catalysed by peroxidase, the peroxide originated from the last step reacts with phenol and 4-aminoantipyrine to form a quinoneimine dye. The concentration of this coloured compound is directly proportional to the concentration of total cholesterol in the sample, which is determined by measuring absorbance at 500 nm.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \text{cholestene-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{quinoneimine dye} + 4\text{H}_2\text{O}
\]

HDL-cholesterol was also measured on the Randox Space AfaWasserMann Analyser (Randox, Co. Antrim UK), using an enzymatic colorimetric method similar to the one described for total cholesterol. The first step consists in the elimination of chylomicron, VLDL-cholesterol and LDL-cholesterol by cholesterol esterase and cholesterol oxidase and subsequently catalase.

\[
\text{Cholesterol ester} \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \text{cholestene-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2
\]

The second step consists of the measurement of HDL-cholesterol following its release by detergents: 4 - aminoantipyrine (4-AA) and N - (2 - hydroxyl - 3 - sulfopropyl) - 3,5 - dimethoxyaniline (HDAOS). The intensity of the quinoneimine dye produced is directly proportional to the HDL-cholesterol concentration in the sample, when measured at 600 nm.
QCs for total and HDL-cholesterol were included at the beginning and end of each assay. For total-cholesterol, intra- and inter-assay CVs were at 4.90 mmol/L: 3.7% and 4.2% and at 6.26 mmol/L: 1.7% and 3.7%, respectively. For HDL-cholesterol, intra- and inter-assay CVs were at 0.96 mmol/L: 5.37% and 9.04% and at 1.80 mmol/L: 1.57% and 2.84%, respectively.

### 2.2.5.5 Analysis of plasma NEFA

NEFA was measured on the Randox Space Alfa WasserMann Analyser (Randox Co., Antrim, UK), using an enzymatic colorimetric method. In a 2-step enzymatically catalysed reaction, hydrogen peroxide is produced from NEFA, which then reacts with N-ethyl-N-(2hydroxy-3-sulf hopropyl) m-toluidine (TOOS) and 4-aminoantipyrine (4-AA) to form a purple adduct.

\[
\text{NEFA} + \text{ATP} + \text{CoA} \xrightarrow{\text{Acyl CoA Synthetase}} \text{Acyl CoA} + \text{AMP} + \text{PPi}
\]

\[
\text{Acyl CoA} + \text{O}_2 \xrightarrow{\text{Acyl CoA Oxidase}} 2,3-,\text{-trans-Enoyl-CoA} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{TOOS} + 4\text{-AA} \xrightarrow{\text{Peroxidase}} \text{purple adduct} + \text{H}_2\text{O}
\]

The concentration of this purple adduct is directly proportional to the concentration of NEFA in the sample, which is determined by measuring absorbance at 550 nm.

QCs were included at the beginning and end of each assay. Intra- and inter-assay CVs were at 1.37 mmol/L: 2.04% and 5.29% and at 0.90 mmol/L: 3.74% and 6.52%, respectively.
2.2.5.6 Analysis of plasma insulin

2.2.5.6.1 Immuno-chemiluminometric assay

Plasma insulin concentrations were measured using a two-site immuno-chemiluminometric assay with the Invitron Insulin Assay kit (Invitron, Monmouth, UK). This assay employs an insulin specific solid phase antibody (immobilised on the microtitre wells) and a soluble antibody labelled with a chemiluminescent acridinium ester (Invitron Limited, 2007). The sample was incubated with the labelled antibody solution in the microtitre well for two hours at 37°C and after that, a wash was performed in an automatic plate washer - Microplate Washer AW1 (Anthos Labtec Instruments, Wals, Austria) to remove unbound labelled antibody. Finally, the bound luminescence was quantified by a microtitre plate luminometer - Microplate Luminometer LB 960 Centro (Berthold Technologies, Wildbad, Germany).

Analysis was performed at room temperature and all standards, QC’s and samples were run in duplicate. Plasma samples were defrosted at 4°C, in the day of the assay, and centrifuged at 1750g (3000 rpm) for 10 minutes at 4°C to spin any fibrin that could otherwise interfere with accurate pipeting. The standards for the assay were supplied with the assay kit and ranged from 0 to 200 mU/L. This assay cross reacts 100% with insulin, 1.2% with intact proinsulin, 1.6% with 32-33 split proinsulin, 0.8% with des 31-32 split proinsulin, 23 % with 65-66 split proinsulin and 44% with des 64-65 split proinsulin and does not cross react with C-peptide.

QCs were included at the beginning of each assay. Intra- and inter-assay CVs were at 36.4 pmol/L: 5.2% and 15.2%, at 364 pmol/L, 3.7% and 6.9% and at 985.1 pmol/L 7.4% and 11.4 %, respectively.

2.2.5.6.2 Radioimmunoassay

Plasma insulin concentrations were also measured by a specific radioimmunoassay (RIA) established at the University of Surrey (Hampton, 1984). This assay uses antisera raised in guinea pig against porcine insulin conjugated to ovalbumin, which cross reacts 100% with human insulin, 61% with des 31-32 biosynthetic proinsulin, 76% with des 64-65 biosynthetic proinsulin and 42% with human biosynthetic proinsulin and less than 0.001% with either plasma C-reactive or proinsulin. Human biosynthetic Insulin (Eli Lilly, Hants, UK) radiolabelled with $^{125}$I
was made at the University of Surrey using the chloramine-T method of iodination (in house method). No extraction of the sample is required and the separation of the bound and free insulin was carried out by solid phase second antibody. Standards were supplied from National Institute of Biological Standard and Controls and consist of natural insulin extract from human pancreas.

Plasma samples were defrosted at 4°C, on the day of the assay, and centrifuged at 1750 g (3000 rpm) for 10 minutes at 4°C to spin any fibrin that could otherwise interfere with accurate pipetting. The samples were assayed for both, specific and non-specific binding. In order to achieve that, a "non-specific binding" (NSB) tube was set up in duplicate for each participant and treatment condition (in addition to a NSB standard and a NSB for each QC) to determine any non-specific binding of the label to the assay tubes used. The assay was carried out on an ice tray and all the assay tubes (LP3 tubes), including samples, standards, QCs and NSBs were run in duplicate.

The assay diluent was 0.04M phosphate buffer (made with 23 g disodium hydrogen orthophosphate anhydrous + 5.97 g sodium dihydrogen orthophosphate in 5 L of distilled water) containing 0.5%(w/v) of BSA that was used throughout the assay and added to all tubes (Table 2.6). Insulin antiserum was diluted 1:20000 (from stock) in assay diluent and 100 µl added to each tube, except NSB tubes. Standards ranging from 1500 pmol/L to 10 pmol/L were then prepared by double dilution from a freeze dried top standard, using assay diluent, and 50 µl of each added to standard curve tubes. Charcoal stripped serum (CSS) was also added to the standard curve (50 µl). Three QCs (low, middle and high) were used and 50 µl of each added to the QC tubes and respective NSB’s. Finally, 50 µl of plasma sample was added to the sample tubes (and to NSB tubes as previously explained). All the tubes were then vortex mixed and incubated for 24 hours at 4°C.

On day two of the assay protocol, 100 µl of the label (diluted to 10,000 counts per minute (cpm) per 100 µl with assay diluent) was added to all tubes. Total tubes, containing only 100 µl of label were also set. All the tubes were incubated for another 24 hours at 4°C.

On the last day of the assay (day three), 100 µl of anti-guinea pig Sac-Cel (IDS, Tyne and Wear, UK) was added to all tubes, except totals, to allow the separation of the
bound and free antibody. The tubes were again vortex mixed and incubated for 30 minutes at room temperature. Then 1 ml of Brij saline solution (1 L water + 9 g of salt + 2 ml Brij 35% solution) was added to all tubes except totals, before centrifugation at 2500 rpm for 30 minutes at 4°C. The supernatant was aspirated under vacuum and the sediment, containing the bound fraction, counted on a gamma counter (Wallac Wizard 1470 with Multicalc level 4.M software). In order to determine the insulin concentration of each plasma samples, each sample was read against the standard curve using specific software on the Wizard 1470.

Table 2.6. Insulin radioimmunoassay protocol

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 1</td>
</tr>
<tr>
<td></td>
<td>Totals NSB St Zero St St NSB QC QC NSB sample Sample</td>
</tr>
<tr>
<td></td>
<td>µl µl µl µl µl µl µl µl µl</td>
</tr>
<tr>
<td>Assay diluent</td>
<td>- 350 250 200 350 250 350 250</td>
</tr>
<tr>
<td>Antiserum</td>
<td>- - 100 100 - 100 - 100</td>
</tr>
<tr>
<td>Standard</td>
<td>- - - 50 - - - -</td>
</tr>
<tr>
<td>CSS</td>
<td>- 50 50 50 - - - -</td>
</tr>
<tr>
<td>QC</td>
<td>- - - - 50 50 - -</td>
</tr>
<tr>
<td>Sample</td>
<td>- - - - - 50 50</td>
</tr>
</tbody>
</table>

Vortex mix all tubes and incubate for 24 hours at 4°C

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 2</td>
</tr>
<tr>
<td>Label</td>
<td>100 100 100 100 100 100 100 100</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Incubate for 24 hours at 4°C</td>
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<table>
<thead>
<tr>
<th>Reagents</th>
<th>Tubes</th>
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<td></td>
<td>DAY 3</td>
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<td>Sac cell</td>
<td>- 100 100 100 100 100 100 100</td>
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<td></td>
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<tr>
<td>Vortex and incubate for 30 minutes at room temperature</td>
<td></td>
</tr>
</tbody>
</table>

| Brij solution  | - 1000 1000 1000 1000 1000 1000 1000 |
|
| Do not vortex, centrifuge all tubes, aspirate and count |

The sensitivity of this assay, based on twice the standard deviation from zero binding, was 2.5 pmol/L. QCs were included at the beginning and end of each assay. Intra- and inter-assay CVs were at 53 pmol/L: 3.7% and 12.2%, at 130 pmol/L: 7.4% and 12.4% and at 362 pmol/L: 1.6% and 9.4%, respectively.
2.2.5.7 Analysis of gut and pancreatic hormones

Total ghrelin, PYY, GLP-1 and PP were measured at the Faculty of Medicine in Imperial College, London (by a person external to this research) using established RIAs. Total ghrelin was measured with a specific and sensitive RIA, which cross-reacts fully with both octanoyl and des-octanoyl ghrelin and does not cross-react with any other known GI or pancreatic hormones (Patterson et al., 2005). PYY was also measured with a specific and sensitive RIA. The assay measured both the hormone fragment (PYY-(3-36)) and the full length hormone (PYY-(1-36)), both of which are biologically active (Adrian et al., 1985). GLP-1- like immunoreactivity was measured by a specific and sensitive RIA, previously established (Kreymann et al., 1987). The antibody was produced in rabbits against GLP-1 coupled to bovine serum albumin. The antibody cross-reacts 100% with all amidated forms of GLP-1 but does not cross react with glycine extended forms [GLP-1 (1-37) and GLP-1 (7-37)] or any other known pancreatic or GI peptide. Plasma PP concentrations were measured with a specific and sensitive RIA, using antisera against human PP raised in rabbits, as previously described (Adrian et al., 1976). This assay cross-reacts fully (100%) with human PP and does not cross-react with any other member of the PP family or GI hormones.

The sensitivity of the assays was 17 pmol/L for ghrelin, 2 pmol/L for PYY, 1 pmol/L for GLP-1 and 2.5 pmol/L for PP. All samples were assayed in duplicate and in one assay to eliminate the effects of inter-assay variation. All the above hormones assayed exhibited an intra-assay coefficient of variation of <10%.

2.2.5.8 Preparation of charcoal stripped serum

Proteins in the plasma may lead to a reduction in the binding between the radiolabel and the antiserum. In order to match this effect also in the standard curve, charcoal stripped serum (CSS) is usually added. CSS was prepared in large quantities in advance to the assay. Approximately 300 ml of fasting blood was taken from a number of volunteers and then left to clot overnight at 4°C. After that, the serum was decanted from each clot, pooled and the total volume measured.

Aragose coated charcoal was prepared previously by the addition of 100 g Noryt A (grade) charcoal (Sigma Chemical Company Ltd, Poole, UK) to 25 g agarose
dissolved in 500 ml of distilled water and heated to 70°C in a water bath. After the addition of the charcoal, the solution was mixed, cooled to 50 °C and poured into 1 L of acetone. This mixture was then stirred and filtered through Whatman No.1 filter paper to remove excess liquid. The resulted charcoal residue was then left to dry overnight. In order to remove any small dust-like particles of charcoal that may interfere with the assay, the agarose coated charcoal was then “de-fined”. This process involves washing the charcoal two to three times in distilled water to remove suspended particles, and drying overnight at 37°C. The preparation of the charcoal should be done at least 24 hours prior to charcoal stripping of serum to allow the charcoal to dry completely.

The agarose coated charcoal was then added to the pooled serum (25 g per 200 ml) and stirred overnight at room temperature. This solution was then centrifuged at 11400 g (10000 rpm) for one hour and the supernatant removed and re-spun following the same procedure. The final supernatant was filtered with Whatman No.1 filter paper to remove any fine residues of charcoal. Finally, 1.5 ml of the CSS was spun at 11400 g (10000 rpm) for 10 minutes in a bench top centrifuge to check for the presence of fine particles, and if necessary the serum was re-filtered. The CSS was then divided into small aliquots and stored at -20 °C.

2.2.6 Statistical analyses

Statistical analysis was carried out using SPSS version 11.0 (SPSS Inc., Chicago, USA) and statistical significance assumed at P<0.05, unless otherwise stated. All variables were checked regarding their normal distribution using the Shapiro-Wilk test. If the derived P value was not significant then the data was assumed to be normally distributed. Parametric tests were used throughout this thesis unless otherwise stated.

The statistical analysis for each individual chapter is described in the methods section of the relevant chapter.
Chapter Three
Chapter 3. Effects of a six-week exercise programme on short-term appetite control in sedentary volunteers

3.1 Introduction

The steady increase in the prevalence of obesity worldwide has been accompanied, on one hand, by an increase in the consumption of energy-dense food and, on the other, by a reduction in PA levels (Varo et al., 2003; World Health Organization, 2003). The role of exercise in the prevention of positive EB and obesity is well-recognised (King, 1999; Moore, 2000) and there is evidence that a sedentary lifestyle predisposes to a failure in appetite regulation, with an uncoupling between EI and EE, most likely due to a failure to down-regulate EI to match the widespread levels of inactivity (Murgatroyd et al., 1999; Moore, 2000). This suggests a link between inactivity and disrupted homeostatic mechanisms involved in appetite control.

The "preload-test-meal" paradigm, where preload energy content is manipulated in order to alter EB, has been widely used in the area of appetite research to study homeostatic feedback control of hunger and satiety (Kissileff, 1985), a process that if not tightly regulated can lead to energy imbalance. The results, despite some variation, tend to show an effect of preloads of different energy content on hunger and subsequent food intake in normal-weight individuals (Hill et al., 1987; Rolls et al., 1994; Goldberg et al., 1998). Appetite responses are thus dependent to some extent on previous EI and sensitive to energy deficits induced through differences in dietary intake. Interestingly, acute energy deficits created by exercise seem to activate distinct pathways and have different effects on appetite control, compensatory responses and EB from those induced by diet (Hubert et al., 1998). Whilst strong compensatory physiological mechanisms seem to be activated when dietary energy deficits disrupt EB (Goldberg et al., 1998; Hill et al., 1987), the majority of the studies show little or no effect of an acute bout of exercise on subjective hunger ratings (Hubert et al., 1998; Imbeault et al., 1997; King et al., 1996; Lluch et al., 1998) or subsequent EI (King and Blundell, 1995; King et al., 1996; King et al., 1997a; Lluch et al., 1998; Westerterp-Plantenga et al., 1997; Blundell and King, 1999; Thompson et al., 1988; Hubert et al., 1998; Imbeault et al., 1997).
However, in contrast to the weak coupling between EE and EI in the short-term in response to acute exercise, physically active individuals, such as long distance runners, usually have a greater EI compared to those with a sedentary lifestyle, but paradoxically have a lower BMI, suggesting that a tight coupling between EI and EE exists at high levels of PA (King et al., 1997b). This raises the hypothesis that exercise may sensitize the physiological mechanisms involved in appetite control, thereby improving the coupling between EI and EE. Support for the role of exercise on appetite control was provided by King and colleagues (1999), who demonstrated that active males are able to detect differences in energy content after an exercise challenge, and to correctly adjust for that at a subsequent meal. A significant suppression of subsequent EI was observed after a high-energy drink, compared with a low-energy drink, with a near perfect energy compensation being achieved (King et al., 1999).

Moreover, it has been shown that active men have a better short-term appetite response to covert preload energy manipulation compared to sedentary men (Long et al., 2002). The sedentary group was unable to adjust subsequent EI in response to a preload energy manipulation, with buffet EI, 60 minutes after either a low- (LEP) or high-energy preload (HEP) remaining essentially the same. In contrast, the active group decreased their subsequent EI following the HEP, demonstrating an almost perfect (90%) compensation. Although these findings provide strong but indirect evidence for the beneficial role of exercise in appetite control, they do not prove causality, since they are based on a cross-sectional design. The better appetite regulation observed in active men could have been due to other factors such as lifestyle or cognitive factors and be unrelated to their activity levels.

### 3.2 Aims

The primary aim of this study was to examine in sedentary normal-weight volunteers, whether increasing habitual PA levels can improve the sensitivity of compensation for previous EI, using a longitudinal design. Secondary aims were to determine whether any improvement in appetite regulation observed with exercise resulted from changes in macronutrient selection and to examine whether the response to exercise differs between genders. Short-term appetite regulation was assessed at baseline and after a six-week exercise intervention, using subjective ratings of
motivation to eat and objective measures of food intake at a buffet meal and over a 24-hour period, following two covertly manipulated preloads of different energy content.

3.3 Methods

3.3.1 Participants

Twenty-nine adult (18-60 years old) healthy sedentary volunteers (15 men and 14 women), with a mean age of 29.8±11.6 years and a BMI of 22.7±2.3 kg/m², who were not dieting to lose weight, were recruited for this study. A sedentary lifestyle was defined as not being engaged in strenuous work or in regular brisk leisure PA more than once a week or engaged in light exercise for more than 20 minutes/day, more than 3 times/week. This was assessed through an exercise history of the three months prior to the study (Appendix VI) and PA and exercise were defined according to the ACSM (American College of Sports Medicine, 2000). Exclusion criteria included a BMI lower than 18 or higher than 27 kg/m², prior or present history of coronary heart disease, type 1 or type 2 diabetes, anaemia, gout, depression or other psychological disorders, eating disorders, drug or alcohol abuse within the last two years and current medication known to affect appetite or induce weight loss and were assessed by a self-certified medical questionnaire (Appendix VII). Participants were also excluded if their measured resting blood pressure was greater than 140/90 or if they smoked more than 10 cigarettes/day.

To measure eating behaviours, all the participants were asked to fill in the DEBQ (van Strien et al., 1986) (Appendix III) and only those scoring <4 in any one of the DEBQ subscales were accepted for the study. Average scores were of 2.2±0.7 for restrained, 2.5±0.7 for emotional and 3.0±0.5 for external eating behaviour, respectively. All the participants had to fill in a health screening questionnaire (Physical Activity Readiness Questionnaire) (Appendix VIII), in order to identify and exclude those who would be at risk of possible harm by participating in an exercise programme.

Participants were unaware of the real purpose of the study and were told that it aimed to investigate the effects of exercise on mood and food choices. All participants gave written consent before enrolling in the study and were debriefed at the conclusion of the study. The study was approved by the University of Surrey Ethics Committee (EC/2004/113/SBMS).
3.3.2 Protocol

Participants underwent a six-week moderate intensity exercise programme. A “preload-test-meal” paradigm, with a LEP and HEP, was used at baseline and after the exercise intervention to assess the ability of participants to regulate their food intake at the test-meal in response to preloading. The effect of the exercise programme on habitual food intake, anthropometry, cardiovascular fitness and plasma levels of fasting hormones and metabolites was also assessed. A scheme of the protocol of the study can be seen in Figure 3.1.

![Exercise Programme Diagram]

Figure 3.1. Schematic representation of the protocol of the study

Participants attended the Clinical Investigation Unit (CIU) at baseline and at the end of the study, after a 12 hour overnight fast, to undergo various assessments including anthropometry, body composition and fitness levels, and to take a fasting venous blood sample. Anthropometric measurements (weight, height, waist and hips) and body composition using bioimpedance (BIA) were performed following standard procedures fully described in Chapter 2 (section 2.2.4.). After that a fasting venous blood sample was taken. Finally, VO\textsubscript{2} max was estimated, as an independent measure of
compliance to the prescribed exercise programme, using the YMCA sub-maximal exercise test on a cycle ergometer (American College of Sports Medicine, 2000), as previously described in Chapter 2 (section 2.2.3). In order to make participants believe that the study aimed to investigate the effects of exercise on mood, mood state was assessed over time using the Profile of Mood States (POMS) questionnaire (Shacham, 1983) (Appendix IX), before participants started the exercise intervention and at the end of every week of the exercise programme. A total mood disturbance index was calculated by adding the scores on the five negative mood states (tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia and confusion-bewilderment) and subtracting the positive vigour-activity score (LeUnes and Burger, 2000).

Participants were asked to maintain their normal diet throughout the study. This was verified by a three-day estimated food diary (including at least one weekend day) using household measures, at baseline and week six of the exercise intervention (Appendix X). In order to standardize the procedure, participants were instructed to include two exercise and one non-exercise day on the three-day food diary of week six. All dietary analysis was performed using WinDiets Professional (The Robert Gordon University, Aberdeen, UK).

### 3.3.2.1 Exercise programme

The exercise programme started at week one and ran until week six. Participants were given two choices: free temporary gym passes, for the duration of the study, or a stationary exercise bicycle delivered to their homes. They were asked to perform 30 to 45 minutes of moderate aerobic exercise (between 65% and 75% of their MHR) at least 4 times per week, continuously or in bouts of at least 10 minutes each, in accordance with the most recent recommendations designed to improve aerobic fitness and body composition (American College of Sports and Medicine, 1998; World Health Organization, 2002; Department of Health, 2004).

To ensure proper monitoring of the exercise intensity and duration and to verify the participants’ compliance to the exercise prescription, participants were asked to use a HR monitor (Polar F1 - Polar Oy, Kempele, Finland) every time they exercised and to record in a daily diary the exercise they performed, including type, duration and intensity (average HR during each bout of exercise as displayed in the HR monitor and
self-recorded by each participant) (Appendix XI). In addition, for those who chose the gym, an independent measure of their frequency of attendance was obtained from the gym staff. Moreover, to check that the prescribed exercise did not result in a compensatory reduction in non-exercise PA participants wore a pedometer (YAMAX SW-200 - Digi Walker, Tokyo, Japan) for a week, before the start of the study (pre-study habitual PA) and at weeks three and six of the exercise programme (Pedometer form - Appendix XII). In order to achieve a good compliance with the prescribed exercise, all participants were contacted by telephone once a week.

3.3.2.2 Appetite challenge days

Using a randomized single-blind crossover design, participants were given a HEP or LEP, fully described in Chapter 2 (2.2.2.3), at baseline on different days of the week (appetite challenge days). These two preloads were at least two days apart in order to prevent participants from becoming bored with the foods presented at the buffet and to prevent any crossover effects. This was again repeated after the six-week exercise intervention, with participants acting as their own controls. A 24-hour food recall was performed prior to each appetite challenge day to verify participants' compliance with the study instructions.

On the morning of each appetite challenge day, participants were asked to consume their usual breakfast before 9.30 am. This was the same on all four appetite challenge days in order to standardize pre-study appetite. After that, participants were instructed not to eat or drink anything except water, which was permitted up to 10.30 am and asked to arrive at the CIU at 11.45 am. Participants were also asked to avoid alcohol consumption and exercise during the 24 hours prior to, and during each appetite challenge day. After arrival, participants were asked to fill in a first set of VAS. Instructions regarding the use of VAS are described in Chapter 2 (section 2.2.2.1). Participants were asked to rate their subjective feelings of hunger, desire to eat and fulness. Seven mood related questions were presented at the beginning to emphasize the image of a mood study, as was explained to the participants (data not analysed) (Appendix XIII). VAS were presented together with food preference lists, as described in Chapter 2 (section 2.2.2.2 and Appendix IV).
Preloads were then presented and participants were asked to consume them within 5 minutes. Further VAS were completed immediately after preload consumption (including the question: "How would you rate the palatability of the milkshake?") and at 20, 40 and 60 minutes afterwards. During this time period, participants stayed in the CIU, but they were free to write, read or watch television. Participants were free to talk to each other, but were asked not to discuss the study or their VAS scores. Each VAS was collected before the next was given to avoid participant of modifying their previous scores.

An ad libitum buffet meal was served 60 minutes after the preload, and participants, each confined to an individual booth, were instructed to eat until they felt comfortably full. Exactly the same type and amount of food was presented to each individual participant on all four appetite challenge days. The food items, amounts and macronutrient composition of the buffet meal, as well as the conditions under which the buffet was presented to participants was fully described in Chapter 2 (section 2.2.2.4.1). Participants were also asked to record in a food diary all they consumed after the buffet lunch until (and including) breakfast of the following day (post-buffet food diary - PBFD) in order to estimate 24 hours cumulative EI (calculated as buffet + PBFD). All nutrient analysis of the diet diaries was performed using WinDiets Professional (The Robert Gordon University, Aberdeen, UK).

### 3.3.2.3 Biochemical analysis

Fasting blood samples were analysed for total and HDL-cholesterol, TAG, NEFA, glucose and insulin. Glucose was analysed using an immobilised enzyme biosensor in the YSI 2300 Stat Plus Glucose & Lactate Analyzer (Yellowsprings, Ohio, USA), insulin using an immunoassay kit (Invitron, Monmouth, UK) and all the other metabolites were quantified using standard methods on the Randox Space Alfa WasserMann Analyser (Randox Co., Antrim, UK). A full description of the above methods, as well as intra- and inter-assay CVs can be found in Chapter 2 (section 2.2.5). Insulin sensitivity (Si) was calculated using the homeostatic model assessment (HOMA model) as (fasting insulin levels (µU/L) x fasting glucose levels (mmol/L)) / 22.5 (Matthews et al., 1985).
3.3.2.4  Assessement of underreporting

To assess whether the reported EI (based on the three-day food diary) is a valid estimate of the actual intake, as measured over that period of time, and as a consequence, to evaluate underreporting of dietary intake, the revised version (Black, 2000) of the Goldberg equation (Goldberg et al., 1991) was used. Briefly, to determine whether a given value of mean EI/BMR in n subjects (at the population or individual level) is acceptable, the following must be satisfied:

$$\frac{EI}{BMR} > PAL \times \exp \left[ SD_{min} \times \frac{S}{100}\right] \frac{\sqrt{V_n}}{n}$$

BMR was calculated using age- and sex-specific formulas (FAO/WHO/UNU, 1985). PAL is the presumed average physical activity level (PAL) for the population (or individual) under study, exp is exponential function (exp(x) or $e^x$), SD_{min} is -2 for 95% lower confidence limit, and S is the overall CV for PAL, taking into account the variability in EI and BMR. S is given by the equation:

$$S = \sqrt{CV_{wei}^2/d + CV_{wb}^2 + CV_{tp}^2}$$

where $CV_{wei}$ is the within-subject variation in EI, d is the number of days of diet assessment, $CV_{wb}$ is the within-subject variation in repeated BMR measurements or the precision of estimated compared with measured BMR, and $CV_{tp}$ is the between-subject variation in PAL. The values used for each factor were of 23% for $CV_{wei}$, 8.5% for $CV_{wb}$ for estimated BMR and 15% for $CV_{tp}$ (Black, 2000).

PAL values of 1.55 and 1.78 for men and 1.56 and 1.64 for women were used in the above calculations, at baseline and end of the study, respectively. These are the PAL values for sedentary and moderate physical activity levels proposed by the WHO (FAO/WHO/UNU, 1985). This resulted in cut-off points, at the population level (n=25) of 1.37 and 1.40 at baseline and 1.58 and 1.47 at the end of the study in men and women, respectively.

The cut-off value of 1.04 and 1.19 for men and 1.05 and 1.10 for women, for baseline and end measurements, respectively, were used to classify individuals as under- or adequate-reporters (using n=1). Participants with an EI/BMR < than these cut-
off values were considered to be under-reporters, whereas those with an EI/BMR ≥ than these cut-off values were considered to be adequate reporters.

### 3.3.3 Statistical analysis

Differences in energy and macronutrient intake in the 24 hours before each appetite challenge day (from data derived from the 24 hour food recalls) were assessed by repeated measures analysis of variance (ANOVA).

Changes in habitual energy and macronutrient intake with the exercise programme (based on the three-day food diaries), as well as in anthropometric variables, fitness and fasting levels of hormones/metabolites, were assessed by paired sample t-tests.

The effect of preload (HEP vs LEP), exercise (pre vs post) and gender on energy and macronutrient (in %) intake, at the buffet lunch and over a 24h period (cumulative EI) was assessed by a mixed between-within subjects ANOVA, with preload and exercise as the within-subject variables and gender as the between-subjects factor. Additionally, paired sample t-tests were performed to investigate differences in buffet EI after each preload before and after the six-week exercise intervention. In order to correct for multiple comparisons, the level of significance (α level) was reduced to P<0.01. Energy compensation, also known as the compensation index, was calculated as the difference in EI between the two study days (HEP vs LEP) divided by the difference in preload energy content and expressed as a percentage (Johnson and Birch, 1994). This was calculated for both EI at the buffet lunch and cumulative EI over a 24-hour period following preload consumption.

Potential correlations between energy compensation and different variables assessed were investigated using Pearson or Spearman correlations, as appropriate.

The effect of preload (HEP vs LEP), exercise (pre vs post) and gender on subjective feelings of hunger and fullness, from immediately before till 60 minutes after preload consumption, were assessed by a mixed between-within subjects ANOVA, with preload and exercise as the within-subject variables, gender as the between-subjects factor and the area under the curve (AUC) as the dependent variable. AUC was calculated using the trapezoidal rule. The same approach was used to look at the effect
of preload, exercise and gender on the energy (kcal) derived from the total number of items selected from the food preference lists, from immediately before till 60 minutes after preload consumption.

Satiety and hunger quotients (SQ and HQ, respectively) were calculated to estimate changes in fulness and hunger ratings in response to the ad libitum buffet lunch in relation to buffet’ EI (Green et al., 1997). SQ was calculated as (post-meal fulness rating – pre-meal fulness rating) / buffet meal EI and HQ as (pre-meal hunger rating – post-meal hunger rating) / buffet meal EI.

3.4 Results

3.4.1 Attrition rate

Of the 29 participants initially recruited, four males did not complete the study due to individual work pressures. BMI, percentage of body fat and fitness levels were not significantly different between the 4 men who withdrew and the 11 who completed the exercise intervention. The results are, therefore, presented for 25 participants (11 males, 14 females).
### 3.4.2 Anthropometry, fitness and metabolic profile

Changes in anthropometry, fitness and metabolic profile with the exercise intervention are shown in Table 3.1.

#### Table 3.1. Anthropometry, fitness levels and metabolic profile at baseline and end of the study in all participant (n=25), men (n=11) and women (n=14)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>End</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>All</td>
<td>Men</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.1±8.7</td>
<td>72.5±7.4</td>
</tr>
<tr>
<td>BMl(kg/m²)</td>
<td>22.7±2.3</td>
<td>23.4±2.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>23.6±7.8</td>
<td>16.3±4.9</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>81.8±7.7</td>
<td>86.3±7.4</td>
</tr>
<tr>
<td>Hips (cm)</td>
<td>99.9±5.1*</td>
<td>100.8±6.2</td>
</tr>
<tr>
<td>Fitness level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>31.1±4.8***</td>
<td>32.7±5.1**</td>
</tr>
<tr>
<td>Pc of VO₂max¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Pc10</td>
<td>3 (21.4%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>10≤Pc&lt;30</td>
<td>6 (42.9%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>30≤Pc&lt;70</td>
<td>4 (28.4%)</td>
<td>1 (9.1%)</td>
</tr>
<tr>
<td>70≤Pc&lt;90</td>
<td>1 (7.1%)</td>
<td>-</td>
</tr>
<tr>
<td>≥Pc90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.6±0.3</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.8±1.0</td>
<td>4.6±1.2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.2±0.3</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>TAG (mmol/L)</td>
<td>1.1±0.5</td>
<td>1.2±0.5</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1±0.4</td>
<td>5.2±0.5</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>62.2±48.7</td>
<td>82.4±66.4</td>
</tr>
<tr>
<td>S₁ (HOMA)²</td>
<td>2.0±1.6</td>
<td>2.8±2.2</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD. Means sharing the same symbol denote significant differences between baseline and end *P<0.05, **P<0.01, ***P<0.0001. S₁—Insulin sensitivity

¹) Percentile of VO₂ max (ml/Kg/min) adjusted for age and gender: classification according to the American College of Sports and Medicine, 2000.

²) HOMA calculated according to Matthews et al., 1985.
No significant changes were observed in any of the anthropometric measures or the plasma levels of hormones/metabolites analysed with the exercise intervention, with the exception of hip circumference that decreased when the group was analysed as a whole (P<0.05) and in women (P<0.05), but not in men, when genders were analysed separately. The majority of the participants had a fitness level, based on their estimated VO$_2$ max, below the 30$^{th}$ percentile at baseline according to the American College of Sports and Medicine (2000), and significant improvements in fitness were observed with the exercise intervention in all participants (P<0.001), men (P<0.01) and women (P<0.05).

As expected, men were significantly heavier (P<0.0001 for both), had a higher waist circumference (P<0.01 for both) and a lower percentage of body fat (P<0.0001 for both) compared with women, at both baseline and end of the study. They also had a significantly higher VO$_2$ max (ml/Kg/min) (P<0.05) at the end and a greater improvement in fitness levels compared to women (5.2±4.7 vs 1.6±2.3, P<0.05).
3.4.3 Exercise compliance

Compliance with the prescribed exercise program was good. Frequency, duration and intensity of exercise were for all participants, men and women of: 4.0±0.8, 3.6±0.6 and 4.4±0.8 times/week, 187.3±74.9, 157.2±29.0 and 210.9±91.3 minutes/week and 69.6±3.1, 70.7±3.9 and 68.8±2.1% MHR, respectively. The only significant difference observed between genders was a higher frequency of exercise in women compared with men (P<0.05).

The number of steps per day in all participants, men and women at baseline, week 3 and week 6 of the study can be seen in Table 3.2. No significant differences were observed overtime or between genders. These indicators suggest that compliance with the exercise intervention was good in the completing subjects.

Table 3.2. Number of steps/day in all participants (n=25), men (n=11) and women (n=14) at baseline, week 3 and week 6 of the study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 3</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>8474±2819</td>
<td>7996±2929</td>
<td>7806±2956</td>
</tr>
<tr>
<td>Men</td>
<td>8223±3546</td>
<td>7810±4032</td>
<td>7196±3760</td>
</tr>
<tr>
<td>Women</td>
<td>8654±2296</td>
<td>8142±1810</td>
<td>8322±2082</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD. No significant differences in the number of steps/day overtime or between genders.
3.4.4 Habitual energy and macronutrient intake

The exercise intervention did not lead to any significant change in habitual energy or % of energy derived from different macronutrients (as assessed by the three-day food diaries), when comparing pre-intervention with week six of the exercise programme, in either all participants, men or women.

Mean EI/BMR in men and women at baseline and end of the study (based on the three-day food diaries) can be seen in Table 3.3.

Table 3.3. EI/BMR in men (n=11) and women (n=14) at baseline and end of the study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>1.42±0.28 (1.11 – 2.15)</td>
<td>1.20±0.21 (0.91 – 1.55)</td>
</tr>
<tr>
<td>Women</td>
<td>1.34±0.19 (1.07 – 1.68)</td>
<td>1.36±0.45 (0.84 – 2.55)</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD (range). No significant differences in EI/BMR were observed between men and women either at baseline or end of the study or between baseline and end when each gender was analysed separately.

No significant differences were observed in EI/BMR between men and women, either at baseline or at the end of the study, or between baseline and end when each gender was analysed separately. The mean EI/BMR ratio was for men, at baseline, of 1.42. This mean value is greater than the lower cut-off limit of 1.37 for the male population in this study (n=11), therefore indicating that underreporting bias was unlikely to be present. For women, at baseline, the mean EI/BMR was of 1.34, slightly lower than the cut-off limit of 1.40 for the female population in this study (n=14). This is likely to indicate some very mild underreporting. At the end of the study, mean EI/BMR was of 1.2 for men and 1.36 for women, both below the lower cut-off limit of 1.58 and 1.47 in the male and female population, respectively. This is likely to indicate moderate to severe underreporting particularly in males at the end of the exercise intervention.

Using the individual cut-off points for underreporting described in the methods section, all men and women were adequate-reporters at baseline, while at the end of the study, 46% of men and 29% of women were under-reporters.
3.4.5 Energy and macronutrient intake over 24 hours

No significant differences were observed in energy or macronutrient intake on the 24 hours preceding each of the four appetite challenge days, in all participants, men or women.

No order effects (of preloading) were found on cumulative EI (kcal) either at baseline (cumulative EI after the HEP: 2299±879 vs 2060±526, P>0.05; cumulative EI after the LEP: 2086±669 vs 2273±580, P>0.05 in those that started first with the HEP and the LEP, respectively) or end of the study (cumulative EI after the HEP: 1859±756 vs 1939±490, P>0.05; cumulative EI after the LEP: 2307±640 vs 2206±723, P>0.05 in those that started first with the HEP and the LEP, respectively).

Cumulative EI for the 24 hours following preload consumption, before and after the six-week exercise intervention, is shown in Figure 3.2. Analysis of variance showed a significant exercise x preload interaction (P=0.023) on 24-hour cumulative EI, but no significant main effects of preload, exercise, gender or other interactions.

![Figure 3.2. Cumulative EI after the HEP and LEP, at baseline and end of the study in all participants (n=25), men (n=11) and women (n=14). Values represent means ±SEM. Analysis of variance showed a significance exercise x preload interaction (P=0.023), but no significant main effects of exercise, preload, gender or other interactions. Columns sharing the same symbol denote significant differences between conditions: ** P<0.01 (assuming a α level<0.01 to correct for multiple comparisons).](image)
Paired t-tests comparing cumulative EI following preload consumption (assuming a α level<0.01 to correct for multiple comparisons) found no significant differences in cumulative EI (kcal) after the HEP and LEP at baseline (2172±785 vs 2163±627, P=0.925). However, after the six-week exercise intervention, buffet EI after the HEP was significantly lower compared with after the LEP (18301±638 vs 2162±594, P=0.007) in all participants. A similar baseline pattern was observed when males and females were analysed separately, with no significant differences in cumulative EI after each preload (HEP vs LEP) at baseline (2770±666 vs 2665±431 kcal, P=0.584; 1745±123 vs 1805± 485 kcal, P=0.475, for men and women respectively). After the 6-week exercise intervention, there was a trend towards a lower cumulative energy intake after the HEP compared with after the LEP (2119±698 vs 2649±707 kcal, P=0.040; 1677±526 vs 1924±428, P=0.051, for men and women respectively).

When energy compensation over a 24 hour period was calculated, a trend towards improved compensation was observed in all participants with the exercise intervention (8.9±118.5% at baseline vs 79.5±146.4% following the exercise intervention, P=0.056). However, when each gender was analysed separately no significant effects were found (men: -8.4±157.7% vs 95.6±190.7%, P=0.150; women: 20.9±87.3% vs 68.4±113.8%, P=0.250, at baseline and end respectively).
No significant effect of exercise, preload, gender or interactions were observed in the percentage of 24 hours cumulative EI provided by each macronutrient (Figure 3.3).

![Graph showing energy intake percentages for different macronutrients at baseline and end of the study for all participants, men, and women.](image)

Figure 3.3. Percentage of 24 hours cumulative EI provided by each macronutrient, at baseline and end of the study in all participants (n=25), men (n=11) and women (n=14). Results expressed as mean ± SEM. No significant effects of exercise, preload, gender or interactions were observed in the % of 24h cumulative energy provided by each macronutrient.

### 3.4.6 Energy and macronutrient intake at the buffet lunch

No significant differences were observed in energy or macronutrient intake on the 24 hours preceding each of the four appetite challenge days, in all participants, men or women.

No order effects (of preloading) were found on buffet EI (kcal) either at baseline (buffet EI after the HEP: 768±345 vs 693±245, P>0.05; buffet EI after the LEP: 838±301 vs 896±296, P>0.05 in those that started first with the HEP and the LEP, respectively) or end of the study (buffet EI after the HEP: 677±293 vs 633±293, P>0.05; buffet EI after the LEP: 850±288 vs 856±285, P>0.05 in those that started first with the HEP and the LEP, respectively).
Buffet lunch EI following each preload, before and after the exercise intervention, is shown in Figure 3.4. Analysis of variance showed a significant effect of preload on buffet EI (kcal), with buffet EI after the LEP being significantly higher compared with after the HEP (858.4±284.2 vs 695.1±294.3 kcal, P<0.0001). No significant main effects of exercise or gender or interactions were observed on buffet EI. Paired t-tests comparing buffet EI following preload consumption (assuming a α level<0.01 to correct for multiple comparisons) found no significant differences in buffet EI (kcal) after the LEP and HEP at baseline either in all participants (864±294 vs 735±302, P=0.015), men (1031±269 vs 920±307, P=0.280) or women (733±248 vs 589±208 P=0.012). However, after the 6-week exercise intervention buffet EI after the HEP was significantly lower compared with after the LEP in all participants (656±288 vs 853±280, P<0.0001) and men (1016±275 vs 759±257, P=0.001), but not in women (724.2±216 vs 573±293, P=0.038).

Mean energy compensation (compensation index), at the buffet meal, increased in all groups with the exercise intervention (all participants - baseline: 35.8±68.7% vs end: 54.6±60.7%, P=0.264; men - baseline: 31±89% vs end: 71±49%, P=0.225 and
women - baseline: 40±51% vs end: 42±68%, P=0.908), however, the differences were not statistically significant.

No significant main effects of exercise, preload or gender were observed in the percentage of energy provided by each macronutrient at the buffet lunch, with the exception of protein that had a significantly lower contribution (as a %) to buffet EI at baseline compared with after the exercise intervention (13.1±2.6% vs 13.9±3.2%, P=0.025 for all participants) (Figure 3.5). A significant preload x gender interaction was observed in the % of energy at the buffet-meal provided by protein (P=0.026), fat (P=0.005) and CHO (P=0.011). When each gender was analysed separately, no main effect of preload, exercise or interaction was found in women. Men, on the other hand, ate proportionally more fat and less CHO after the LEP compared with after the HEP (fat - HEP: 31.0±6.1% vs LEP: 33.0±6.2%, P=0.009 and CHO - HEP: 54.8±5.1% vs LEP: 53.2±5.0%, P=0.009) and more protein after the exercise intervention (baseline: 14.1±2.6 vs end: 15.0±2.6, P=0.03).

Figure 3.5. Percentage of energy provided by each macronutrient at the buffet lunch after the HEP and LEP, at baseline and end of the study, in all participants (n=25), men (n=11) and women (n=14). Results expressed as mean ± SEM. No significant main effects of exercise, preload or gender were observed in the % of energy provided by fat and CHO at the buffet lunch. A significant effect of exercise (P=0.025) was found in the % of energy provided by protein and a significant preload x gender interaction in the % of energy provided by protein (P=0.026), fat (P=0.005) and CHO (P=0.011).
3.4.7 Short-term energy compensation

Seven men and eight women improved in short-term energy compensation, at the buffet meal, with the exercise intervention (improvement was defined as a reduction in the absolute distance to perfect compensation (100%)), while four men and six women got worse. One outlier (male) was found with an improvement of 243 units (3.3 SDs away from the mean) and was, therefore, excluded from the analysis. When those who improved in short-term energy compensation were compared with those who did not improve in relation to anthropometry, fitness levels, number of steps per day (derived from pedometer readings), eating behaviour, exercise compliance and metabolic profile, only their change in fat mass was significantly different (P=0.016). Those who improved in energy compensation, lost on average, 0.73±1.25 kg of fat mass, while those who did not improve gained, on average, 0.31±0.67 kg of fat mass. Moreover, when the analysis was performed in men, the number of steps per day before the start of the study and the duration of exercise (min/week) were found to be significantly different between those who improved and did not improve in energy compensation. Men who improved in their ability to compensate reported a significantly higher number of steps/day before the start of the study (10628±3515 vs 5681±1356, P<0.05) and a higher duration of exercise (min/week) (175±19 vs 130±25 minutes/week, P<0.05), during the six-week exercise programme, compared with those who did not improve. Finally, women who improved in energy compensation, at the buffet meal, with the exercise intervention, had a significantly lower BMI and body weight, both at baseline and end of the study (21.0±1.5 vs 23.7±2.1 kg/m², P<0.05; 58.5±4.7 vs 64.6±5.9 kg, P<0.05; 21.0±1.3 vs 24.0±2.2, P<0.01 and 58.6±4.4 vs 65.2±6.5 kg, P<0.05, respectively), and a higher fat mass loss (-0.93±1.06 vs 0.21±0.87, P<0.05) compared with those who did not improve.
3.4.8 Changes in subjective ratings of hunger/fullness and preload palatability

The AUC for hunger and fullness, from immediately before until 60 minutes after preload intake, is shown in Figure 3.6 and Figure 3.7. Analysis of variance showed no significant effects of preload, exercise, gender or interactions on AUC for hunger. When each gender was analysed separately, no significant effect of preload, exercise or interaction were observed in women, but in men a preload x exercise interaction was observed (P=0.026) (subjective ratings of hunger/fullness over time, after the HEP and LEP, at baseline and end of the study in all participants, men and women can be seen in Appendix XIV).

Figure 3.6. AUC (0-60 minutes) for hunger after the HEP and LEP, at baseline and end of the study, in all participants (n=25), men (n=11) and women (n=14). Values represent means ± SEM. Analysis of variance in all participants showed no significant effect of exercise, preload, gender or interactions.

A similar pattern to that described for subjective hunger was observed with motivation to eat (ie response to the question “How much do you think you can eat”). A significant effect of gender was observed on AUC for motivation to eat, with men showing higher values compared with women (448±112 vs 368±129 cm/min, P=0.038), but no effect of preload, exercise or interactions were found. When each gender was
analysed separately, a preload x exercise interaction was observed in men (P=0.028): motivation to eat was higher after the LEP compared with the HEP at baseline, while paradoxically, after the exercise intervention the opposite was observed.

A significant effect of preload was observed in the AUC for fullness, with higher scores after the HEP compared with after the LEP (378±133 vs 331±110 cm/min, P=0.008). An exercise x preload interaction (P=0.046) was also observed on the AUC for fullness, but no main effects of exercise or gender or other interactions were found. When each gender was analysed separately, no significant effect of preload, exercise or interaction were observed in women, while in men a significant effect of preload was found (P=0.038).

Figure 3.7. AUC (0-60 minutes) for fullness after the HEP and LEP, at baseline and end of the study, in all participants (n=25), men (n=11) and women (n=14). Values represent means ± SEM. Analysis of variance showed a main effect of preload (P=0.008) and an exercise x preload interaction (P=0.046).
ANOVA revealed no significant effects of preload, exercise, gender or interactions on hunger ratings immediately before the buffet meal, but a significant effect of exercise on fulness ratings, which increased with the exercise intervention (P=0.002). Multiple paired samples t-tests showed no significant differences in hunger scores immediately before the buffet meal either between preloads (HEP vs LEP) or within preloads overtime (baseline vs end). The same happened for fulness scores, except at baseline where fulness ratings were found to be significantly higher after the HEP compared with after the LEP (Table 3.4).

Table 3.4. Hunger and fulness ratings immediately before the buffet test-meal following preload consumption, at baseline and after the exercise intervention in all participants (n=24)

<table>
<thead>
<tr>
<th></th>
<th>HEP</th>
<th>LEP</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger rating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.4±2.2</td>
<td>6.3±2.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>End</td>
<td>6.1±2.1</td>
<td>6.1±2.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Fulness rating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.9±2.0</td>
<td>3.4±2.0</td>
<td>0.001</td>
</tr>
<tr>
<td>End</td>
<td>4.9±2.4</td>
<td>4.0±2.1</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values represent mean±SD. Significance was assumed at P<0.01 to correct for multiple comparisons.

Moderate to large positive correlations, statistically significant, were found in all participants between hunger scores immediately before the buffet lunch and buffet EI after the HEP both at baseline and end and after the LEP at the end of the study (r=0.666, n=25, P<0.0001; r=0.557, n=25, P=0.005 and r=0.441, n=25, P=0.031, respectively). Moderate to large negative correlations, also statistically significant, were found between fulness scores before buffet and buffets' EI after the HEP at baseline and after the LEP at the end of the study, in all participants (r=-0.526, n=25, P=0.007 and r=-0.428, n=25, P=0.037).

No significant effect of preload, exercise, gender or interactions were observed on preload palatability.
3.4.9 Food preference lists

The AUC for the total amount of energy (kcal) derived from the food preference lists, from immediately before until 60 minutes after preload intake, is shown in Figure 3.8. Analysis of variance showed a significant main effect of preload (P=0.019) and gender (P=0.012), but no effect of exercise or any interactions. The energy derived from the food preference lists was significantly higher after the LEP compared with after the HEP (86956±67264 vs 73366±62216 kcal/min, P=0.019) and significantly higher in men compared with women (113079±77553 vs 54297±36236 kcal/min, P=0.012). When each gender was analysed separately, a significant effect of preload was observed only in women (HEP: 46552±29447 vs LEP: 62042±41028 kcal/min, P=0.014) (total energy (kcal) over time derived from the food preference lists, after the HEP and LEP, at baseline and end of the study in all participants, men and women can be seen in Appendix XV).

Figure 3.8. AUC (0-60 minutes) for total amount of energy (kcal) derived from food preference list, at baseline and end of the study, in all participants (n=25), men (n=11) and women (n=14). Values represent means ± SEM. Analysis of variance showed a main effect of preload (P=0.019) and gender (P=0.012).
Moderate to large positive correlations, which were statistically significant, were found in all participants between the total amount of energy (kcal) selected from the food preference list immediately before the buffet meal and buffet EI after the HEP at baseline and after the LEP at baseline and end of the study ($r=0.520$, $n=24$, $P=0.009$; $r=0.549$, $n=23$, $P=0.007$ and $r=0.782$, $n=19$, $P<0.0001$, respectively).
3.4.10 Satiety and hunger quotients

No significant main effect of preload, exercise, gender or interactions were observed in the satiety quotient. Hunger quotient in relation to buffet lunch following each preload, before and after the exercise intervention, is shown in Figure 3.9. Analysis of variance showed a significant effect of preload on hunger quotient, with higher values after HEP compared with after the LEP (0.008±0.0039 vs 0.0065±0.0035 cm/kcal, P=0.039) and a significant effect of exercise with higher values after the exercise intervention compared with baseline (0.008±0.004 vs 0.0066±0.0034 cm/kcal, P=0.040). Paired t-tests (assuming a α level<0.01 to correct for multiple comparisons) found no significant differences in hunger quotient after the LEP and HEP at baseline (0.0066±0.0038 vs 0.0066±0.0030 cm/kcal, P=0.993). However, after the six-week exercise intervention hunger quotient was significantly higher after the HEP compared with after the LEP (0.0093±0.0034 vs 0.0065±0.0033 cm/kcal, P=0.009). While hunger quotient after the LEP did not change with the exercise intervention (0.0066±0.0038 vs 0.0065±0.0033 cm/kcal, P=0.974), it increased significantly after the HEP (0.0066±0.0030 vs 0.0095±0.0044 cm/kcal, P=0.005).

Figure 3.9. Hunger quotient in relation to buffet meal after the HEP and LEP, at baseline and end of the study, in all participants (n=25), men (n=11) and women (n=14). Values represent means ± SEM. ANOVA showed a main effect of preload (P=0.039) and exercise (P=0.040). Columns sharing the same symbol denote significant differences between conditions: ** P<0.01 (assuming a α level<0.01).
3.4.11 Mood states

Total mood disturbance index derived from the POMS in all participants, men and women throughout the study period can be seen in Figure 3.10. Analysis of variance showed, in all participants, a significant effect of time ($P=0.001$) and gender ($P=0.022$), but no time x gender interaction on total mood disturbance index. This index increased from the baseline to the end of week 1 and decreased throughout until the end of the study and was higher in men compared with women. When each gender was analysed separately a significant effect of time on total mood disturbance index was observed in women, but not in men, with scores following a similar pattern to the one described for all participants. Similar results were found for total negative mood score (excluding vigour) which decreased throughout the study period.

![Figure 3.10](image)

Figure 3.10. Total mood disturbance index in all participants ($n=25$), men ($n=11$) and women ($n=14$) throughout the study period. Values represent means ± SEM. Analysis of variance showed a significant effect of time ($P=0.001$) and gender ($P=0.022$), but no time x gender interaction.
3.5 Discussion

The main purpose of this study was to examine, using a longitudinal design, the effect of a six-week exercise programme on short-term appetite control in sedentary individuals. The present study supports the cross-sectional evidence previously reported by Long and colleagues (2002), and more recently by van Wallegheen and collaborators (2007), describing a more accurate energy compensation in active versus sedentary individuals, and shows, for the first time, that exercise improves short-term appetite regulation by leading to a more sensitive eating behaviour in response to previous EI.

A significant improvement in the compensatory response to preload energy manipulation, for the 24 hour period following preload intake, was observed with the exercise intervention, as evidenced by the significant exercise x preload interaction. While at baseline participants did not distinguish between the two preloads and had a similar cumulative EI over the next 24 hours, after the six-week exercise intervention they significantly down-regulated cumulative EI after the HEP compared with after the LEP. All macronutrients seem to have contributed to this improvement in compensation over a 24-hour period, since no preload x exercise interaction was observed in the percentage of 24 hours cumulative EI provided by each macronutrient. Moreover, an almost significant ($P=0.056$) improvement in energy compensation (expressed as a % of compensation over the 24h period), from an average of 8.7% to 79.5%, was observed with the exercise programme.

This improvement in compensation during the first 24-hours following the preload intake, was not, however, reflected acutely at the buffet meal. Analysis of variance did not reveal an exercise x preload interaction on buffet EI. However, secondary analysis showed that while buffet EI after each preload was very similar at baseline, whether the analysis was performed in all participants, males or females separately, after the exercise intervention buffet EI after the HEP was significantly lower compared with after the LEP in all participants and males, but not in females. There is evidence in the literature supporting a gender difference in the response to exercise. Studies that have assessed the effects of acute exercise on subsequent EI, have highlighted a gender effect, at least, in normal-weight volunteers, with women up-regulating their EI in response to exercise, in contrast to men (Imbeault et al., 1997; Thompson et al., 1988; Pomerleau et al., 2004). The effect of chronic exercise on body
weight is also modulated by gender, with men losing more weight than women (Garrow and Summerbell, 1995; Donnelly et al., 2003; Saris et al., 2003), probably due to differences in energy compensation (upregulation of habitual EI in order to match the increased EE) (Westerterp et al., 1992; Tremblay et al., 1984; Woo and Pi-Sunyer, 1985; Tremblay et al., 1984). Although our findings are consistent with a gender difference in the response to exercise, with men showing a better acute compensatory response (at the buffet meal) to preload energy manipulation, an exercise x preload x gender interaction was not found, excluding the existence of a significant effect of gender on energy compensation at the buffet lunch.

The trend towards an improvement in acute appetite regulation in response to preloading (from secondary analysis) was not, however, paralleled by any significant improvement in the compensation index (energy compensation expressed as a %). However, this study was not powered to examine changes in the compensation index. It is probable that the large inter-individual variation in energy compensation observed in this study, a finding that has been observed by others (Johnson and Birch, 1994; Cecil et al., 2005), had contributed to the absence of a significant improvement in the compensation index (at the buffet meal) with the exercise intervention. This large inter-individual variation clearly underlines the complexity of the compensation mechanism and the involvement of factors other than exercise in short-term appetite control.

It is important to compare the present results with the cross-sectional data reported in Long’s study since both followed a similar protocol. Baseline energy compensation, at the buffet test-meal, was in this study of 31% in males, a value much higher than the 7% reported in inactive men in Long and colleagues’ study (2002). In Long’s study, participants were classified as active or inactive based on an exercise history of the previous three months. A similar approach was used to recruit participants in the present study. Fitness levels at baseline were, in the majority of the participants of this study, below the 30th percentile, which together with average baseline pedometer readings below 10000 steps per day, the accepted cut point for an active lifestyle (Tudor-Locke & Bassett, Jr. 2004), confirms their sedentary lifestyle. However, other factors may help to explain these differences between the two studies, namely eating behaviour and body weight, both previously shown to be key determinants in the compensation for previous EI (Rolls et al., 1994; Almeras et al., 1995; Spiegel et al.,

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1989; Ruderman, 1986). Although the restrained score, derived from the DEBQ, and BMI were very similar in this and Long’s male inactive group (2.0±0.8 vs 1.9±0.5 and 23.4±2.4 vs 24.3±3.0kg/m², respectively), the slightly higher BMI of Long’s male inactive group may help to explain their lower baseline compensation. The slightly better baseline energy compensation observed in this study in sedentary women, when compared with inactive men (average values of 40 and 31%, respectively), is unlikely to be explained by differences in the levels of restrained eating behaviour, very similar in both genders, but may be derived, at least partially, from their lower BMI.

After the six-week exercise programme, males presented an average acute energy compensation, at the buffet-meal, of 71%, a figure that is lower than the 90% compensation reported in Long’s male active group (Long et al., 2002). As previously explained, in Long’s study, participants’ activity levels were based on an exercise history of the previous three months. In the present study, the exercise programme was of only six weeks duration, what means that at the end of the study, participants would still be classified as inactive according to Long’s criteria (Long et al., 2002). Therefore, a longer intervention would probably be needed in order to attain that level of energy compensation. This is supported by the findings that those men who improved in energy compensation, acutely at the buffet meal, were the more active ones before the start of the intervention (based on pedometer readings) and those who exercised for longer periods during the six-week exercise programme. This, again, reinforces the beneficial role of PA on short-term appetite control.

A very recent investigation (van Wallegheghen et al., 2007), described, in a similar way to Long’s study (2002), a more accurate energy compensation in active versus sedentary individuals, but over the course of a day instead of acutely, at a test-meal. Van Wallegheghen and collaborators (2007) measured energy compensation, in normal-weight unrestrained young and older adults (both genders), using a non-preload versus preload condition and measuring EI 30 minutes later at a buffet meal and for the rest of the day. They reported a significantly better energy compensation (%), over the course of the day, in active compared with sedentary individuals, independently of the age group. Although the present investigation was not powered to look at differences in energy compensation (%), as previously discussed, the findings reported here expand and confirm the cross-sectional data by van Wallegheghen and colleagues (2007), by
showing that a six-week exercise intervention improves the sensitivity of compensation in response to previous EI, over a 24h period, in previously sedentary volunteers.

According to Blundell and collaborators (2003), the effects of exercise on appetite control can be explained by its impact on macronutrient preferences and food choices, in the hedonic response (pleasure) to food or, alternatively, in the sensitivity of the satiety cascade system. No significant changes in macronutrient selection were observed with exercise, when the three-day food diaries were analysed, which is consistent with the majority of the literature (Tremblay and Drapeau, 1999; Westerterp et al., 1992; Donnelly et al., 2003). Moreover, no significant changes in macronutrient selection, at the buffet test-meals, during the appetite challenge days, or on the following 24 hours were observed with exercise, with the exception of the % of energy provided by protein, at the buffet meal, which increased with the exercise intervention in men. Estimation of the hedonic response to food, on the other hand, was beyond the scope of this study.

The mechanisms targeted by exercise that are likely to explain its role in appetite regulation probably fall into three categories: long-term, including leptin and insulin; intermediate, including post-absorptive signals associated with macronutrient oxidation such as glucose and free fatty acids levels and, finally, short-term mechanisms involving post-ingestive, but pre-absorptive, signals arising from the GI tract in response to food intake (Blundell, 1991). GI hormones involved in short-term appetite regulation, such as CCK, GLP-1 and PYY are potential candidates (de Graaf et al., 2004). No significant changes in fasting insulin, glucose or NEFA levels, or in insulin sensitivity, were found with the exercise intervention. This may result from the short duration of the study and/or from the fact that it was performed in normal-weight subjects with a normal glucose metabolism. Short-term post-ingestive satiety signals released by the GI tract are, therefore, likely to be involved in the improvement in short-term appetite control observed in this study. Unfortunately, the effect of long-term exercise on the plasma levels of GI hormones involved in appetite control (Murphy and Bloom, 2004; Batterham and Bloom, 2003), remain largely unknown. Two isolated studies have shown that chronic exercise has no effect on fasting CCK levels in active men (Bailey et al., 2001) but slightly increases fasting and postprandial peak of PP plasma levels in previously sedentary males (Hurley et al., 1991). Although some of
these peptides, such as CCK, may remain elevated for three to five hours after a large meal, most start to fall after the first 2 to 3 hours (de Graaf et al., 2004). Changes in the plasma levels of these gut peptides could therefore explain the trend towards the improvement in acute compensation at the buffet meal, presented 60 minutes after preload consumption, but are unlikely to account for the significant improvement in energy compensation over 24 hours.

Interestingly, although this six-week exercise intervention did not result in a significant change in body weight or body composition, those who improved in short-term energy compensation, at the buffet meal, lost fat mass (0.73±1.25 kg), while those who did not improve gained fat (0.31±0.67 kg), a difference that was statistically significant. Even though this difference is relatively small and based on a technique - BIA that is not particularly robust in detecting changes in body composition (Forbes et al., 1992), the above findings may suggest that adiposity signals are involved in the improved appetite regulation, observed in some individuals in response to exercise. In this context, it is possible that changes in the plasma levels of interleukin-6 and adiponectin, putative appetite-related adipokines (Trayhurn and Bing, 2006) may help to explain the increase in the sensitivity of compensation in response to previous EI observed in some participants with the exercise intervention. Adiponectin is, unlike most adipokines, inversely related to adiposity and there is evidence suggesting that adiponectin may act centrally in the control of appetite and EB (Trayhurn and Bing, 2006) and that chronic exercise that results in improved fitness levels and reduced body weight may increase resting adiponectin levels (Kraemer and Castracane, 2007).

An increase in the sensitivity to hunger or satiety signals does not seem to have been involved in the improvement in short-term appetite regulation with the exercise intervention. The down-regulation in buffet EI after the HEP with the exercise programme did not result from changes in hunger or fulness ratings (either AUC from before preload till before lunch or ratings immediately before the buffet test-meal). This is in line with cross-sectional findings reporting no differences in the ratings of hunger and fulness, following preload consumption, between physically active and sedentary individuals (van Wallegheen et al., 2007; Long et al., 2002). Interestingly, the hunger quotient in relation to the buffet lunch after the HEP increased significantly with the exercise intervention, paralleling the down-regulation in buffet EI. This increase in the
hunger quotient resulted from a decrease in buffet EI paralleled by a bigger suppression of hunger in response to buffet consumption.

Rather unexpectedly, hunger and fulness ratings and the total amount of energy derived from the food preference list, immediately before the buffet test-meal was presented, were not always correlated with buffet EI, suggesting an uncoupling between subjective feelings of appetite and food intake. However, the association between subjective appetite and food intake has been shown not to be linear (Hill et al., 1987; Mattes, 1990; Flint et al., 2000; Stubbs et al., 2000), and hunger and fulness ratings not always to predict EI (Blundell and Rogers, 1980; Hill et al., 1995b).

Compliance with the exercise programme was very good, with average values greater than the prescribed 30 to 45 minutes of aerobic exercise, at least 4 times per week and at an intensity between 65% and 75% of MHR. However, measurement of compliance is probably the major limitation of this study, since it is largely based on self-reporting. A significant 10% improvement in estimated VO₂ was observed overall with the six-week exercise intervention in this study. This is in accordance with other studies in sedentary normal-weight men and women, where a 5.5% and 7.3-9.5% improvement in VO₂ max was observed after a six- and 24-week supervised, or partially supervised, moderate exercise program (Nishida et al., 2001; Mayer-Davis et al., 1998; Asikainen et al., 2002). Although the prediction of VO₂ max, based on sub-maximal exercise tests, has considerable limitations, a decrease in HR for a fixed workload overtime is likely to reflect an improvement in fitness levels (American College of Sports Medicine 2000). Despite the good compliance with the prescribed six-week exercise programme, an increase in total EE presupposes that normal activity throughout the rest of the day remains unchanged or increases (Wilmore, 1996), a question that remains controversial (Meijer et al., 1991; Goran and Poehlman, 1992). However, it was shown here that normal activity throughout the day, as assessed by pedometers, remained unchanged over time, strongly suggesting a genuine increase in total EE with the exercise programme.

In the present investigation, the six-week exercise intervention was not followed by changes in either body weight or body composition. This is rather unexpected given the increase in total EE, since no compensatory increase in estimated habitual EI (based on the three-day food diaries) was observed with the exercise programme. However, a
closer analysis of the data using the Goldberg cut-offs (Goldberg et al., 1991; Black, 2000) revealed a moderate to severe degree of under-reporting of habitual EI, derived from the three-day food diaries, at the end of the study, with 46% of men and 29% of women being classified as under-reporters, but not at baseline. These findings may explain the absence of a difference in estimated habitual EI between baseline and the end of the study and suggest that a higher EI, derived from the three-day food diaries, should be expected at the end of the exercise intervention. This compensatory increase in EI with exercise would explain the lack of changes in body weight and/or body composition in response to the exercise intervention. Although it can be argued that three-day food diaries are not sensitive enough to pick out small changes in EI and that longer periods of time are needed to provide a more accurate estimation of habitual EI (Nelson et al., 1989), the finding of significant underestimation of EI after the six-week exercise intervention, but not at baseline, suggests misreporting, likely due to “good response” beliefs (Stubbs et al., 1998), since participants were asked not to change their normal diet throughout the study. Even though underestimation of habitual EI by a fraction of the participants is unavoidable in all methods of dietary assessment, estimation of habitual EI and the measurement of compliance with the exercise intervention are likely to be the two main limitations of this study.

Although the impact of exercise on mood states was not under the scope of this study, it was interesting to find that a six-week exercise programme in previously sedentary individuals improved mood states. Sakuragi & Sugiyama (2006) reported similar results, also using the POMS, with a reduction in negative mood scores after a four-week walking programme (Sakuragi and Sugiyama, 2006). Overall, exercise has been shown to have a significant anti-depressive, anti-anxiety and mood enhancing effect (Fox, 1999; Byrne and Byrne, 1991).

In conclusion, this is the first study showing that increasing habitual PA levels, in previously sedentary individuals, improves short-term appetite regulation. PA may, therefore, not only increase EE, but also lead to a more sensitive eating behaviour in response to previous EI. These findings have important implications in terms of the steady increase in the prevalence of obesity in the UK and the current failure to meet the Department of Health PA recommendations (Department of Health, 2004), which were very similar to the ones used in this research. Further studies are needed to elucidate the
optimal intensity and duration of exercise necessary and the mechanisms whereby exercise improves short-term appetite control.
Chapter Four
Chapter 4. Effects of acute exercise on gut peptides, food intake and appetite

4.1 Introduction

Obesity has become a worldwide epidemic (World Health Organization, 2006) and the UK is no exception, with an almost threefold increase in obesity prevalence in the last two decades and present numbers indicating that over 60% of the population are overweight (HSE, 2005). This picture is undeniably linked to a decrease in PA over the past few decades, driven by dramatic changes in lifestyle (Varo et al., 2003; World Health Organization, 2003).

Although the role of PA in preventing weight gain is widely accepted (Martinez-Gonzalez et al., 1999; Haapanen et al., 1997), its impact on weight-loss in the absence of energy restriction seems to be only modest (Miller et al., 1997). The ability of exercise in creating a negative EB relies directly on its impact on EE, but also indirectly on its potential to modulate EI (King et al., 1997b). It has been suggested that the relative inefficacy of exercise on weight-loss, may originate from the energy deficit created by exercise being partially compensated by an increase in EI (Blundell and King, 1999).

The effects of acute exercise on appetite sensations and subsequent food intake remain controversial (Blundell and King, 1999). Although most of the studies show no impact of acute exercise on appetite (Hubert et al., 1998; Imbeault et al., 1997; King et al., 1996; Lluch et al., 1998) or subsequent EI (King and Blundell, 1995; King et al., 1996; King et al., 1997a; Lluch et al., 1998; Westerterp-Plantenga et al., 1997; Blundell and King, 1999; Thompson et al., 1988; Hubert et al., 1998; Imbeault et al., 1997), some have shown a suppression (King et al., 1994; King and Blundell, 1995; Tsofliou et al., 2003) or even an increase in appetite sensations (Maraki et al., 2005), in addition to both an increase (Verger et al., 1992; Pomerleau et al., 2004) or decrease in EI (Westerterp-Plantenga et al., 1997). This lack of consistency is probably due to differences in methodology, namely the intensity of exercise (Thompson et al., 1988), nutritional state (Durrant et al., 1982), gender (Imbeault et al., 1997), macronutrient composition of the test-meal (Tremblay et al., 1994; King and Blundell, 1995) and the time lag between exercise and eating (Verger et al., 1992).
Satiety, the inhibition of eating at a subsequent meal, reflects a complex cascade of physiological responses, where different signals operate at different times: long-term signals including leptin and insulin; intermediate signals including post-absorptive signals associated with macronutrient oxidation and, finally short-term post-ingestive signals generated by the GI tract in response to feeding (Blundell, 1991; Blundell and Halford, 1994). Whilst fasting increases the production of the orexigenic hormone ghrelin (Ariyasu et al., 2001), nutrients in the GI tract stimulate the production and release of satiety hormones such as CCK, GLP-1, PYY and PP (de Graaf et al., 2004; Cummings et al., 2001; Kissileff et al., 1981). The response to these short-term signals is modulated by long-term regulators such as leptin and insulin (Blundell and Naslund, 1999; Blundell and Halford, 1994; de Graaf et al., 2004).

How these appetite-related hormones respond to exercise, a major determinant in the EB equation, is therefore, of extreme importance. However, very little research has been done in this area. Ghrelin appears to be resistant to both acute (Schmidt et al., 2004; Dall et al., 2002; Kraemer et al., 2004; Zoladz et al., 2005) and chronic exercise in the absence of weight loss (Foster-Schubert et al., 2005; Kraemer and Castracane, 2007), however, only one of these studies had a control condition (Kraemer et al., 2004) and on that study, exercise was performed in the fasted state. The available evidence suggests that acute exercise increases fasting plasma levels of CCK (Bailey et al., 2001) and GLP-1 (O'Connor et al., 1995; O'Connor et al., 2006), although the last two studies were performed in athletes. Acute exercise has also been shown to increase plasma PP levels, not only in fasting (Sullivan et al., 1984; Hilsted et al., 1980; O'Connor et al., 1995) but also postprandially (Greenberg et al., 1986), despite being dependent on the intensity of exercise (Holmqvist et al., 1986). Chronic exercise, on the other hand, has been shown not to increase fasting CCK levels in active men (Bailey et al., 2001) and to induce a slight increase in fasting PP plasma levels, as well as on its postprandial peak (Hurley et al., 1991), in previously sedentary males. However, most of these studies looked only at the impact of exercise on the fasting levels of these gut peptides. CCK, GLP-1 and PP are satiety hormones released in the postprandial state and, therefore, changes in fasting levels provide very little information. Moreover, appetite was not the primary outcome in any of the previous studies.
It was shown in the previous chapter that long-term exercise not only increases EE, but also improves appetite control, with a more sensitive eating behaviour in response to previous EI. It was hypothesised that this improvement in short-term appetite regulation might result from changes in the release of post-ingestive satiety signals released by the GI tract. However, to test this hypothesis, another long-term intervention was needed, and this could not be performed due to time constraints. As explained above, research in this area is almost non-existent. Moreover, at the acute level, it remains uncertain if, and to what extent, exercise impacts on postprandial plasma levels of appetite related hormones and whether alterations in the release of these peptides can provide a better understanding of how exercise might influence subjective and objective measures of appetite and net EB.

4.2 Aims

The purpose of this study was, therefore, to investigate the effects of an acute bout of moderate intensity exercise, when performed in the fed-state, on the plasma levels of appetite related hormones (ghrelin, PYY, GLP-1, PP, insulin) and metabolites (glucose, NEFA, TAG) in normal weight volunteers and to correlate potential alterations with changes in subjective feelings of hunger/fulness and prospective food intake at a subsequent meal.
4.3 Methods

4.3.1 Sample size calculation

This study was powered to look at the effects of acute exercise, when performed in the fed state, on postprandial plasma ghrelin levels, an area that had not been investigated before. Based on data from Robertson and colleagues (2004), showing a significant suppression in postprandial ghrelin plasma levels in response to a period of overfeeding (Robertson et al., 2004), it was estimated that a total of 11 participants would be needed to detect a 250 pmol/L difference in postprandial ghrelin levels between the exercise and the control condition, with a variation (SD) of 260 pmol/L, at 80% power and a significance level of 0.05.

4.3.2 Participants

Twelve adult (18-60 years old) healthy volunteers (six male, six female), who were not currently dieting to lose weight, were recruited for this study. Their mean age was 25.9±4.6 years and their mean BMI was 22.0±3.2 kg/m². All participants were asked to fill in the DEBQ (van Strien et al., 1986) (Appendix III) and only those scoring <3.5 in restrained and <4 on the other two subscales of the DEBQ were accepted for the study. Average scores were of 2.4±0.8 for restrained, 2.2±0.6 for emotional and 2.7±0.6 for external eating behaviour, respectively. Participants also had to fill in a health screening questionnaire (Physical Activity Readiness Questionnaire) (Appendix VIII), in order to identify and exclude those who would be at risk of possible harm by performing a bout of moderate intensity exercise.

The exclusion criteria included a BMI lower than 18 or higher than 27 kg/m², history of coronary heart disease, type 1 or type 2 diabetes, anaemia, gout, depression or other psychological disorders, eating disorders, drug or alcohol abuse within the last two years, current medication known to affect appetite or induce weight loss and hypertension. These were assessed by a self-certificated medical questionnaire (Appendix VII). Regular smokers (> 10 cigarettes/day) and those with a highly active lifestyle (performing more than 1 hour of moderate to intense exercise per day, on every
day of the week, based on a three-month exercise history) (Appendix VI), were also excluded.

All participants gave written consent before enrolling in the study and were debriefed at the conclusion of the study. The study was approved by the University of Surrey Ethics Committee (EC/2005/35/SBMS).

4.3.3 Study protocol

Postprandial levels of appetite related hormones and metabolites in response to rest and exercise, as well as subjective ratings of hunger/fulness and subsequent EI at a test-meal, were investigated using a randomised crossover design. Subjects acted as their own controls and were assigned to the two experimental conditions (resting and exercise), one week apart, in a counter-balanced order. A scheme of the protocol of the study can be seen in Figure 4.1.

![Figure 4.1. Schematic representation of the protocol of the study](image)

To reduce the inherent variability, for 24h prior to each investigation, subjects were instructed to refrain from moderate to heavy exercise and from consuming alcohol. They were required to complete 24h food records, which were analysed for energy and macronutrient intake using WinDiets Professional (The Robert Gordon University,
Aberdeen, UK). Participants were also provided with a standardised evening meal (pasta ready-meal containing 521 kcal, 22 g of protein, 26 g of fat and 51 g of CHO) and asked to eat its full amount at around 8.00 pm on the day preceding each of the study days.

On each of the two study days, participants arrived at the CIU at approximately 8.00 am, after an overnight fast of at least 12 hours. An intravenous cannula was inserted into an antecubital vein and two fasting blood sample were taken (-30 and 0 minutes). Serial blood samples (20 ml) were then taken at regular intervals (30, 60, 80, 100, 120, 150 and 180 min) for the following three hours.

Participants were given a standard breakfast at time = zero (hot chocolate drink: 500 Kcal, 15% protein, 38% fat and 47% CHO) (Robertson et al., 2003) (for full details see Chapter 2 – section 2.2.2.3), asked to drink it within five minutes and were randomly allocated to a resting or exercise intervention which began at time = 1 hour. During the resting condition participants remained seated, whilst allowed to read/write quietly. During the exercise condition, participants cycled on a cycle ergometer at 65% of their age-estimated MHR for 60 minutes. Participants were provided with water *ad libitum* throughout the study.

A set of VAS for hunger, fullness and prospective food consumption was completed after each blood sample, as well as immediately before and after breakfast, as described previously (Hill et al., 1987; Flint et al., 2000). Instructions regarding the use of VAS are described in Chapter 2 (section 2.2.2.1). After the resting/exercise intervention (time = 120 – 180 min), participants stayed in the CIU, but were free to write/read quietly. An *ad libitum* buffet meal was served one hour after the end of the exercise/resting intervention (t=180 min) and participants, each confined to an individual booth, were instructed to eat until comfortably full. Exactly the same type and amount of food was presented to each participant on both study days. The food items, amounts and macronutrient composition of the buffet meal, as well as the conditions in which the buffet was presented to participants are fully described in Chapter 2 (section 2.2.2.4.1).
4.3.3.1 Exercise protocol

The exercise intervention consisted of intermittent cycling on a cycle ergometer - Cateye Fitness EC-C400 Upright exercise bike (Cateye, Dallas, USA) at 65% of each participant's age-estimated MHR for approximately 60 minutes (2 minutes warming up + 17 minutes exercise + 3 minutes break + 17 minutes exercise + 3 minutes break + 17 minutes exercise + 2 minutes cooling down). HR was measured continuously using a HR monitor (Polar F1 – Polar Electro Oy, Finland). Average heart rate during the 60 minutes of cycling was 129±6bpm, corresponding to 66.4±2.5% of MHR.

The energy expended during the exercise and resting sessions (180 minutes) was calculated using metabolic equivalents (MET), as previously described (Ainsworth et al., 2000).

4.3.3.2 Hormone and metabolites measurement

Blood samples were analysed for TAG, NEFA, glucose, insulin, ghrelin, PYY, GLP-1 and PP. Plasma TAG and NEFA were measured colorimetrically on the Randox Space Alfa WasserMann automated analyser (Randox Co., Antrim, UK) and glucose using an immobilised enzyme biosensor (YSI 2300 Stat Plus Glucose & Lactate Analyzer). Total ghrelin, PYY, GLP-1, PP and insulin were quantified using established RIAs (Adrian et al., 1976; Hampton, 1984; Kreymann et al., 1987; Adrian et al., 1985; Patterson et al., 2005). A full description of the above methods, as well as intra- and inter-assay CVs can be found in Chapter 2 (section 2.2.5). Fasting SI was calculated using the homeostatic model assessment (HOMA model) as (fasting insulin levels (μU/L) x fasting glucose levels (mmol/L)) / 22.5 (Matthews et al., 1985).

Hematocrit (Hct) was measured in duplicate with a microhematocrit centrifuge - Haematospin 1400 (Hawksley, Lancing, UK) and haemoglobin (Hb) was measured using a co-oximeter (Instrumentation Laboratory, Warrington, UK).

4.3.4 Statistical analysis

Differences in the plasma levels of metabolites and hormones and appetite/hunger sensations between the two conditions (control and exercise) were assessed by a two-way repeated measures ANOVA using treatment and time as
independent variables. Relative energy intake (REI) was calculated by subtracting the estimated EE during the exercise and rest sessions (over 180 minutes) from their respective buffet EI. Differences in absolute and relative EI at the buffet lunch, as well as in the percentage of energy provided by each macronutrient, between the two experimental conditions were assessed using paired sample t-tests.

AUC were calculated using the trapezoidal rule. Potential correlations between the metabolites and hormones measured, appetite sensations and EI at the buffet meal, as well as between energy compensation and different variables assessed, were carried out using Pearson’s or Spearman’s correlations as appropriate.

Energy compensation was calculated as the difference in buffet EI between the exercise and the control condition divided by the energy expended during the one hour exercise bout and expressed as a percentage.
4.4 Results

No significant differences between study legs were observed in energy or macronutrient intake on the 24 hours prior to each trial.

4.4.1 Plasma metabolites and hormones

No significant changes in either Hct or Hb were observed between legs (rest vs exercise) or over time (Figure 4.2 and Figure 4.3). Therefore, as there was no evidence for haemoconcentration in response to exercise, all metabolites and hormones measured are expressed in pmol/L of plasma instead of in terms of blood volume.

Figure 4.2. Haemoglobin levels (mg/dl) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed no significant main effects of time, condition or a time x condition interaction.
Figure 4.3. Haematocrit (%) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed no significant main effects of time, condition or a time x condition interaction.
No significant main effects of time or condition were observed in TAG plasma levels, but a significant time x condition interaction (P=0.011) was found (Figure 4.4). Mean TAG plasma levels were significantly elevated during the one hour of exercise (60-120 minutes) compared with rest, despite returning to levels similar to those observed during the rest condition once the exercise had ceased.

Figure 4.4. Plasma TAG concentrations (mmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed no significant main effects of time or condition but a significant time x condition interaction (P=0.011).
Plasma NEFA levels followed a similar pattern to that described for TAG. No significant main effects of time or condition were observed on NEFA plasma levels, but a significant time x condition interaction ($P<0.0001$) was found (Figure 4.5). Mean NEFA levels started to increase 20 minutes after the start of the exercise bout ($t=80$ minutes) and became significantly elevated throughout until the end of the study ($t=180$ minutes) in the exercise compared with the resting condition.

![Figure 4.5](image)

Figure 4.5. Plasma NEFA concentrations (mmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed no significant main effects of time or condition but a significant time x condition interaction ($P<0.0001$).
A significant effect of time was observed in glucose plasma levels (P<0.0001), which increased after breakfast until t=30 minutes and decreased afterwards until the end of the study (t=180 minutes) (Figure 4.6). No significant effect of condition or time x condition interaction was found on glucose plasma levels.

Figure 4.6. Plasma glucose concentrations (mmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time (P<0.0001) but no significant effect of condition or a condition x time interaction.
No significant effect of condition was found in PYY plasma levels, but a significant effect of time (P<0.0001) and a time x condition interaction were observed (P=0.038) (Figure 4.7). PYY plasma levels increased throughout from breakfast intake until the end of the study (t=180 minutes) and were significantly elevated during the exercise bout (60-120 minutes) compared with resting, despite returning to levels similar to those observed during the rest condition once the exercise had ceased.

Figure 4.7. Plasma PYY concentrations (pmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time (P<0.0001) and a significant condition x time interaction (P=0.038).
A significant effect of time (P<0.0001) and condition (P=0.011), and a condition x time interaction (P=0.001), were observed in GLP-1 plasma levels (Figure 4.8). GLP-1 plasma levels increased from breakfast until t=120 minutes and decreased afterwards until the end of the study (t=180 minutes) and were significantly elevated during exercise compared with resting, despite returning to levels similar to those observed during the rest condition once the exercise had ceased.

Figure 4.8. Plasma GLP-1 concentrations (pmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time (P<0.0001), condition (P=0.011) and a condition x time interaction (P=0.001).
A significant effect of time (P<0.0001) and condition (P=0.001), and a condition x time interaction (P<0.0001), were observed in PP plasma levels (Figure 4.9). PP plasma levels increased from breakfast until t=100 minutes and decreased afterwards until the end of the study (t=180 minutes) and were significantly elevated during exercise (60-120 minutes) compared with resting, an increase that was maintained during the post-exercise period.

Figure 4.9. Plasma PP concentrations (pmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time (P<0.0001) and condition (P=0.001) and a condition x time interaction (P<0.0001).
A significant effect of time (P<0.0001), but no significant effect of condition or a time x condition interaction were observed in ghrelin plasma levels (Figure 4.10), which decreased throughout the study period.

Figure 4.10. Plasma ghrelin concentrations (pmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time (P<0.0001), but no effect of condition or a time x condition interaction.
No significant effect of time, condition (P=0.066) or a time x condition interaction (P=0.069) were observed in insulin plasma levels. However, a tendency was observed towards decreased insulin levels in the exercise compared with the control leg, especially during the exercise bout (60-120 minutes) (Figure 4.11).

Figure 4.11. Plasma insulin concentrations (pmol/L) over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed no significant effect of time, and a trend towards a significant effect of condition (P=0.066) and a time x condition interaction (P=0.069).

No significant differences were observed in the plasma levels of any of the metabolites and hormones measured immediately before buffet lunch (t=180 min) between the two conditions, with the exception of NEFA plasma levels that were significantly higher in the exercise compared with the control leg (0.69±0.28 vs 0.40±0.31mmol/L, P=0.001).
4.4.2 Subjective ratings of hunger and fulness

Hunger and fulness scores during the exercise and the control legs are shown in Figure 4.12 and Figure 4.13. Analysis of variance showed a significant effect of time ($P<0.0001$) on hunger scores, which decreased after breakfast and increased thereafter until the end of the study period. No significant effect of condition was observed, but a time x condition interaction ($P=0.004$) was found, with subjective ratings of hunger being significantly decreased during the one hour of exercise (60-120 minutes) compared with rest, despite returning to levels similar to those observed during the rest condition once the exercise had ceased.

Figure 4.12. Subjective ratings of hunger over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time ($P<0.0001$) and a condition x time interaction ($P=0.004$).
A significant effect of time (P<0.0001), but no significant effect of condition or a time x condition interaction were observed in fullness scores. The temporal pattern was the reverse of that described for hunger scores; fullness ratings increased after breakfast and decreased afterwards until the end of the study period.

Figure 4.13. Subjective ratings of fullness over time, after a 500 kcal breakfast, during the exercise and the control trials. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time (P<0.0001), but no effect of condition or a time x condition interaction.

A significant effect of time (P<0.0001), but no significant effect of condition or a time x condition interaction were observed on desire to eat. Ratings for desire to eat followed a similar temporal pattern to that described for hunger scores (data not shown).

No significant differences were observed in hunger, fullness or desire to eat ratings, immediately before the buffet meal (t=180 min) between the exercise and the resting conditions. No significant correlations were observed between hunger, fullness or desire to eat (AUC) and any of the hormones or metabolites measured (AUC), either during the all study period (180 minutes) or during the 60 minutes of exercise/rest intervention (60-120 minutes).

An inverse temporal pattern was observed, during the one hour of exercise/control intervention (60<t<120 min), between hunger and desire to eat scores.
(derived from VAS) and the plasma levels of the satiety hormones measured: PYY, GLP-1 and PP.

### 4.4.3 Energy and macronutrient intake at the test-meal

Absolute energy and macronutrient intake at the buffet lunch and REI during the exercise and control trials can be seen in Table 4.1.

Table 4.1. Absolute energy and macronutrient intake (%) at the buffet lunch and relative energy intake during the exercise and control trials

<table>
<thead>
<tr>
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<th>Exercise</th>
<th>Control</th>
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<tbody>
<tr>
<td>Absolute</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>913±363*</td>
<td>762±252*</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>14±2</td>
<td>15±3</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>36±7</td>
<td>33±6</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>51±6</td>
<td>53±5</td>
</tr>
<tr>
<td>REI (kcal)</td>
<td>421±302*</td>
<td>565±226*</td>
</tr>
<tr>
<td>EE (kcal)</td>
<td>492±92*</td>
<td>197±37*</td>
</tr>
</tbody>
</table>

EE – energy expenditure; REI – relative energy intake. Values are means ± SD for 12 participants. Means sharing the same symbol denote significant differences between trials: * P<0.05.

Absolute EI at the buffet meal was significantly higher in the exercise compared with the control intervention (P=0.04), with no significant differences being observed in the percentage of energy provided by protein, fat or CHO. However, REI was significantly lower during the exercise compared with the control leg (P=0.038).

No significant correlations were observed between EI at the buffet lunch and subjective appetite ratings or the metabolites and hormones measured (plasma levels immediately before the buffet meal (t=180 min) or total AUC) in either the exercise or the control leg. The only exceptions were a significant large positive correlation between EI at the buffet lunch and total AUC for desire to eat in the control leg (r=0.615, n=12, P=0.033) and between EI at the buffet lunch and hunger and desire to eat in the exercise leg (r=0.748, n=12, P=0.005; r=0.728, n=12, P=0.007).
4.4.4 Energy compensation

Five participants (four women and one men) fully compensated for the energy expended during the exercise bout (104, 145, 154, 182 and 208%) and five (four men and one women) partially compensated (15, 18, 29, 29 and 34%). The remaining two (one men and one women) under compensated (-27 and -154%), that meaning that they ate less at the buffet lunch following the exercise condition compared with following the control condition, and that the difference in buffet EI was greater than the energy expended during the exercise period. Those who fully compensated for the energy expended during the exercise bout (energy compensation >100%) and were, therefore, unable to achieve a short-term negative EB were compared with those who compensated partially or under compensated (energy compensation <100%) and were, therefore, able to achieve a short-term negative EB, in relation to anthropometry, eating behaviour, intensity of the exercise bout (as a % of MHR), plasma levels of the different metabolites and hormones measured and subjective feelings of appetite (fasting, immediately before the buffet lunch (t=180min) and AUC).

Those who achieved a short-term negative EB had significantly lower scores for external and emotional eating derived from the DEBQ (2.3±0.2 vs 3.2±0.1, P=0.007 and 1.8±0.1 vs 2.7±0.2, P=0.003, respectively) compared with those who did not achieve this. Moreover, they also had significantly lower insulin levels, both in fasting (control leg: 22.8±4.0 vs 35.0±3.0 pmol/L, P=0.049; exercise leg: 19.5±1.9 vs 28.47±3.2 pmol/L, P=0.030) and immediately before the buffet meal (t=180min) (control leg: 35.6±4.1 vs 54.1±8.3 pmol/L, P=0.033; exercise leg: 22.4±2.9 vs 53.1±8.3 pmol/L, P=0.017), lower insulin AUC during the control trial (15086±1690 vs 32829±7019 pmol/L*min, P=0.016) and lower fasting S1, based on the HOMA model (control leg: 0.60±0.29 vs 1.00±0.24 μU/L*mmol/L, P=0.033; exercise leg 0.50±0.16 vs 0.79±0.22 μU/L*mmol/L, P=0.021).
4.5 Discussion

The main purpose of this study was to investigate whether acute exercise could affect the postprandial levels of appetite-related hormones and metabolites, thereby providing a potential mechanism for changes in appetite sensations and EI in response to exercise. The present investigation showed, for the first time, that an acute bout of moderate intensity exercise, performed in the fed-state, significantly increases the plasma levels of the satiety hormones: PYY, GLP-1 and PP, has no impact on ghrelin (an orexigenic peptide) and leads to a temporary reduction in self-reported hunger.

PYY and GLP-1 are both secreted from endocrine L-cells of the distal ileum and colon in response to feeding (Bottcher et al., 1984) and PP, a member of the PP peptide family, which also includes PYY and NPY, is produced by endocrine cells of the pancreatic islets (Adrian et al., 1976). These satiety hormones inhibit food intake by altering central nervous system appetite circuits, within the ARC of the hypothalamus or area postrema (Batterham and Bloom, 2003; Gutzwiller et al., 1999). However, the inhibitory effect of PP on food consumption seems to be indirectly regulated via vagal nerves in part by decreasing gastric emptying rates (Katsuura et al., 2002).

GLP-1 had already been shown to increase with marathon running (O'Connor et al., 1995), and in response to a two-hour running session (O'Connor et al., 2006). However, both studies were performed in the fasting state, involved high-intensity exercise and were carried out in athletes. An increase in PP plasma levels had also been reported before, both in fasting (Sullivan et al., 1984; Hilsted et al., 1980) and postprandially (Greenberg et al., 1986). Greenberg and colleagues (1986) followed a protocol similar to the one described in this study. Exercise consisted of 45 minutes of moderate intensity cycling on a cycle ergometer at 50% VO2max and was performed 30 minutes after a 425 kcal breakfast (20% protein, 38% fat and 42% CHO), therefore supporting the results of this study. However, this is the first study to address the effects of acute exercise on PYY plasma levels in humans and its potential role on subjective appetite and subsequent EI.

Hunger ratings and the plasma levels of satiety gut peptides showed an inverse temporal pattern during the one hour of exercise/control intervention; while mean levels of PYY, GLP-1 and PP increased (in relation to the control leg), theoretically inducing a
higher satiety effect, hunger scores were suppressed. The phenomenon of “exercise-induced anorexia”, despite traditionally associated with intense exercise (King et al., 1994; King and Blundell, 1995), has also been reported in response to moderate intensity exercise (Tsofliou et al. 2003), which is consistent with our findings. Even though several mechanisms have been proposed to explain the phenomenon of “exercise induced anorexia” including: hyperthermia, already dismissed (Westerterp-Plantenga et al., 1997); increased levels of lactic acid, tumor necrosing factor (King et al., 1997b) and catecholamines (Leon et al., 1979), increased activity of the sympathetic nervous system, with consequent reduction in intestinal tract motility or, alternatively, metabolic changes (Westerterp-Plantenga et al., 1997), the reason for this phenomenon remains unknown. We have demonstrated here that the phenomenon of “exercise induced anorexia” could be due to increased secretion of satiety peptides.

The inverse temporal pattern observed between hunger scores and the plasma levels of the satiety hormones measured does not, however, prove causality. A significant increase in the plasma levels of PYY, GLP-1 and PP, together with a significant suppression in hunger scores (compared with infusion of saline) has been reported in studies where these hormones were infused to normal-weight healthy volunteers (Batterham et al., 2003a; Flint et al., 1998; Degen et al., 2005). However, the satiety effects of these hormones are generally seen only at pharmacological levels, with the exception of GLP-1 where these effects can be observed at the top end of the physiological level. Although comparisons among studies are limited due to differences in RIA protocols, the peak level of PYY, GLP-1 and PP observed during exercise in this study was of 30.1±9.2, 66.1±30.3 and 42.6±29.8 pmol/L, respectively, much lower than the peak level of PYY and PP associated with a significant reduction in hunger in exogenous hormone infusion studies: 87.5 pmol/L and 285.5 pmol/L, respectively (Batterham et al., 2003a; Degen et al., 2005), but similar to the peak level of GLP-1: 60-90 pmol/L (Flint et al. 1998b).

Even though the observed increase in PYY, GLP-1 and PP plasma levels with exercise was probably too small to have significant effects on hunger when considered in isolation, PYY and GLP-1 were already shown to inhibit food intake additively when infused together (Neary et al., 2005) and the anorexic effects observed with PP infusion seem to be independent of changes in PYY, GLP-1 or indeed other gut peptides.
Batterham et al., 2003a). The concomitant increase in the plasma levels of these three satiety peptides yields, therefore, a potential explanation for the phenomenon of "exercise induced anorexia". However, the precise role of these gut peptides in the suppression of hunger in response to acute exercise can only be fully investigated when specific antagonists of these hormones, in humans, become available.

The mechanisms by which acute exercise increases the plasma levels of satiety gut peptides (PYY, GLP-1 and PP) remain unknown. It is possible that metabolic/hormonal changes in response to exercise act as a stimulus for the release of these hormones. It has been suggested that the activation of the sympathetic nervous system (adrenergic) observed with exercise, with the consequent raise in catecholamine levels, may explain the increase in PP plasma levels observed with exercise (Gingerich et al., 1979). The finding that PP release in response to a bout of exercise is significantly lower after chronic training supports this hypothesis, since a greater elevation in catecholamines in response to exercise has been reported in untrained versus trained subjects (Gingerich et al., 1979).

In contrast to the hormones previously discussed, ghrelin is the only peripheral hormone with orexigenic properties, and is likely to be involved in meal initiation (Cummings et al., 2001). The effect of exercise on ghrelin levels was recently reviewed by Kraemer & Castracane (2007) who concluded that acute exercise of moderate to high intensity, does not elicit an increase in fasting ghrelin levels. No significant effect of acute exercise, when performed in the fed state, has also been reported on plasma ghrelin levels both in normal normal-weight (Zoladz et al., 2005) and overweight volunteers (Borer et al., 2005). It is important to emphasize that these two papers were published after we started the study described in this chapter. The finding that one hour of moderate intensity cycling does not impact on the postprandial levels of ghrelin in normal-weight subjects is, therefore, in line with previous research.

Although it was shown in this study that exercise in the fed-state induces compensatory neuroendocrine reflexes needed for the regulation of metabolic fuels (Coyle, 2000), with increases in both NEFA and TAG plasma levels, no similar compensatory responses regarding ghrelin, PYY, GLP-1 and PP, GI hormones involved in appetite regulation, that would defend the body against a negative EB were observed. There is no previous evidence that acute exercise can trigger physiological adaptations
that would lead to an increase in hunger sensations or subsequent EI, with the exception of a study in overweight post-menopausal women, where an increase in pre-meal fasting ghrelin levels was described after two bouts of exercise inducing a 800 kcal energy deficit (Borer et al., 2005). The significant increase in buffet EI observed with exercise in the present study was not explained by differences in hunger sensations or changes in any of the appetite related hormones studied immediately before the buffet meal. This apparent uncoupling between subjective feelings of hunger and food intake has, however, been reported before (Mattes, 1990; Flint et al., 2000). It can be hypothesized that the increase in absolute EI observed in response to acute exercise in this study may be the result of cognitive factors including attitudes and beliefs associated with exercise, such as “food rewards for exercising” and the belief that “exercise increases appetite”, as previously suggested (King, 1999). The absence of significant differences in the plasma levels of PYY, GLP-1 and PP, between the two legs, immediately before the test-meal does not mean, however, that the temporary increase in satiety gut peptides observed during exercise did not modulate EI at lunch time. It is possible that these peptides had already acted within the hypothalamus and exerted their satiety properties, which would help to explain the reduction in REI observed in the exercise compared with the control leg and the attainment of a short-term negative EB.

The significant increase in absolute EI observed in this study in response to one hour of moderate intensity exercise, with all the macronutrient being equally targeted, is not a new finding. Although the majority of the studies report no effect of acute exercise on subsequent EI (King and Blundell, 1995; King et al., 1996; King et al., 1997a; Lluch et al., 1998; Westerterp-Plantenga et al., 1997; Blundell and King, 1999; Thompson et al., 1988; Hubert et al., 1998; Imbeault et al., 1997), an increase in EI has been reported by some (Verger et al., 1992; Pomerleau et al., 2004). However, when the acute effects of exercise on appetite, EI and EB are under investigation, it is extremely important to measure not only absolute EI, but also REI (King et al., 1997b; King et al., 1994). Several studies have failed to show any positive effect of acute exercise on EB simply because they do not account for the energy cost associated with it (King et al., 1997a). In fact, if EE during exercise is taken into account, a significant decrease in REI is usually observed, allowing the attainment of a short-term negative EB (Maraki et al., 2005; Verger et al., 1994). This pattern was reproduced here. Despite a significant increase in absolute EI at the buffet lunch during the exercise leg, once the energy
expended during the one hour of exercise was taken into account a negative EB was achieved, with REI being significantly lower during the exercise compared with the control leg. Even though the use of METs to estimate EE has some limitations, since it does not take into account subjects’ fitness level or gender (two important predictors of EE), similar results were obtained when EE was estimated using an equation (Hiilloskorpi et al., 2003) specific for each gender which incorporates HR, an accurate predictor of EE. However, the estimation of the energy expended during the exercise and control trials, instead of its measurement by indirect calorimetry, is clearly the main limitation of this study.

Although one hour of moderate intensity exercise resulted in an overall short-term negative EB, some participants fully compensated for the energy expended during the exercise bout. It is of extreme importance to identify who can and who cannot benefit from exercise in terms of EB. Those who fully compensated for the extra energy expended during the one hour of exercise were more insulin resistant compared with those who did not fully compensate. This may suggest a link between $S_1$ and compensatory responses (increase in EI) to exercise. However, this study was not powered to look at the impact of $S_1$ on energy compensation in response to exercise and it did not include individuals with real insulin resistance. If this link is proven, that will mean a lower efficacy of exercise in creating a negative EB in insulin resistant individuals. Significant differences in eating behaviour were also observed between compensators and non compensators, with the first scoring higher in external and emotional eating, derived from the DEBQ. This may suggest a disadvantage, in terms of EB in response to exercise, in those with a greater susceptibility to external and emotional cues that regulate food intake.

In conclusion, our findings that acute exercise, performed in the fed-state, significantly increases PYY, GLP-1 and PP plasma levels but has no effect on ghrelin levels, together with the short-lived suppression of hunger and a significant reduction in REI, strengths the ability of exercise in creating a negative EB and reinforces its role in appetite control and weight maintenance. The phenomenon of “exercise induced anorexia” may be potentially linked to the increased PYY, GLP-1 and PP plasma levels observed during exercise although more research is needed in this area.
Chapter Five
Chapter 5. Effect of dietary restraint on postprandial metabolic and hormonal responses

5.1 Introduction

An increased number of the population in Western countries try conscientiously to limit their food intake in order to achieve and/or maintain a desirable body weight. This concept is known as dietary or cognitive restraint and has been defined as a self-imposed resistance to the internal and external cues that regulate eating behaviour (Herman and Mack, 1975). However, over-reliance on cognitive control of eating behaviour increases a person’s vulnerability to uncontrolled eating when these cognitive processes are disrupted (Herman and Polivy, 1980). Excessive restraint can, therefore, have a counterproductive effect and eventually lead to weight gain, with alternate periods of dieting and overindulgence.

An unexpected finding from data generated in the previous chapter (Chapter 4) concerned a possible relationship between PYY plasma levels and restraint. Secondary analysis of the data showed lower fasting PYY plasma levels, at the limit of significance (P=0.05), in moderately restrained individuals, compared with unrestrained eaters (using the middle point of the restraint subscale of the DEBQ (2.5) as the cut-off point) and a trend (P=0.07) towards lower levels of PYY in the postprandial state in the control leg (see Appendix XIV). PYY is a satiety hormone released in response to feeding that inhibits subsequent food intake by modulating the activity of orexigenic and anorexigenic neurons within the hypothalamus (Batterham and Bloom, 2003). Lower PYY plasma levels in restrained eaters, particularly in the postprandial state, would generate less fulness (and greater hunger) following a meal, compared with unrestrained eaters, and therefore, the need to rely on strict cognitive control over eating in order not to gain weight. However, this previous study was not designed to look at the effect of restraint on PYY plasma levels and highly restrained participants (>3.5 in the restraint subscale of the DEBQ) were excluded from the study, therefore, resulting in a relatively narrow range of restraint. PYY plasma levels have been shown to be sensitive to acute and chronic food restriction (Tovar et al., 2004), to be modulated by gender, with higher levels in females (Kim et al., 2005), and also body weight, with
lower fasting and postprandial levels in obese subjects (Batterham et al., 2003b; le Roux et al., 2006), although these last findings have not been demonstrated by all investigators (Kim et al., 2005). These variables are, however, unlikely to explain the differences in PYY plasma levels, between restrained and unrestrained eaters, observed in chapter 4, since both groups had a similar male/female ratio, and no significant differences were found in body weight or energy or macronutrient intake on the 24 hours before the study.

In addition to this unexpected finding, a substantial amount of evidence has been accumulating over the last three decades suggesting a link between restrained eating behaviour and altered physiological pathways, with restrained eating having been shown to impact on metabolic (Laessle et al., 1989b; Hibscher and Herman, 1977), as well as endocrine functions (Keim and Horn, 2004; Pirke et al., 1990; Burton-Freeman, 2005). Dietary restraint seems to play a role in the magnitude of cephalic phase reflexes, with restrained women showing a larger cephalic phase insulin (Teff and Engelman, 1996) and salivary responses compared with unrestrained women, in some (Tepper, 1992), although not all studies (Crystal and Teff, 2006). Increased levels of fasting TAG (Laessle et al., 1989b) and NEFA (Hibscher and Herman, 1977) and lower fasting insulin (Keim and Horn, 2004; Pirke et al., 1990) and leptin levels, even after controlling for fat mass (von Prittwitz et al., 1997; Adami et al., 2002; Laessle et al., 2000) have also been reported in restrained eaters. Moreover, lower total EE (TEE) has been described in restrained individuals (Tuschl et al., 1990) and higher levels of cortisol, indicative of increased psychological stress, probably in association with “eating behaviour”, has also been found in some (Anderson et al., 2002; McLean et al., 2001), but again, not in all studies (Beiseigel and Nickols-Richardson, 2004).

The metabolic and endocrine responses to a meal seem also to be affected by the level of restraint. Restrained women were shown to have reduced diet-induced thermogenesis (Westerterp-Plantenga et al., 1992) and increased CHO oxidation after a mixed meal (Keim and Horn, 2004), as well as reduced secretion of noradrenaline (Pirke et al., 1990). It was proposed that this change in fuel oxidation may be related to an increased insulin sensitivity in restrained individuals (Keim and Horn, 2004). Another study, however, found, that high disinhibition, not high restraint was associated with a lower thermic effect of food (Lawson et al., 1995). Finally, restraint has been
shown to impact on the postprandial release of CCK, a satiety hormone involved in the control of appetite and food intake, with a blunted release, in response to a 40% fat meal, reported in restrained eaters (Burton-Freeman, 2005). However, apart from this investigation, no studies have looked at the impact of restraint on appetite-related hormones.

The previously described pattern of reduced leptin levels, reduced TEE, reduced ability to oxidize fat and reduced levels and/or a blunted release of satiety hormones may put restrained individuals at an increased risk for weight gain and could explain why they need to cognitively restrict their food intake, in order to maintain their body weight (Pirke et al., 1990).

5.2 Aims

The primary aim of this study was to investigate the effect of dietary restraint on fasting and postprandial levels of appetite-related hormones and metabolites, with special emphasis to PYY, in normal-weight volunteers and to correlate potential alterations with changes in subjective feelings of hunger/fulness and prospective food intake at a subsequent meal. Secondary aims were to determine whether restrained eaters need a bigger energy stimulation in order to achieve a similar postprandial response to that observed in unrestrained individuals.

5.3 Methods

5.3.1 Sample size calculation

Based on pilot data described in the introduction (originated from Chapter 4), it was estimated that a total of 38 participants would be needed to detect a 23% reduction in PYY plasma levels, in the postprandial state, in restrained eaters, with a variation (SD) of 25%, at 80% power and at a significance level of 0.05. Because the pilot data was originated from a sample that did not include highly restrained individuals, we considered it likely that the real difference between groups would be even larger, when a wider variation in restraint was studied. For that reason, it was decided to recruit a sample of 32 participants (16 in each group).
5.3.2 Participants

Thirty-three adult (18-60 years old) normal-weight healthy volunteers (twelve men and twenty one women) not currently dieting to lose weight and weight stable (variation of less than three kg on the last two months) were recruited for this study. Their mean age was 28.3±8.8 years and their mean BMI was 21.9±2.0 kg/m². This sample was originated from an initial pool of 133 people (47 male and 86 female) that showed an interest in taking part in the study, following a formal interview to assess their suitability in taking part in the study (see flowchart in 0). The exclusion criteria included a BMI lower than 18 or higher than 25 kg/m², prior or present history of coronary heart disease, type 1 or type 2 diabetes, anaemia, gout, depression or other psychological disorders, eating disorders, drug or alcohol abuse within the last two years and current medication known to affect appetite or induce weight loss. These were assessed by a self-certificate medical questionnaire (Appendix VII). Regular smokers (> 10 cigarettes/day) and elite athletes (club level or above) were also excluded. PA was assessed by the Baecke Questionnaire (Baecke et al., 1982) (Appendix XV). Those with a haematocrit < 35% (measured with a microhematocrit centrifuge - Haematospin 1400 (Hawksley, Lancing, UK)) were also excluded.

All the potential participants were asked to fill in the RRS (Herman and Polivy, 1980) (Appendix I), the TFEQ-18R (Karlsson et al., 2000) (Appendix II) and the DEBQ (van Strien et al., 1986) (Appendix III) and classified as restrained if scoring on the top third of the scales (restraint subscale of the TFEQ-18R≥18 and/or of the DEBQ≥3.7) and unrestrained if scoring on the bottom third of the scales (restraint subscale of the TFEQ-18R≤12 and of the DEBQ≤2.3). Due to difficulties in recruiting restrained males (since men score lower in restraint compared with women), the cut-off point for restraint had to be lowered in men (restraint subscale of the TFEQ-18R≥16 and/or of the DEBQ≥3.1). Those scoring in the middle were not accepted for the study. This resulted in the recruitment of seventeen unrestrained (six men and eleven women) and sixteen restrained participants (six men and ten women).

Participants were unaware of the real purpose of the study and were told that it aimed to investigate the effects of different eating behaviours on mood, appetite sensations and metabolic responses. All participants gave written consent before
enrolling in the study and were debriefed at the conclusion of the study. The study was approved by the University of Surrey Ethics Committee (EC/2006/43/SBMS).

5.3.3 Study protocol

Plasma levels of appetite-related hormones and metabolites, subjective ratings of hunger/fulness and subsequent EI at a test-meal in response to two breakfasts differing in their energy content, were investigated, in restrained and unrestrained eaters, using a randomised crossover design. Participants acted as their own controls and were assigned to the two experimental conditions (500 kcal vs 1000 kcal breakfast), one week apart, in a counter-balanced order. A scheme of the protocol of the study can be seen in Figure 5.1.

![Blood samples](Blood samples)

![Visual Analogue Scales](Visual Analogue Scales)

Rest

-30 min 0 60 min 120 min 180 min

**Cannulation**

**Breakfast**

500 kcal vs 1000 kcal

**Pasta lunch**

Figure 5.1. Schematic representation of the protocol of the study

To reduce interpersonal variability, participants were instructed to refrain from moderate to heavy exercise and from consuming alcohol for the 24 hours prior to each investigation. They were required to complete 24 hour food records, which were analysed for energy and macronutrients using WinDiets Professional (Robert Gordons University, Aberdeen, UK). Participants were also provided with a standardised evening meal (pasta ready-meal containing 521 kcal, 22 g of protein, 26 g of fat and 51 g of CHO) and asked to eat it at around 8.00 pm on the day preceding each of the study days.
On each of the two study days, participants arrived at the CIU at approximately 8.00 am, after an overnight fast of at least 12 hours. An intravenous cannula was inserted into an antecubital vein and two fasting blood sample were taken (-30 and 0 minutes). Participants were then randomly assigned to one of two preload breakfasts with a similar energy density, but double the volume: 250 ml (500 kcal) or 500 ml (1000 kcal) (hot chocolate drink: 15% protein, 38% fat and 47% CHO) (Robertson et al., 2003) and asked to drink it within 5 to 10 minutes (for full details about the preloads see Chapter 2 – section 2.2.2.3). Serial blood samples were then taken postprandially at regular intervals: every 15 minutes on the first hour and every 30 minutes on the second and third hour.

A set of VAS for hunger, fullness and prospective food consumption was completed after each blood sample (except at t=15 and t=45 minutes), as well as immediately before and after breakfast, as previously described (Hill et al., 1987; Flint et al., 2000). Cognitive and emotional states associated with restraint and disinhibition (“How rebellious and defiant about dieting do you feel??”, “Do you feel resigned and like giving up your diet??”, “Do you feel out of control of your eating??”, “Are you preoccupied with thoughts of food??”, “Do you feel an overpowering urge to eat??”, “Do you feel guilty” and “Do you feel preoccupied with thoughts of dieting??”) (Ogden, 1990) were also assessed using VAS and presented before the appetite questions. Instructions regarding the completion of VAS are described in Chapter 2 (section 2.2.2.1).

Participants had to stay in the CIU during the full duration of the study, but were allowed to read/write quietly and were provided with water ad libitum throughout the study. A pasta-based lunch was served at the end (t=180 min) and participants, each confined to an individual booth, were instructed to eat until comfortably full. Exactly the same type and amount of food was presented to each participant on both study days. The ingredients, amounts and macronutrient composition of the pasta meal, as well as the conditions in which lunch was presented to participants have been fully described in Chapter 2 (section 2.2.2.4.2).

Participants were also asked to record in a food diary everything they consumed after the pasta lunch until (and including) breakfast of the following day (post-buffet food diary - PBFD) in order to estimate 24 hour cumulative EI (calculated as pasta meal
All nutrient analysis of the diet diaries was performed using WinDiets Professional (The Robert Gordon University, Aberdeen, UK).

### 5.3.4 Hormone and metabolites measurement

Blood samples were analysed for TAG, glucose, insulin and PYY (all time points for glucose and insulin, all except t=15 and t=45 minutes for TAG and PYY). Plasma TAG and glucose were measured calorimetrically on the ILAB 650 (Instrumentation Laboratory, Milan, Italy), an automated analyser. Insulin was measured using an immunoassay kit (Invitron, Monmouth, UK) and PYY using an established RIA (Adrian et al., 1985). A full description of the above methods, as well as intra- and inter-assay CVs can be found in Chapter 2 (section 2.2.5).

### 5.3.5 Measurement of insulin sensitivity

Fasting insulin sensitivity (SI), as a percentage of a normal reference population (%S), and fasting insulin resistance (IR), as the reciprocal of %S (100/%S), were calculated using the HOMA2 Calculator version 2.2 (University of Oxford, Oxford, UK).

Postprandial SI was assessed using a minimal model index (Caumo et al., 2000). This model provides an estimate of SI following CHO ingestion specific for each individual and following a particular meal. This method utilizes cumulative integrated AUC measures of both insulin and glucose concentration assuming that the total glucose disposal from the system after 240 minutes (or when basal values have been reached) equals the glucose entering the peripheral circulation allowing for first-pass extraction by the liver. Insulin-independent mechanisms also contribute to total glucose disposal and a constant rate of glucose effectiveness (GE) has been assumed for the whole time interval. The following equation was used to calculate postprandial SI:

$$\text{SI (oral)} = \frac{\int X D_{\text{oral}} \frac{\text{AUC} \left( \frac{\Delta g(t)}{g(t)} \right)}{\text{AUC} \left( \Delta g(t) \right)} - \text{GE} \times \text{AUC} \left( \frac{\Delta g(t)}{g(t)} \right)}{\text{AUC} \left( \Delta i(t) \right)} \quad (\text{Caumo et al., 2000})$$
D_{oral} is the dose of ingested CHO/unit of body weight (mg/kg) and f is the fraction of ingested CHO reaching the peripheral circulation as glucose. AUC was calculated from time zero until the end of the test, and GE was fixed at 0.0024 litres/kg x min. Since the preloads provided in this study did not contain any starch and a digestibility of 100% would, therefore, be expected from the CHO used, a nominal value for f of 1 was chosen for all participants.

5.3.6 Statistical analysis

Differences in energy and macronutrient intake in the 24h preceding each study day (500 kcal vs 1000 kcal) were assessed using a paired sample t-test.

The effect of restraint (unrestrained vs restrained), preload (500 kcal vs 1000 kcal) and time on the plasma levels of metabolites and hormones were assessed by a mixed between-within subjects analysis of variance (ANOVA), with preload and time as the within-subject variables and restraint as the between-subjects variable.

The AUC for the plasma levels of metabolites and hormones, appetite and hunger sensations and cognitive and emotional state was calculated using the trapezoidal rule, from before the preload until immediately before the pasta lunch (three-hour period). AUC for TAG and cognitive and emotional variables was not normal distributed and, therefore, a Log10 transformation was applied. The effect of preload, restraint and gender on these variables, as well as on EI at the pasta lunch and cumulative EI over a 24-hour period, was assessed using a mixed between-within subjects ANOVA, with preload as the within-subject variable and restraint and gender as the between-subjects factors.

Postprandial S_{i} in each individual after the 500 kcal and 1000 kcal preloads were considered as replicates and an average value was, therefore, used. Differences in fasting and postprandial S_{i} between restrained and unrestrained eaters were assessed by independent sample t-test. Energy compensation was calculated as the difference in EI between the two study days (500 kcal vs 1000 kcal) divided by the difference in preload energy and expressed as a percentage. This was performed for both EI at the pasta lunch and cumulative EI over a 24-hour period following preload consumption. Potential correlations between the metabolites and hormones measured, appetite sensations and
EI at the pasta meal, as well as between short-term energy compensation, at the pasta lunch, and the different variables assessed, were carried out using Pearson’s or Spearman’s correlations as appropriate.

5.4 Results

5.4.1 Anthropometry, physical activity and eating behaviour

Anthropometry, physical activity levels and eating behaviour in restrained and unrestrained eaters is shown in Table 5.1.

Table 5.1. Anthropometry, physical activity and eating behaviour in restrained and unrestrained eaters, in all participants (n=33), men (n=6) and women (n=21)

<table>
<thead>
<tr>
<th></th>
<th>Restrained (n=16)</th>
<th>Unrestrained (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Male</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.3±9.3</td>
<td>71.6±5.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4±2.0</td>
<td>23.3±2.4</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>82.2±9.0</td>
<td>89.0±6.4</td>
</tr>
<tr>
<td>Hips (cm)</td>
<td>98.7±4.9</td>
<td>102.0±3.0</td>
</tr>
<tr>
<td>Physical activity 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>8.1±0.9</td>
<td>8.1±1.1</td>
</tr>
<tr>
<td>Work</td>
<td>2.1±0.5</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>Leisure</td>
<td>3.0±0.5</td>
<td>3.0±0.6</td>
</tr>
<tr>
<td>Sport</td>
<td>3.0±0.7</td>
<td>3.1±0.8</td>
</tr>
<tr>
<td>RRS</td>
<td>17.3±4.1**</td>
<td>14.7±1.9**</td>
</tr>
<tr>
<td>TFEQ-18R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restrained eating</td>
<td>16.1±2.3**</td>
<td>14.0±1.3**</td>
</tr>
<tr>
<td>Uncontrolled</td>
<td>19.2±4.2</td>
<td>20.5±4.2</td>
</tr>
<tr>
<td>Emotional</td>
<td>6.4±2.1*</td>
<td>5.7±2.3</td>
</tr>
<tr>
<td>DEBQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restrained eating</td>
<td>3.7±0.6**</td>
<td>3.2±0.4**</td>
</tr>
<tr>
<td>External</td>
<td>2.9±0.7</td>
<td>2.8±0.9</td>
</tr>
<tr>
<td>Emotional</td>
<td>2.7±0.9*</td>
<td>2.4±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. Means sharing the same symbol denote significant differences between restrained and unrestrained eaters within each group: ** P<0.0001, * P<0.01.

1) Based on the Baecke questionnaire (Baecke et al., 1982)
No significant differences were observed in anthropometry or any of the PA scores derived from the Baecke questionnaire between restrained and unrestrained eaters in any of the groups. This study was designed so that restrained eaters had significantly higher restraint scores compared with their unrestrained counterparts, what was confirmed: the RRS score and the restrained eating scores derived from the TFEQ-18R and DEBQ were significantly higher in restrained compared with unrestrained eaters, in all participants, men and women (P<0.0001 for all). Emotional eating scores derived from both the TFEQ-18R and DEBQ were also significantly higher in restrained compared with unrestrained eaters, in all participants and women (P<0.01 for both), but not in men. No significant differences were observed in uncontrolled eating, derived from the TFEQ-18R, or external eating, derived from the DEBQ, between restrained and unrestrained individuals in any of the groups.

As expected, men had a significantly higher body weight (P<0.0001) and BMI (P<0.05) compared with women, but no significant differences between genders were observed in PA levels, derived from the Baecke questionnaire, or in any of the parameters related to eating behaviour.

5.4.2 Twenty-four hours food recalls

No significant differences between study legs were observed in energy or macronutrient intake on the 24 hours prior to each trial (500 kcal vs 1000 kcal breakfast) in all participants, men or women or when restrained and unrestrained eaters were analysed separately. No significant differences were observed between restrained and unrestrained in EI on the 24 hours prior to each trial, either in all participants, men or women.
5.4.3 Plasma metabolites and hormones

5.4.3.1 Fasting levels

One outlier was found on fasting insulin plasma levels in the 500 kcal leg, with a value of 171 pmol/L (3.8 SDs away from the mean) and was, therefore, excluded from the analysis. The fasting plasma levels of the metabolites and hormones measured in restrained and unrestrained eaters can be seen in Table 5.2.

Table 5.2. Fasting plasma levels of TAG, glucose, insulin and PYY in the 500 kcal and 1000 kcal leg, in restrained and unrestrained eaters, in all participants (n=33), men (n=6) and women (n=21)

<table>
<thead>
<tr>
<th></th>
<th>Restrainted (n=16)</th>
<th>Unrestrained (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=16)</td>
<td>Male (n=6)</td>
</tr>
<tr>
<td>TAG (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 kcal</td>
<td>0.82±0.33</td>
<td>0.98±0.40</td>
</tr>
<tr>
<td>1000 kcal</td>
<td>0.83±0.28</td>
<td>1.04±0.20</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 kcal</td>
<td>4.74±0.42</td>
<td>4.85±0.33</td>
</tr>
<tr>
<td>1000 kcal</td>
<td>4.71±0.39</td>
<td>4.90±0.35</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 kcal</td>
<td>28.4±22.9*</td>
<td>36.6±28.3</td>
</tr>
<tr>
<td>1000 kcal</td>
<td>27.7±23.7†</td>
<td>46.6±28.2</td>
</tr>
<tr>
<td>PYY (pmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 kcal</td>
<td>14.5±8.6</td>
<td>8.3±3.7</td>
</tr>
<tr>
<td>1000 kcal</td>
<td>16.1±8.3</td>
<td>10.5±4.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. Means sharing the same symbol denote significant differences between restrained and unrestrained eaters within each group: * and †, P<0.05.

No significant differences, between restrained and unrestrained eaters, were observed in the fasting plasma levels of any of the metabolites and hormones measured in either all participants, men or women. The only exception was in fasting insulin levels, which were significantly higher in unrestrained compared with restrained eaters in all participants in both legs (500 kcal leg: 40.8±16.9 vs 28.4±22.9 pmol/L, P=0.032 and 1000 kcal leg: 43.2±18.9 vs 27.7±23.7 pmol/L, P=0.048, respectively) and in women in the 1000 kcal leg (46.0±16.7 vs 18.2±14.8 pmol/L, P=0.0001, respectively), despite a trend in the same direction also in the 500 kcal leg (P=0.056).
No significant correlations were observed between the RRS, or the restraint subscales of the DEBQ and TFEQ-18R, and fasting TAG or PYY plasma levels in all participants, men or women. Correlations between fasting glucose/insulin and the RRS, and restraint subscales of the DEBQ and TFEQ-18R, can be seen in Table 5.3.

Table 5.3. Correlations between fasting glucose/insulin plasma levels and the RRS and restraint subscales of the DEBQ and TFEQ-18R, in the 500 kcal and 1000 kcal leg

<table>
<thead>
<tr>
<th></th>
<th>RRS</th>
<th>DEBQ</th>
<th>TFEQ-18R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 500 kcal</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- 1000 kcal</td>
<td>-</td>
<td>r=-0.369, P=0.037</td>
<td>r=-0.489, P=0.005</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 500 kcal</td>
<td>r=-0.399, P=0.024</td>
<td>r=-0.467, P=0.007</td>
<td>r=-0.414, P=0.018</td>
</tr>
<tr>
<td>- 1000 kcal</td>
<td>r=-0.432, P=0.014</td>
<td>r=-0.349, P=0.050</td>
<td>r=-0.392, P=0.027</td>
</tr>
</tbody>
</table>

Glucose levels in the 1000 kcal, but not in the 500 kcal leg, were negatively correlated, with statistical significance, with the restraint subscale of the DEBQ and TFEQ-18R in all participants, but not when each gender was analysed separately. Fasting insulin levels were also negatively correlated with the RRS and restraint subscales of the DEBQ and TFEQ-18R, in both the 500 kcal and 1000 kcal legs, in all participants and women, but not in men.
5.4.3.2 Postprandial levels

A significant main effect of time (P<0.0001), but no effect of preload, restraint or interactions, was observed on TAG plasma levels, which increased over time (Figure 5.2). This was observed in all participants, men and women. No significant effect of preload, restraint or interactions was observed in the AUC for TAG, but a significant effect of gender was found with significantly higher levels in men compared with women (214±105 vs 137±60 mmol/L*min, P=0.008).

![Figure 5.2. Plasma TAG concentrations (mmol/L) over time, after a 500 kcal and 1000 kcal breakfast, in restrained and unrestrained eaters. Values represent means ± SEM for 33 participants (16 restrained and 17 unrestrained). ANOVA showed a significant main effect of time (P<0.0001), but no effect of preload, restraint or interactions.](image-url)
A significant main effect of time (P<0.0001), preload (P=0.012) and restraint (P=0.045), and a preload x time interaction (P<0.0001), were found in glucose plasma levels. Glucose plasma levels increased from breakfast until t=30 minutes and decreased afterwards until the end of the study and were significantly higher in the 1000 kcal compared with the 500 kcal leg and in unrestrained compared with restrained individuals (Figure 5.3).

![Figure 5.3. Plasma glucose concentrations (mmol/L) over time, after a 500 kcal and 1000 kcal breakfast, in restrained and unrestrained eaters. Values represent means ± SEM for 33 participants (16 restrained and 17 unrestrained). ANOVA showed a significant main effect of time (P<0.0001), preload (P=0.012) and restraint (P=0.045) and a preload x time interaction (P<0.0001).](image)

In women, a significant effect of time (P<0.0001) and a restraint x time interaction (P=0.016) were found in glucose plasma levels, together with a trend (P=0.071) towards higher glucose levels in the 1000 kcal compared with the 500 kcal leg. In men, only a significant effect of time (P<0.0001) was observed. When each leg was analysed separately, a significant effect of time (P<0.0001) was observed on glucose plasma levels in the 500 kcal leg, while in the 1000 kcal leg a significant effect of time (P<0.0001) and restraint (P=0.032) were found.
One outlier was found in the AUC for glucose in the 500 kcal leg, with a value of 396 pmol/L*min (3.6 SDs away from the mean) and was, therefore, excluded from the analysis. A significant effect of preload (P<0.0001) and a trend towards an effect of restraint (P=0.054), but no effect of gender or interactions, was observed in the AUC for glucose, with high glucose levels in the 1000 kcal leg and in unrestrained compared with restrained eaters.

A significant main effect of time (P<0.0001), preload (P<0.0001) and restraint (P=0.015), and a preload x time interaction (P=0.002) were observed in insulin plasma levels. Insulin plasma levels increased from breakfast until t=30 minutes and decreased afterwards until the end of the study and were significantly higher in the 1000 kcal compared with the 500 kcal leg and in unrestrained compared with restrained individuals (Figure 5.4).

![Figure 5.4. Plasma insulin concentrations (pmol/L) over time, after a 500 kcal and 1000 kcal breakfast, in restrained and unrestrained eaters. Values represent means ± SEM for 33 participants (16 restrained and 17 unrestrained). ANOVA showed a significant main effect of time (P<0.0001), preload (P<0.0001) and restraint (P=0.015), and a time x preload interaction (P=0.002).](image)

In women, a significant effect of time (P<0.0001) and preload (P=0.013), and a trend towards an effect of restraint (P=0.072), were found in insulin plasma levels, with higher insulin levels in the 1000 kcal compared with the 500 kcal leg and in
unrestrained compared with restrained women. In men, a significant effect of time (P<0.0001) and preload (P=0.007), and a preload x time interaction (P=0.033), were also found in insulin plasma levels, but no effect of restraint was observed. When each leg was analysed separately, a significant effect of time and restraint were observed in both the 500 kcal (P<0.0001 and P=0.02) and 1000 kcal leg (P<0.0001 and P=0.038). A significant effect of preload (P<0.0001) and restraint (P=0.01), but no effect of gender or interactions, was observed in the integrative AUC for insulin, with high insulin levels in the 1000 kcal leg and in unrestrained eaters.

A significant main effect of time (P<0.0001) and preload (P<0.0001) and a time x preload interaction (P<0.0001), but no effect of restraint or other interactions, were observed on PYY plasma levels. PYY plasma levels increased throughout the study period and were significantly higher in the 1000 kcal compared with the 500 kcal leg (Figure 5.5). The same was observed when each gender was analysed separately.

![Figure 5.5. Plasma PYY concentrations (pmol/L) over time, after a 500 and 1000kcal breakfast, in restrained and unrestrained eaters. Values represent means ± SEM for 33 participants (16 restrained and 17 unrestrained). ANOVA showed a significant main effect of time (P<0.0001) and preload (P<0.0001) and a time x preload interaction (P<0.0001), but no effect of restraint or other interactions.](image-url)
A significant effect of preload (P<0.0001) and gender (P=0.02), but no effect of restraint or interactions, were observed in the AUC for PYY. The AUC for PYY was higher in the 1000 kcal compared with the 500 kcal leg and in women compared with men (4650±1634 vs 3369±1758 pmol/L*min, P=0.02).

No significant correlations were observed between the RRS, or the restraint subscales of the DEBQ and TFEQ-18R, and the AUC for TAG and PYY, or integrative AUC for insulin, in all participants, men or women. However, a significant negative correlation was observed, in all participants, between glucose AUC and the restrained subscale of the DEBQ (r=-0.365, n=33, P=0.040 and r=-0.361, n=33, P=0.043 in the 500 kcal and 1000 kcal leg, respectively) and a trend between glucose AUC and the restraint subscale of the TFEQ-18R (r=-0.346, n=33, P=0.053 and r=-0.313, n=33, P=0.081, in the 500 kcal and 1000 kcal leg, respectively).
### Table 5.4. Fasting and postprandial insulin sensitivity, and fasting insulin resistance, in restrained and unrestrained eaters, in all participants (n=33), men (n=6) and women (n=21)

<table>
<thead>
<tr>
<th></th>
<th>Restrained (n=16)</th>
<th>Unrestrained (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=16)</td>
<td>Male (n=6)</td>
</tr>
<tr>
<td>Fasting $S_i$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 kcal</td>
<td>511±794†</td>
<td>199±121</td>
</tr>
<tr>
<td>1000 kcal</td>
<td>679±960‡</td>
<td>144±105</td>
</tr>
<tr>
<td>Fasting IR</td>
<td>0.6±0.5*</td>
<td>0.8±0.6</td>
</tr>
<tr>
<td>500 kcal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 kcal</td>
<td>0.6±0.5§</td>
<td>1.0±0.6</td>
</tr>
<tr>
<td>Postprandial $S_i$</td>
<td>3.9±1.4††</td>
<td>4.1±1.4††</td>
</tr>
</tbody>
</table>

IR – insulin resistance, $S_i$ – insulin sensitivity. Values are means ± SD. Means sharing the same symbol denote significant differences between restrained and unrestrained eaters within each group: *, †, ‡ and §§, $P<0.05$; ** and ††, $P<0.01$.
1) As a percentage of a normal reference population.
2) Values for postprandial $S_i$ are $x 10^ {-3}$ and expressed as dl glucose/kg.min/uU insulin.ml
Correlations between fasting and postprandial $S_i$ and restraint levels according to the different questionnaires (RRS, DEBQ and TFEQ-18R) can be seen in Table 5.5.

Table 5.5. Correlations between fasting and postprandial insulin sensitivity ($S_i$) and restraint levels according to different questionnaires, in all participants (n=33), men (n=12) and women (n=21)

<table>
<thead>
<tr>
<th></th>
<th>RRS</th>
<th>DEBQ</th>
<th>TFEQ-18R</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting $S_i$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 kcal</td>
<td>$r=0.403$, $P=0.02$</td>
<td>$r=0.448$, $P=0.009$</td>
<td>$r=0.415$, $P=0.016$</td>
</tr>
<tr>
<td>1000 kcal</td>
<td>$r=0.422$, $P=0.016$</td>
<td>$r=0.335$, $P=0.061$</td>
<td>$r=0.382$, $P=0.031$</td>
</tr>
<tr>
<td><strong>Postprandial $S_i$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 kcal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 kcal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Men (n=12)</strong></td>
<td>$r=0.562$, $P=0.057$</td>
<td>$r=0.702$, $P=0.011$</td>
<td>$r=0.595$, $P=0.041$</td>
</tr>
<tr>
<td>500 kcal</td>
<td>$r=0.475$, $P=0.03$</td>
<td>$r=0.469$, $P=0.032$</td>
<td>$r=0.444$, $P=0.044$</td>
</tr>
<tr>
<td>1000 kcal</td>
<td>$r=0.706$, $P&lt;0.001$</td>
<td>$r=0.572$, $P=0.007$</td>
<td>$r=0.522$, $P=0.015$</td>
</tr>
<tr>
<td><strong>Women (n=21)</strong></td>
<td>$r=0.385$, $P=0.085$</td>
<td>$r=0.436$, $P=0.048$</td>
<td>$r=0.477$, $P=0.029$</td>
</tr>
</tbody>
</table>

Significant positive correlations, or a trend towards it, were observed between fasting $S_i$ and restraint levels when the RRS, DEBQ and TFEQ-18R were used to classify restraint, in all participants in both legs. These correlations become stronger and more significant in women, especially in the 1000 kcal leg, but no significant correlations were observed between fasting $S_i$ and measures of restraint in men, in either the 500 kcal or 1000 kcal leg. Positive correlations, highly significant, were also observed between postprandial $S_i$ and measures of restraint, using the RRS, DEBQ or TFEQ-18R, in all participants. These correlations become less significant, or lost significance, when each gender was analysed separately.

No significant correlations were observed between fasting or postprandial $S_i$ and anthropometric variables (weight, BMI or waist circumference), body fat (%), age, total score derived from the Baecck questionnaire, or from its subscales, or energy or macronutrients intake on the 24 hours prior to each study leg or derived from the PBFD in all participants, men or women. The only exceptions were postprandial $S_i$ that was

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negatively correlated with body weight (r=-0.418, n=33, P=0.016, r=-0.481, n=21, P=0.027 in all participants and women, respectively) and fasting Si that was also negatively correlated with body weight in all participants in the 500 kcal (r=-0.356, n=33, P=0.042), but not in the 1000 kcal leg. The sport and total PA index were positively correlated with fasting Si (r=0.670, n=12, P=0.017 and r=0.730, n=12, P=0.007, respectively) in men in the 500 kcal, but not in the 1000 kcal leg.

5.4.5 Subjective ratings of hunger and fulness

A significant effect of preload (P<0.0001) and gender (P=0.026), but no effect of restraint, were observed in the AUC for hunger, with significantly higher hunger levels in the 500 kcal compared with the 1000 kcal leg (743±401 vs 497±352 cm*min, P<0.0001) and in men compared with women (571±456 vs 235±251 cm*min, P=0.026) (Figure 5.6).

Figure 5.6. AUC (0-180 minutes) for hunger after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant effect of preload (P<0.0001) and gender (P=0.026).

When each gender was analysed separately, a significant effect of preload (P<0.0001) and restraint (P=0.045) was found in the AUC for hunger in women, with higher hunger ratings in unrestrained compared with restrained eaters (629±348 vs
385±234 cm*min, P=0.045), while in men only a significant effect of preload (P=0.007) was observed. Similar results were obtained for prospective food consumption, with a significant effect of preload (P<0.0001) and gender (P=0.021), and a trend towards an effect of restraint (P=0.069), in the AUC for prospective food consumption (data not shown). Prospective food consumption ratings were significantly higher in the 500 kcal compared with the 1000 kcal leg (831±396 vs 590±361 cm*min, P<0.0001) and in men compared with women (907±441 vs 606±327 cm*min, P=0.021), with a trend towards higher values in unrestrained compared with restrained eaters (814±388 vs 601±379 cm*min, P=0.069). When each gender was analysed separately, a significant effect of preload (P<0.0001) and restraint (P=0.019) was found in the AUC for prospective food consumption in women, with higher ratings in unrestrained compared with restrained eaters (736±348 vs 464±239 cm*min, P=0.019). In men, only an effect of preload (P=0.05), at the limit of significance, was observed.

A significant effect of preload (P<0.0001) and restraint (P=0.033), but no significant effect of gender, was observed in the AUC for fulness (Figure 5.7).

Figure 5.7. AUC (0-180 minutes) for fulness after a 500 kcal and 1000 kcal breakfast in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant effect of preload (P<0.0001) and restraint (P=0.033).
Fulness ratings were significantly higher in the 1000 kcal compared with the 500 kcal leg (950±345 vs 698±336 cm*min, P=0.001) and in restrained compared with unrestrained eaters (954±384 vs 696±305 cm*min, P=0.033). When each gender was analysed separately, a significant effect of preload (P=0.002) and restraint (P=0.001) was found in the AUC for fulness in women, with higher ratings in restrained compared with unrestrained eaters (1038±333 vs 670±275 cm*min, P=0.001). In men, a significant effect of preload (P=0.030), but no effect of restraint, was found in the AUC for fulness.

Subjective ratings of hunger/fullness over time, after the 500 kcal and 1000 kcal breakfast, in restrained and unrestrained eaters, in all participants, men and women can be seen in Appendix XVI.

No significant correlations were observed between the AUC for hunger, fulness or prospective food consumption and the AUC for any of the metabolites and hormones measured, in the 500 kcal or 1000 kcal leg, in all participants or when restrained and unrestrained eaters were analysed separately.
5.4.6 Cognitive and emotional state

A significant effect of preload and restraint, but no effect of gender, were observed in the AUC for “Resigned and like giving up your diet” scores, which were higher in the 500 kcal compared with the 1000 kcal preload (212±208 vs 169±195, P=0.025) and in restrained compared with unrestrained eaters (256±213 vs 132±173, P=0.025) (Figure 5.8).

Figure 5.8. AUC (0-180 minutes) for “resigned and like giving up” scores after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant effect of preload (P=0.025) and restraint (P=0.025)
No significant effect of preload, restraint or gender, but a significant preload x restraint x gender interaction (P=0.041), was observed in the AUC for “Out of control of your eating” scores (Figure 5.9). Ratings were higher in restrained women, compared with men, but only after the 1000 kcal preload.

Figure 5.9. AUC (0-180 minutes) for “out of control of your eating” scores after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant preload x restraint x gender interaction (P=0.042)
A significant effect of restraint was found in the AUC for “Preoccupation with thoughts of dieting” scores, but no effect of preload, gender or interactions, with higher ratings in restrained eaters (P=0.001) (Figure 5.10).

![Graph showing AUC for preoccupation with thoughts of dieting](image)

Figure 5.10. AUC (0-180 minutes) for “preoccupation with thoughts of dieting” after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant effect of restraint (P=0.001), but no effect of preload, gender or interactions.

A significant effect of preload, but no effect of restraint, gender or interactions, was observed in the AUC for “Preoccupation with thoughts of food” and an “Overpowering urge to eat” scores, which were higher in the 500 kcal leg (P=0.002 and P<0.0001, respectively) (Figure 5.11 and Figure 5.12). No significant effect of preload, restraint, gender or interactions were found in the AUC for “Guilt” or “Rebellious/defiant about dieting” scores (data not shown).
Figure 5.11. AUC (0-180 minutes) for “preoccupation with thoughts of food” after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant effect of preload (P<0.002), but no effect of restraint, gender or interactions.

Figure 5.12. AUC (0-180 minutes) for “overpowering urge to eat” after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed only a significant effect of preload (P<0.0001).
5.4.7 Energy intake at the test-meal

No order effects (of preloading) were found on lunch EI (kcal) (lunch EI after the 500 kcal preload: 938.2±432.1 vs 903.5±448.3, P>0.05; lunch EI after the 1000 kcal preload: 711.7±380.4 vs 754.0±406.7, P>0.05 in those that started first with the 500 kcal and the 1000 kcal preload, respectively).

EI at the pasta lunch after each preload (500 kcal vs 1000 kcal) in restrained and unrestrained eaters can be seen in Figure 5.13.

![Energy Intake Chart](image)

Figure 5.13. Energy intake (kcal) at the pasta lunch after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant effect of preload (P=0.003) and gender (P<0.0001), but no effect of restraint or interactions.

Analysis of variance showed a main effect of preload and gender on EI at lunch. Lunch EI was significantly higher after the 500 kcal preload compared with the after the 1000 kcal preload (754±333 vs 558±281 kcal, P=0.003) and significantly higher in males compared with females (869±274 vs 527±276 kcal, P<0.0001). Although no main effect of restraint was found, a tendency was observed with restrained participants eating less than unrestrained at lunch time, regardless of the preload (590±334 vs 701±300 kcal, P=0.098). When each gender was analysed separately, a significant effect of preload, but no effect of restraint, was observed in women, with a higher lunch EI.
after the 500 kcal compared with after the 1000 kcal preload (607±448 vs 300±130 kcal, P=0.014, respectively), while in men there was only a trend towards an effect of preload (P=0.063).

No significant correlations were observed between EI at the pasta lunch, after the 500 kcal or 1000 kcal breakfast, and the AUC (0-180 min) for hunger, prospective food consumption or fulness, in unrestrained eaters. However, a significant positive correlation was observed between EI at the pasta lunch and hunger (r=0.515, n=17, P=0.034 and r=0.518, n=17, P=0.033) and prospective food consumption at t=180 minutes (r=0.503, n=17, P=0.039 and r=0.562, n=17, P=0.019) after the 500 kcal and 1000 kcal preload, respectively. In restrained eaters, significant correlations were observed between EI at the pasta lunch after the 500 kcal breakfast and the AUC (0-180 min) for hunger (r=0.552, n=16, P=0.027), prospective food consumption (r=0.547, n=16, P=0.028) and fulness (r=0.628, n=16, P=0.009), but not between EI at the pasta lunch and subjective ratings of hunger/fulness immediately before the meal (t=180 min). The correlations between EI at the pasta lunch and the AUC for subjective hunger/fulness became stronger in the 1000 kcal leg and EI at the pasta lunch was also correlated with hunger/fulness immediately before the meal. EI at the pasta lunch after the 1000 kcal breakfast was significantly correlated with hunger (r=0.808, n=15, P<0.0001 and r=0.781, n=15, P=0.001), prospective food consumption (r=0.772, n=15, P=0.001 and r=0.754, n=14, P=0.002) and fulness (r=-0.733, n=15, P=0.002 and r=-0.765, n=15, P<0.0001) in restrained eaters, for AUC and scores immediately before the meal was presented, respectively.

No significant correlations were observed between EI at the pasta lunch, after the 500 kcal or 1000 kcal breakfast, and the AUC for any of the metabolites and hormones measured or their plasma levels in fasting or immediately before the meal was presented, in either restrained or unrestrained eaters.

Although mean short-term energy compensation (%), at lunch time, was higher in restrained compared with unrestrained eaters, no significant differences were observed between the two groups, in all participants, men or women (35.2±48.1 vs 28.0±57.5, P>0.05; 47.7±56.4 vs 16.0±43.5, P>0.05 and 35.2±48.1 vs 28.0±57.5, P>0.05, respectively). No significant correlations were observed between short-term energy compensation, at the pasta meal, and any of the eating behaviour constructs.
measured (RRS, or any of the subscales derived from the DEBQ or TFEQ-18R) in all participants, men or women.

### 5.4.8 Cumulative EI over a 24h period

No order effects (of preloading) were found on cumulative EI (kcal) over a 24h period (cumulative EI after the 500 kcal preload: 2955±794 vs 2945±434 kcal, P>0.05; cumulative EI after the 1000 kcal preload: 2718±850 vs 2640±429 kcal, P>0.05 in those that started first with the 500 kcal and the 1000 kcal preload, respectively).

Cumulative EI, over a 24h period, after each preload (500 kcal vs 1000 kcal) in restrained and unrestrained eaters can be seen in Figure 5.14.

![Figure 5.14. Cumulative energy intake (kcal), over a 24h period, after a 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters, in all participants (n=33), men (n=12) and women (n=21). Values represent means ± SEM. ANOVA showed a significant effect of preload (P=0.034) and gender (P=0.001), but no effect of restraint or interactions.](image)

Analysis of variance showed a main effect of preload and gender on cumulative EI. 24h cumulative EI was significantly higher after the 500 kcal preload compared with the after the 1000 kcal preload (2697±601 vs 2511±629 kcal, P=0.034) and significantly higher in men compared with women (3086±577 vs 2360±469 kcal, P=0.001). When
each gender was analysed separately, a trend towards an effect of preload was observed in women (P=0.07), but not in men.

No significant differences in energy compensation (%) over a 24h period were observed between restrained and unrestrained eaters in all participants, men or women (27.0±94.0 vs 46.6±84.8, P>0.05; 62.9±65.1 vs 11.7±86.5, P>0.05 and 12.6±102.7 vs 72.8±78.6, P>0.05, respectively).

5.5 Discussion

The primary aim of this study was to investigate the effect of dietary restraint on fasting and postprandial levels of appetite-related hormones and metabolites, in a mixed sample of normal-weight volunteers, and to correlate potential alterations with changes in subjective feelings of hunger/fulness and prospective food intake at a pasta lunch. Opposite to our expectations, no significant effect of restraint was observed on fasting or postprandial levels of TAG or PYY. However, restrained eaters presented with significantly lower insulin plasma levels and were more insulin sensitive, in the fasting state. The present investigation also showed, for the first time, significantly lower postprandial plasma levels of glucose and insulin and higher fulness ratings, in the postprandial state, in restrained eaters, independently of the preload. When a minimal model index was used to calculate postprandial Si, restrained eaters presented with significantly higher Si compared with unrestrained eaters. However, no significant effect of restraint was observed on EI at the pasta meal, with both restrained and unrestrained eaters showing a regulatory eating behaviour in response to preloading.

Fasting insulin levels were significantly lower in restrained eaters, in all participants, in both study legs, and in women in the 1000 kcal leg, despite a trend in the same direction also in the 500 kcal leg. Using the HOMA model, restrained eaters were also shown to be more insulin sensitive, in the fasting state, independently of the study leg, in all participants and women. Lower fasting insulin plasma levels had already been reported in restrained women (Pirke et al., 1990; Keim and Horn, 2004), as well as lower IR, also using the HOMA model (Keim and Horn, 2004). However, body weight was a confounder in this last study, since restrained women had significantly lower body weight than unrestrained women. The findings of the present investigation are, therefore, in line and reinforce these earlier findings.
Moreover, this is the first study to report lower plasma glucose and insulin levels, in the postprandial state, in restrained eaters. Despite the significantly lower fasting insulin levels reported in restrained women in Keim and Horn (2004) and Pirke and colleagues (1990) studies, no significant effect of restraint had been reported before on postprandial levels of glucose and insulin in women (Pirke et al., 1990; Keim and Horn, 2004) or in a mixed sample of men and women (Burton-Freeman, 2005). It might be argued that the lower energy content of the preload used by Keim & Horn (2004) (338±30kcal in restrained and 403±26kcal in unrestrained, depending on body weight), compared with the ones used in the present investigation (500 kcal and 1000 kcal preload), was not enough to detect an effect of restraint on the postprandial plasma levels of glucose and insulin; however the percentage of CHO was in fact higher than the one used in the present study (60 vs 47%). It is, therefore, unlikely that differences in the energy content or composition of the preloads explain the absence of an effect of restraint on postprandial glucose and insulin plasma levels in Keim & Horn’s study. It is more likely that their failure to detect any differences reflects the use of an inappropriate time frame for blood sampling (fasting, one, two and three hours postprandially only) (Keim and Horn, 2004). Both glucose and insulin plasma levels peak around 30 minutes after a meal and, depending on the CHO content of a meal, may return to baseline levels as early as one hour after meal consumption. For that reason, in the present study, blood samples were taken intensively during the first postprandial hour (every fifteen minutes) and every 30 minutes on the second and third hours. In Pirke and colleagues’ (1990) study, blood sampling times were very similar to the ones used in this study and, therefore, an inappropriate sampling time frame cannot be an explanation for the lack of effect of restraint on postprandial glucose and insulin plasma levels in their study. However, although Pirke and colleagues (1990) had used a preload with the same energy content as one of the preload used in this study (500 kcal), it contained a much lower percentage of CHO (30 vs 47%) which may have been insufficient to enable an effect of restraint to be found. In Burton-Freeman’s study (2005), on the other hand, insulin was only measured on the first 45 minutes postprandially and despite preloads of 47% and 81% CHO had been used, men received on average only 230 kcal and women 224 kcal, resulting, therefore, in a much lower “net” energy and CHO load compared with the one used in the present investigation.
The reason for the lower glucose and insulin plasma levels, in the postprandial state, found in restrained eaters in the present study is not known. The increased $S_i$ observed in restrained eaters offers a plausible explanation. Moreover, noradrenaline may also be involved. Noradrenalin plays a key role in energy metabolism, by increasing glycolysis (Krarup, 1992), with consequent release of glucose into the bloodstream. Significantly lower noradrenalin plasma levels have been reported in restrained normal-weight women after a 500 kcal meal (Pirke et al., 1990), therefore offering another potential mechanism for the lower postprandial glucose plasma levels observed in restrained women in the present study. Lower glucose plasma levels, in the postprandial state, would demand less insulin to be released and, therefore, explain the lower postprandial levels of insulin.

This is also the first study to show increased $S_i$ in restrained eaters, in the postprandial state, when a minimal model index was used to calculate $S_i$. $S_i$ has been shown to be modulated by several factors. Excess adiposity is unquestionably the most important determinant of insulin resistance, with increasing body weight and body fat, especially when centrally distributed, being associated with reduced $S_i$. Weight loss and increased levels of PA, on the other hand, have been associated with improved $S_i$. In terms of individual nutrients, a diet high in CHO and fibre and low in fat has been shown to reduce IR (McAuley and Mann, 2006). Although $S_i$ was found to be negatively correlated with body weight (fasting $S_i$ only in the 500 kcal but not in the 1000 kcal leg), in the present investigation, all participants were weight stable on the three months prior to the study and no significant differences were observed, between restrained and unrestrained eaters, on body weight or body composition (% of body fat), waist circumference, a measure of abdominal adiposity, PA levels, derived from the Baecck questionnaire, or macronutrient intake on the 24 hours prior to the investigation or derived from the PBFD. Although one day is clearly not enough to estimate habitual food intake accurately (Nelson et al., 1989), studies looking at the impact of restraint on macronutrient consumption, in free living conditions, based on self-recorded food diaries, have yielded inconclusive results. Restraint was found not to impact on macronutrient intake in some studies (Lawson et al., 1995; Beiseigel and Nickols-Richardson, 2004), while others have reported a lower intake of fat (Rideout et al., 2004; De Castro, 1995) and CHO (De Castro, 1995) and an increased consumption of protein (Laessle et al., 1989b) in restrained eaters or, in opposition, a higher intake of fat.
and lower of CHO (Klesges et al., 1992). The reasons for the increased $S_t$ in restrained eaters remain, therefore, unknown and this is clearly an area deserving further investigation.

The absence of a significant effect of restraint on fasting and postprandial levels of TAG and PYY was rather disappointing since previous research has supported a link between restraint and fasting TAG (Laessle et al., 1989b), as well as between restraint and the postprandial release of CCK, an hormone with similar satiety properties to PYY (Burton-Freeman, 2005). Moreover, pilot data (see chapter 4) pointed to lower fasting levels of PYY in restrained eaters and a trend towards lower levels in the postprandial state. However, most of the studies showing an association between restrained eating behaviour and altered physiology, described in detail in the introduction of this chapter, are limited by the fact that they use the median split of a particular scale as the cut-off point for restraint. The study by Laessle and collaborators (1989), showing increased levels of fasting TAG on restrained eaters, used the median point (score=10.4) of the TFEQ and the study by Burton-Freeman (2005), showing a blunted release of CCK in restrained eaters in the postprandial state, used an arbitrary point (score=10) of the same questionnaire as the cut-off point for restraint. If restrained eating behaviour is a continuous rather than a dichotomic variable, as previously suggested (Tomarken and Kirschenbaum, 1984; Herman and Polivy, 1979), using a single cut-off point to classify restraint results in the arbitrary inclusion on either the restrained or unrestrained category of those scoring in the middle of the scale. For this reason, only those scoring very low or very high in restraint were accepted in the present investigation, an approach that although not very common, has been previously adopted by others (Lawson et al., 1995; McLean et al., 2001). Moreover, by excluding those scoring in the middle of the scale, a smaller sample size is needed to find potential differences between groups.

Although significantly higher fasting TAG levels were previously reported in normal-weight restrained women (Laessle et al., 1989b), no significant differences between restrained and unrestrained eaters were observed in the present study, in either all participants, or when men or women were analysed separately. However, a gender effect was found, with significantly higher TAG levels in men, a finding that is well supported by the literature (Couillard et al., 1999). In Laessle and collaborators (1989)
study, BMI and disinhibition scores (derived from the TFEQ) were significantly higher in restrained compared with unrestrained women, something that did not occur in the present study. It is, therefore, possible that the higher body weight and disinhibition scores, and not restraint in itself, contributed to the increased fasting TAG plasma levels reported by Laessle and colleagues (1989) in restrained women. These authors also reported lower EI (derived from seven-day food-diaries) in restrained women and suggested that although chronic energy deprivation was unlikely to occur in restrained eaters, intermittent energy restriction might have contributed to the increased levels of fasting TAG observed in restrained females (Laessle et al., 1989b). Although a single day is clearly not long enough to estimate habitual EI accurately (Nelson et al., 1989), no significant difference in EI on the 24 hours prior to each trial was observed between restrained and unrestrained eaters, either in all participants, men or women, in the present investigation. It has also been suggested by Keim & Horn (2004) that the higher fasting TAG levels observed in restrained women in Laessle et al (1989) study could be the result of a prompt esterification of mobilized fatty acids.

A blunted release of CCK after a 40% fat meal was reported in a mixed sample of normal-weight restrained eaters (men and women) (Burton-Freeman, 2005). While unrestrained eaters had a significantly higher CCK release after 40% fat preloads (with different fatty acid composition) compared with a control low fat preload (4% fat), with an identical energy content, restrained eaters had a similar release of CCK regardless of the preload. This blunted release of CCK, in restrained eaters, in response to high fat preloads was not associated, however, in increased hunger and/or reduced fullness ratings or higher EI at a subsequent meal (45 minutes after preloading), compared with unrestrained eaters. Although CCK was not measured in the present study, no significant effect of restraint was observed on PYY plasma levels in response to a meal (38% fat), in all participants, men or women. However, a significant effect of preload was observed, with higher levels after the larger preload, which was expected, since PYY secretion from endocrine L-cells of the distal ileum and colon is known to be proportional to the energy content of the meal (Bottcher et al., 1984). A tendency was also observed towards higher PYY plasma levels (AUC) during the three hours after preloading in women, which is in line with previous research (Kim et al., 2005). Although PYY has a suppressive effect on appetite and inhibits further food intake, similar to CCK, their secretion and duration of action patterns are very different. PYY
peaks later and remains elevated for longer after a meal suggesting that, while CCK is probably involved in satiation or meal termination, PYY may be more involved in satiety (Batterham and Bloom, 2003). It is, therefore, possible that restraint impacts on the release of CCK, or other satiety peptides, but not on PYY and more research is needed to clearly establish the impact of restraint on the release of appetite-related hormones.

Another striking finding of this study was increased feelings of fullness in restrained eaters. Restrained eaters reported feeling fuller, in the postprandial period, than unrestrained eaters (independent of the preload), and restrained women experienced not only increased fullness, but also reduced feelings of hunger and prospective food consumption throughout the study period. This was unexpected, since no main effect of restraint was observed on PYY plasma levels and postprandial glucose and insulin plasma levels were significantly lower in restrained eaters. It was proposed more than half a century ago that circulating levels of glucose could modulate hunger and fullness and regulate food intake in the short-term: a decrease in glucose utilization would represent a stimulus for meal initiation while a rise in glucose utilization would stimulate the onset of satiety (British Nutrition Foundation 1999). It seems, therefore, a paradox that restrained eaters presented with lower glucose levels but felt fuller than unrestrained eaters after a meal. However, the impact of glucose plasma levels on appetite remains controversial and the evidence linking high blood glucose levels with lower appetite ratings seems to be rather weak (de Graaf et al., 2004). De Graaf and colleagues (2004) have concluded that even though "transient and dynamic declines in blood glucose concentration within a short time frame (5 min) are strongly related to meal initiation", "absolute glucose concentrations have no straightforward relation to appetite" (page 953).

Although insulin has a well-recognized role as a key glucostatic regulator, recent studies have shown that insulin also has powerful central effects on appetite control, in synergy with leptin. Insulin plasma levels, both fasting and postprandially, are directly proportional to the amount of adipose tissue, functioning, therefore, as an adiposity signal (Woods et al., 2006). It has been shown that a small fraction of circulating insulin reaches the brain and modulates the activity of neuropeptides within the hypothalamus providing a negative feedback signal in the regulation of body fat by reducing food
intake. Apart from this direct effect, insulin together with leptin, also act indirectly by increasing the sensitivity of the brain to meal-related satiety signals and, therefore, modulating the amount of food eaten at an individual meal (Woods et al., 2006; Schwartz et al., 1999). It was, therefore, rather unexpected that even though restrained eaters presented with reduced levels of insulin, both in fasting and postprandially, they did not report increased feelings of hunger or ate more at the pasta meal. On the contrary, restrained eaters reported increased feelings of fullness and ate the same amount of pasta at the test-meal, with no significant correlations being observed between glucose or insulin (AUC) and hunger or fullness (AUC) in all participants, restrained or unrestrained eaters. However, the relationship between insulin and glucose plasma levels and subjective sensations of hunger/fullness and subsequent food intake remains controversial. In a recent meta-analysis by Flint and collaborators (2007), postprandial insulin responses were associated with decreased hunger in normal-weight and obese subjects and with increased fullness in normal-weight subjects, but only with lower subsequent EI in the obese group using meta-regression analysis. Multivariate regression analysis showed similar results, with postprandial insulin levels being associated with hunger, fullness and subsequent EI in the normal-weight group and with subsequent EI in all subjects. Regarding glucose, only the multivariate regression analysis was able to show an inverse association between postprandial glucose plasma levels and subsequent EI (Flint et al., 2007).

It was originally proposed, almost two decades ago, that restrained eaters suffer from a weak sensitivity to physiological cues that regulate food intake (Heatherton et al., 1989). This initial model was, however, shortly dismissed by Ogden & Wardle (1990), who showed that restrained eaters are not less sensitive to internal cues, but do have an increased sensitivity to external cues, compared with unrestrained eaters. The increased fullness in restrained compared with unrestrained eaters after the same meal, reported in the present study, may, therefore, be a result of an increased sensitivity to external cues that regulate food intake (the two preloads carried a different cognitive message: one or two mugs of hot chocolate). However, more studies are needed to clearly establish if restrained eaters do have an increased sensitivity to internal, external or both cues.
The impact of dietary restraint on subjective ratings of hunger and fullness has not been extensively studied. Restraint, disinhibition and hunger scores, derived from the TFEQ, were found not to be associated with appetite sensations (desire to eat, hunger, fullness and prospective food consumption) after a test-meal in a mixed sample of normal-weight and obese men and women (Drapeau et al., 2005). Moreover, no significant differences in hunger, desire to eat, gastric fullness or prospective food consumption were reported between restrained and unrestrained eaters during eight hours following a high- or low-fat meal either correctly or incorrectly labelled (Chapelot et al., 1995). However, while in unrestrained eaters, fullness was not affected by any condition; restrained eaters reported feeling fuller after the “actual high-fat” compared with the “actual low-fat” meal. Interestingly, Ogden & Wardle (1991) reported a greater decrease in hunger and increase in fullness, in restrained compared with unrestrained eaters, after a HEP; suggesting, according to the authors, that restrained eaters may be more sensitive to the satiety effects of high-energy food. No significant differences in baseline hunger or fullness were observed between restrained and unrestrained individuals in the present study, as well as in hunger or prospective food consumption in the postprandial period, but restrained eaters reported significantly higher fullness ratings and restrained women higher fullness and lower hunger and prospective food consumption ratings in the fed state, throughout the study period.

Although the lower EI reported in restrained eaters, in a free-living environment (Laessle et al., 1989b; De Castro, 1995; McLean et al., 2001), may, at least to a certain extent, be a result of underreporting, a phenomenon that has been shown to be especially important in this population (Rennie et al., 2006), the evidence is unclear. Tuschl and collaborators (1990), for instance, found a good correlation between reported EI, based on 14-day food diaries, and measured EE, based on the doubly labelled water technique, for both restrained and unrestrained eaters, suggesting according to De Castro (1995) that if underreporting occurs, it occurs in both groups and to the same extent. Moreover, there is some evidence suggesting that restrained eaters do actually need less energy than unrestrained eaters. A lower TEE, using the doubly-labelled water method (Tuschl et al., 1990) and RMR, using indirect calorimetry (Platte et al., 1996), has been reported in restrained women, although the last finding remains controversial (Lawson et al., 1995). More importantly, the lower TEE and RMR reported in restrained women were not the result of lower PA levels or weight
cycling, respectively (Tuschl et al., 1990; Platte et al., 1996). The increased fullness, in the postprandial state, reported by restrained eaters in the present study, may, therefore, offer an additional explanation for the lower habitual EI in restrained individuals.

Interestingly, despite a significantly increased fullness in restrained eaters (men and women) and higher fullness and lower hunger and prospective food consumption ratings in restrained women throughout the study period, no significant effect of restraint was observed on EI at the pasta meal. However, a tendency was observed for restrained participants to eat less than their unrestrained counterparts at lunch time, regardless of the preload, which is in line with their increased fullness ratings.

Restrained eating behaviour has been associated, in the laboratory environment, with counter-regulatory eating behaviour (Herman and Mack, 1975; Rotenberg and Flood, 2000; Woody et al., 1981; Polivy et al., 1988; Spencer and Fremouw, 1979; Hibbscher and Herman, 1977). However, this phenomenon has been only found when the RRS is used to measure restraint and a single, highly palatable and “diet-breaking” food is used as the test-meal. The present study added support to this view by showing that both restrained and unrestrained eaters presented with a regulatory eating behaviour in response to preloading, when a pasta meal was offered, eating more after the 500 kcal compared with the 1000 kcal preload, an effect that was maintained outside the laboratory for the next 24 hours. Despite the absence of disinhibition in restrained eaters, feelings of “giving up diet” and “preoccupation with thoughts of food” were significantly higher in restrained eaters, regardless of the preload and feelings of “loss of control of eating” were also increased in restrained women, after the 1000 kcal preload.

It needs to be emphasized that although restrained eating is a behaviour traditionally associated with females, and the findings of the present investigation are consistent with a gender difference (restrained women, but not men, showed lower postprandial glucose levels, reduced hunger and prospective food consumption and increased fullness ratings), a gender x restraint interaction was never found, therefore, not supporting the claim that women respond differently than men.

In conclusion, restrained eating behaviour does not seem to impact on PYY or TAG plasma levels, but is likely to exercise an important role in glucose metabolism.
This study showed, for the first time, that normal-weight restrained individuals experience increased feelings of fullness, compared with unrestrained eaters, after the same meal, although this did not impact on subsequent food intake at a test-meal. However, increased fullness in restrained eaters may explain, at least partly, the lower habitual EI reported by this group. Further studies are needed to elucidate if similar findings are to be expected for preloads with different energy, macronutrient composition, physical state and cognitive message and the mechanisms behind increased fullness and $S_f$ in restrained eaters.
Chapter Six
Chapter 6. A comparison of different questionnaires to assess restrained eating in their ability to predict disinhibition

6.1 Introduction

The concept of dietary restraint was first developed in the 70’s in the context of obesity aetiology (Nisbett, 1972) and has since been the object of extensive research. Dietary restraint refers to the extent to which individuals are concerned with their body weight and attempt to control it by dieting. It was originally defined as a self-imposed resistance to the internal and external cues that regulate eating behaviour motivated by the desire to maintain or suppress body weight (Herman and Mack, 1975).

There is a considerable amount of evidence showing that restrained eaters when challenged with certain events (collectively known as disinhibitors) such as preloading, exhibit, unlike their unrestrained counterparts, a “counter-regulatory behaviour”, eating more after preloading compared with a no preload condition (Herman and Mack, 1975; Herman and Polivy, 1980; Polivy, 1976; Woody et al., 1981; Rotenberg and Flood, 2000; Hibscher and Herman, 1977). However, a closer analysis of the literature reveals that only a few studies have been able to show a true “counter-regulatory eating behaviour” in response to preloading in restrained eaters (Herman and Mack, 1975; Rotenberg and Flood, 2000; Woody et al., 1981; Polivy et al., 1988; Spencer and Fremouw, 1979; Hibscher and Herman, 1977). Significantly, all these studies have used a single, highly palatable and “diet-breaking” food (ice cream) as the test-meal and measured restraint by the RRS (Herman and Polivy, 1975). The response to preloading, in terms of eating behaviour, seems, therefore, to be dependent on both the questionnaire used to measure restraint and the type of test-meal presented.

Although several studies have tried to identify the best questionnaire to predict disinhibition in the laboratory (Jansen et al., 1988; Dritschel et al., 1993; Ridgway and Jeffrey, 1998; van Strien et al., 2000; Ouwens et al., 2003), they have produced inconclusive results and are limited by the fact that all have used a single, highly palatable food, usually seen as “diet breaking”, as the test-meal. The question remains, therefore, as to the best questionnaire to predict disinhibition in restrained eaters in the laboratory environment.
6.2 Aims

The primary aim of this study was to investigate the predictive validity of three scales that assess dietary restraint: the RRS, a shortened version of the TFEQ - the TFEQ revised 18 items (TFEQ-18R) and the DEBQ in their ability to predict disinhibited eating behaviour in the laboratory by using a HEP and LEP and assessing EI one hour later at an ad libitum buffet lunch. Secondary aims were to correlate the extent of energy compensation, at the buffet test-meal, with different constructs of eating behaviour and to extend the investigation of the impact of restraint on food intake outside the laboratory, in a free-living environment, by assessing food intake in the following 24 hours.

6.3 Methods

6.3.1 Participants

Fifteen normal-weight female volunteers, aged 18-60 years old, not currently dieting to lose weight and weight stable (variation of less than three kg in the last two months) were recruited for this study. Their mean age was 24.1±7.3 years and their mean BMI was 21.2±1.8 kg/m². The exclusion criteria included a BMI lower than 18 or higher than 25 kg/m², prior or present history of coronary heart disease, type 1 or type 2 diabetes, anaemia, gout, depression or other psychological disorders, eating disorders, drug or alcohol abuse within the last two years and current medication known to affect appetite or induce weight loss; these were assessed by a self-certificate medical questionnaire (Appendix VII). Regular smokers (>10 cigarettes/day) and elite athletes (club level or above) were also excluded.

Participants were unaware of the real purpose of the study and were told that it aimed to investigate the effects of concerns with body weight on mood state and how mood can influence what they eat. All participants gave written consent before enrolling in the study and were debriefed at the conclusion of the study. The study was approved by the University of Surrey Ethics Committee (EC/2004/113/SBMS).
6.3.2 Measurement of eating behaviour

Participants were asked to fill in the RRS (Herman and Polivy, 1980) (Appendix I), the TFEQ-18R (Karlsson et al., 2000) (Appendix II) and the DEBQ (van Strien et al., 1986) (Appendix III) and were classified as restrained or unrestrained eaters based on a median split of their scores obtained with each questionnaire: 13, 12 and 2.2, respectively. Two restraint subscales derived from the TFEQ: flexible and rigid control of eating behaviour were also used (Westenhoefer et al., 1999). The first subscale is associated with low levels of disinhibition and the second with high levels of disinhibition.

6.3.3 Study protocol

Subjective feelings of appetite, cognitive and emotional state and EI at a test-meal, following a HEP and LEP, clearly labelled with their exact energy content, were investigated in restrained and unrestrained women, according to different questionnaires, using a randomised crossover design. Participants acted as their own controls and were assigned to the two experimental conditions (appetite challenge days), at least two days apart, in a counterbalanced order. A scheme of the protocol of the study can be seen in Figure 6.1.

![Figure 6.1. Schematic representation of the protocol of the study](image-url)
To reduce the inherent variability, for the 24 hours prior to each appetite challenge day, participants were instructed to refrain from moderate to heavy exercise and from consuming alcohol. They were required to complete 24 hours food records, which were analysed for energy and macronutrients using WinDiets Professional (Robert Gordons University, Aberdeen, UK).

On the morning of each appetite challenge day, participants were asked to consume their usual breakfast before 9.30 am. This was the same on both appetite challenge days in order to standardize pre-study appetite. After that, participants were instructed not to eat or drink anything except water, which was permitted up to 10.30 am and asked to arrive at the CIU at 11.45 am. After arrival, participants were asked to fill in a first set of VAS. Participants were asked to rate their subjective feelings of hunger, prospective food consumption and fullness. Mood ("Do you feel depressed?", "Do you feel anxious?") and cognitive and emotional state associated with restraint and disinhibition ("How rebellious and defiant about dieting do you feel?", "Do you feel resigned and like giving up your diet?", "Do you feel out of control of your eating?", "Are you preoccupied with thoughts of food?", "Do you feel an overpowering urge to eat?", "Do you feel guilty" and "Do you feel preoccupied with thoughts of dieting?") (Ogden, 1990) were also assessed using VAS. These were presented before the appetite questions in order to conceal the primary purpose of the study to the participants. Instructions regarding the completion of VAS are described in Chapter 2 (section 2.2.2.1).

A HEP or LEP, fully described in Chapter 2 (2.2.2.3), clearly labelled with their exact energy content (607 and 246 kcal, respectively), was then presented and participants were asked to consume it within 5 minutes. Further VAS were completed immediately after preload consumption (including the question: "How would you rate the palatability of the milkshake?") and at 20, 40 and 60 minutes afterwards. During this time period, participants stayed in the CIU, but they were free to write or read quietly. Participants were free to talk to each other, but were asked not to discuss the study or their VAS scores. Each VAS was collected before the next was given to avoid participants being influenced by, or modifying their previous scores.

An *ad libitum* buffet meal was served 60 minutes after preload intake, and participants, each confined to an individual booth, were instructed to eat until they felt...
comfortably full. Exactly the same type and amount of food was presented to each individual participant on each appetite challenge day. The food items, amounts and macronutrient composition of the buffet meal, as well as the conditions under which the buffet was presented to participants is fully described in Chapter 2 (section 2.2.2.4.1). Participants were asked to maintain their normal diet throughout the study period and to record in a food diary all they consumed after the buffet lunch until (and including) breakfast of the following day (PBFD) in order to estimate 24 hours cumulative EI (calculated as buffet + PBFD). All nutrient analysis of the diet dairies was performed using WinDiets Professional (The Robert Gordon University, Aberdeen, UK).

6.3.4 Statistical analysis

Differences in energy and macronutrient intake in the 24h prior to each appetite challenge day (HEP vs LEP) were assessed using a paired sample t-test.

The effect of preload and restraint, according to the different questionnaires, on total energy, percentage of energy from each food item and healthy/unhealthy options, macronutrient (in %) intake at the buffet lunch, and cumulative EI during a 24h period, was assessed by a mixed between-within subjects analysis of variance (ANOVA), with preload (HEP vs LEP) as the within-subjects variable and restrained/unrestrained (classified based on the median split of each questionnaire), as the between-subjects variable. Sandwiches, salad, fruit and yoghurt were considered healthy food choices while crisps, biscuits, cake and sauces were considered unhealthy options. Energy compensation was calculated as the difference in energy intake at the buffet lunches between the two study days (LEP vs HEP) divided by the difference in preload energy and expressed as a percentage (Johnson and Birch, 1994).

The AUC for hunger, prospective food consumption, fullness and cognitive and emotional state was calculated using the trapezoidal rule, from before the preload until immediately before the buffet lunch (one-hour period). The effect of preload and restraint on subjective hunger, prospective food consumption and fullness, as well as cognitive and emotional state, was assessed using a mixed between-within subjects ANOVA, with preload (HEP vs LEP) as the within-subjects variable, restrained/unrestrained as the between-subjects factor and the AUC as the dependent
variable. AUC for cognitive variables was not normally distributed and, therefore, a $\log_{10}$ transformation was applied.

Potential correlations (Pearson or Spearman, as appropriated) were investigated between short-term energy compensation at the buffet meal and the different eating behaviours measured: cognitive restraint according to the different questionnaires, emotional and external eating derived from the DEBQ, uncontrolled and emotional eating derived from the TFEQ-18R and the flexible and rigid dimensions of restraint.

All the analysis performed in this chapter was repeated three times with restraint being classified according to the three different questionnaires (the RRS, the DEBQ and the TFEQ-18R).

6.4 Results

6.4.1 Eating behaviour scales

The mean/median scores obtained with each of the questionnaires used to measure eating behaviour are shown in Table 6.1.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRS</td>
<td>11.8</td>
<td>13.0</td>
<td>5.9</td>
<td>3.0-26.0</td>
</tr>
<tr>
<td>DEBQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restrained eating</td>
<td>2.4</td>
<td>2.2</td>
<td>0.9</td>
<td>1.4-4.1</td>
</tr>
<tr>
<td>Emotional</td>
<td>2.5</td>
<td>2.5</td>
<td>0.7</td>
<td>1.4-3.7</td>
</tr>
<tr>
<td>External</td>
<td>3.2</td>
<td>3.1</td>
<td>0.5</td>
<td>2.2-4.2</td>
</tr>
<tr>
<td>TFEQ-18R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restraint eating</td>
<td>12.7</td>
<td>12.0</td>
<td>4.2</td>
<td>6.0-22.0</td>
</tr>
<tr>
<td>Uncontrolled</td>
<td>17.5</td>
<td>18.0</td>
<td>3.9</td>
<td>11.0-25.0</td>
</tr>
<tr>
<td>Emotional</td>
<td>6.5</td>
<td>6.0</td>
<td>2.5</td>
<td>3.0-10.0</td>
</tr>
<tr>
<td>Flexible control</td>
<td>5.1</td>
<td>4.0</td>
<td>3.5</td>
<td>1.0-12.0</td>
</tr>
<tr>
<td>Rigid control</td>
<td>3.8</td>
<td>3.0</td>
<td>2.6</td>
<td>1.0-11.0</td>
</tr>
</tbody>
</table>
Using the median of the participants’ responses to the different scales, as the cut-off point to classify restraint, as described in the methods section, eight females were classified as restrained and seven as unrestrained based on either the RRS, or the restraint scale of the DEBQ and TFEQ-18R. Of the seven woman classified as unrestrained according to the RRS, six were also classified as unrestrained according to the DEBQ and five according to the TFEQ. Of the eight women who were identified as restrained through the RRS, seven were also classified as restrained through the DEBQ and six through the TFEQ (Table 6.2).

Table 6.2. Number of restrained and unrestrained eaters based on the RRS, DEBQ and TFEQ-18R

<table>
<thead>
<tr>
<th></th>
<th>RRS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Restrained (≥13)</td>
</tr>
<tr>
<td>DEBQ-Restraint subscale</td>
<td></td>
</tr>
<tr>
<td>Restrained (≥2.2)</td>
<td>7</td>
</tr>
<tr>
<td>Unrestrained (&lt;2.2)</td>
<td>1</td>
</tr>
<tr>
<td>TFEQ-18R-Restraint subscale</td>
<td></td>
</tr>
<tr>
<td>Restrained (≥12)</td>
<td>6</td>
</tr>
<tr>
<td>Unrestrained (&lt;12)</td>
<td>2</td>
</tr>
</tbody>
</table>
6.4.2 Anthropometry and eating behaviour

Anthropometry and eating behaviour scores in restrained and unrestrained women, according to the different questionnaires, are shown in Table 6.3.

Table 6.3. Anthropometry and eating behaviour scores in restrained (n=8) and unrestrained women (n=7), according to the different questionnaires

<table>
<thead>
<tr>
<th></th>
<th>Restrained</th>
<th>Unrestrained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRS</td>
<td>DEBQ</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.8±7.7</td>
<td>57.6±7.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4±2.0</td>
<td>21.1±2.0</td>
</tr>
<tr>
<td>RRS</td>
<td>16±4.4***</td>
<td>14.6±6.4*</td>
</tr>
<tr>
<td>-Restraint</td>
<td>2.9±0.9**</td>
<td>3.0±0.8***</td>
</tr>
<tr>
<td>-Emotional</td>
<td>2.7±0.8</td>
<td>2.7±0.8</td>
</tr>
<tr>
<td>-External</td>
<td>3.4±0.5</td>
<td>3.4±0.5</td>
</tr>
<tr>
<td>TFEQ-18R</td>
<td>14.6±4.1*</td>
<td>15.4±3.7**</td>
</tr>
<tr>
<td>-Restraint</td>
<td>19.3±3.5</td>
<td>19.5±3.3*</td>
</tr>
<tr>
<td>-Uncontrolled</td>
<td>7.6±2.3</td>
<td>7.3±2.5</td>
</tr>
<tr>
<td>Flexible control</td>
<td>7.1±3.4**</td>
<td>7.1±3.4**</td>
</tr>
<tr>
<td>Rigid control</td>
<td>4.9±3.2</td>
<td>5.1±3.0*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Means sharing the same symbol denote significant differences between groups (restrained vs unrestrained) according to the same questionnaire: *** P<0.001, ** P<0.01, * P<0.05.

No significant differences were observed in anthropometry (body weight or BMI) between restrained and unrestrained women, regardless of the questionnaire used to measure restraint. When the RRS was used to classify restraint, as expected, restrained women had a significantly higher RRS score, but also higher restraint scores, derived both from the DEBQ and TFEQ-18R, and flexible control of eating score, compared with unrestrained eaters. When the DEQB was used to classify restraint, significantly higher RRS scores and restraint scores, derived from both the DEBQ and TFEQ-18R, uncontrolled scores derived from the TFEQ-18R and flexible and rigid control of eating scores were observed in restrained compared with unrestrained...
women. Finally, when restraint was classified based on the TFEQ-18R, no significant difference was observed on the RRS score between groups, but restrained women had a significantly higher restraint score derived from the DEBQ and higher restraint, uncontrolled and emotional eating scores derived from the TFEQ-18R, as well as flexible control of eating scores.

6.4.3 Twenty-four hour food recalls

EI was significantly higher on the 24h prior to the LEP leg compared with the HEP leg (2016±569 vs 1821±492 kcal, P=0.003), but no difference was observed in the percentage of energy provided by each macronutrient on the 24h prior to each appetite challenge day (HEP vs LEP).
6.4.4 Energy and macronutrient intake at the buffet meal

No order effects (of preloading) were found on buffet EI (kcal) (buffet EI after the HEP: 424.8±194.0 vs 341.0±216.5, P>0.05; buffet EI after the LEP: 580.3±322.2 vs 741.7±136.8, P>0.05 in those that started first with the HEP and the LEP, respectively).

EI at the buffet lunch after each preload (HEP vs LEP) in restrained and unrestrained women, according to the different questionnaires can be seen in Figure 6.2. Analysis of variance showed a main effect of preload on EI at the buffet lunch, with a significantly higher EI after the LEP compared with after the HEP, independently of the questionnaire used to measure restraint (RRS: 645±270 vs 361±200 kcal, P<0.01; DEBQ: 645±270 vs 391±200 kcal, P<0.01 and TFEQ-18R: 645±270 vs 391±200 kcal, P<0.01, respectively). Although no main effect of restraint or interaction was found, a tendency was observed for restrained women to eat more than unrestrained after the HEP and less after the LEP. This pattern was apparent regardless of the questionnaire used to measure restrained eating behaviour.

![Figure 6.2. Energy intake (kcal) at the buffet lunch after the HEP and LEP, in restrained (Rest) (n=8) and unrestrained (Unr) (n=7) women, according to different questionnaires. Results expressed as means ± SEM. Analysis of variance showed a main effect of preload (P<0.01 for all), but no main effect of restraint or interaction.](image-url)
No significant main effect of preload or restraint, or interaction, was found in the percentage of energy provided by each macronutrient, at the buffet meal, independently of the questionnaire used to measure restraint.

Although mean short-term energy compensation (%), at the buffet meal, was higher in unrestrained compared with restrained women, no significant difference was observed in short-term energy compensation (%) between the two groups, independently of the questionnaire used to classify restraint (RRS: 95.3±40.9 vs 48.4±83.3%, P=0.200; DEBQ: 93.4±38.5 vs 50.0±85.3%, P=0.239 and TFEQ-18R: 86.8±44.4 vs 55.8±85.7%, P=0.405, respectively).

6.4.5 Food choices at the buffet meal

No significant main effect of restraint or preload, or interaction, was found on the percentage of energy at the buffet lunch provided by healthy or unhealthy options, as well as by each food item, regardless of the questionnaire used to assess restraint. The only exception was in the percentage of energy, at the buffet meal, provided by cake, which was significantly higher after the LEP compared with after the HEP, independently of the questionnaire used to measure restraint (P<0.05 for all).
6.4.6 Cumulative energy intake

Cumulative energy intake, over a 24h period, after each preload (HEP vs LEP) in restrained and unrestrained women, according to the different questionnaires can be seen in Figure 6.3. Analysis of variance showed no main effects of preload or restraint, or interaction, on cumulative EI independently of the questionnaire used to classify restraint.

Figure 6.3. Cumulative energy intake (kcal) over a 24h period, after the HEP and LEP, in restrained (Rest) (n=8) and unrestrained (Unr) (n=7) women, according to different questionnaires. Results expressed as means ± SEM. Analysis of variance showed no main effect of preload or restraint, or interaction.
6.4.7 Subjective ratings of hunger and fullness

The AUC for hunger and fullness, from immediately before until 60 minutes after preload intake, in restrained and unrestrained women according to the different questionnaires, can be seen in Figure 6.4 and Figure 6.5. No significant main effect of preload or restraint, or interaction, was found in the AUC for hunger, independently of the questionnaire used to measure restraint. However, the magnitude of the mean difference in hunger ratings (AUC) between the two legs (LEP-HEP) was greater in restrained women, although this did not reach statistical significance (RRS: 82±165 vs 19±60 cm\*min, P>0.05; TFEQ-18R: 87±139 vs 14±108 cm\*min, P>0.05 and DEBQ: 72±160 vs 31±81 cm\*min, P>0.05 in restrained and unrestrained women, respectively).

Figure 6.4. AUC (0-60 minutes) for hunger after the HEP and LEP, in restrained (Rest) (n=8) and unrestrained (Unr) (n=7) women, according to different questionnaires. Results expressed as mean ± SEM. Analysis of variance showed no main effect of preload or restraint, or interaction.
No significant main effect of preload or restraint, or interaction, was observed in the AUC for fullness when restraint was measured by the RRS or the TFEQ-18R. However, a main effect of restraint was found in the AUC for fullness when the DEBQ was used, with higher fullness ratings being reported by restrained compared with unrestrained women (395±146 vs 205±157 cm*min, P=0.023), despite no main effect of preload or interaction. The magnitude of the difference in fullness ratings (AUC) between the two legs (HEP-LEP) was higher in restrained women, despite never reaching statistical significance (RRS: 84±108 vs -19±117 cm*min, P>0.05; TFEQ-18R: 71±104 vs -4±134 cm*min, P>0.05 and DEBQ: 70±104 vs -3±133 cm*min, P>0.05 in restrained and unrestrained women, respectively).

Figure 6.5. AUC (0-60 minutes) for fullness after the HEP and LEP, in restrained (n=8) (Rest) and unrestrained (n=7) (Unr) women, according to different questionnaires. Results expressed as mean ± SEM. Analysis of variance showed no main effect of preload or restraint, or interaction, when the RRS or the TFEQ-18R were used, but a main effect of restraint (P=0.023) when the DEBQ was used to measure restraint.

Subjective ratings of hunger/fullness over time, after the HEP and LEP, in restrained and unrestrained women, according to different questionnaires can be seen in Appendix XVII.
No significant main effect of preload or restraint, or interaction, was observed on prospective food consumption, regardless of the questionnaires used to measure restraint (data not shown). Moreover, no significant effect of preload, restraint, or interaction, was observed on preload palatability.

6.4.8 Cognitive and emotional state

From all the cognitive and emotional states assessed in this study, a significant main effect of preload or restraint, or interaction, was only found on the AUC for “guilt” (Figure 6.6).

![Figure 6.6. AUC (0-60 minutes) for “guilt” after the HEP and LEP, in restrained (Rest) (n=8) and unrestrained (Unr) (n=7) women, according to different questionnaires. Results expressed as means±SEM. Analysis of variance showed a main effect of preload when the RRS (P=0.032) and TFEQ-18R (P=0.031) were used and a main effect of preload (P=0.043) and restraint (P=0.029) when the DEBQ was used to classify restraint.](image)

A significant main effect of preload on the AUC for “guilt” was found when the RRS and TFEQ-18R were used (P=0.032 and P=0.031, respectively), with higher overall ratings in the HEP compared with the LEP condition. Despite no significant main effect of restraint or preload x restraint interaction, examination of the means revealed higher guilt ratings in restrained compared with unrestrained eaters, when the
RRS (114±128 vs 51±54 cm*min, P>0.05) and the TFEQ-18R (104±117 vs 62±86 cm*min, P>0.05) were used to classify restraint, especially after the HEP. When the DEBQ was used, a significant main effect of preload (P=0.043) and restraint (P=0.029) was found in guilt scores, with higher ratings being observed in restrained compared with unrestrained eaters (123±123 vs 40±51 cm*min, P=0.029) and after the HEP compared with after the LEP (109±133 vs 60±58 cm*min, P=0.043). The magnitude of the difference in guilt ratings (AUC) between the two legs (HEP-LEP) was higher in restrained women, despite never reaching statistical significance (RRS: 91.4±155.3 vs 0.3±17.7 cm*min, P>0.05; TFEQ-18R: 52.2±148.3 vs 45.1±89.1 cm*min, P>0.05 and DEBQ: 83.6±160.5 vs 9.2±11.8 cm*min, P>0.05 in restrained and unrestrained women, respectively).

6.4.9 Correlations between energy compensation, EI at the buffet lunch and cumulative EI, and eating behaviours

A significant negative correlation was observed between short-term energy compensation, at the buffet meal, and restraint when the RRS (r=-0.653, n=15, P<0.01), but not other scales (DEBQ: r=-0.261, n=15, P=0.347 and TFEQ-18R: r=-0.150, n=15, P=0.593) were used to classify restraint. No significant correlations were observed between energy compensation and the emotional or external subscales of the DEBQ, the uncontrolled and emotional subscales of the TFEQ-18R or the flexible and rigid dimensions of restraint.

No significant correlations were observed between measures of restraint: RRS, restraint subscales of the DEBQ and TFEQ-18R or flexible and rigid dimensions of restraint, and EI at the buffet lunch or cumulative EI after the HEP or LEP.

6.5 Discussion

The aim of the present study was to compare psychometric tools used to measure dietary restraint in their ability to predict disinhibited eating in response to preloading, in the laboratory, using a more “real” setting, closer to everyday life, by presenting the preloads clearly labelled with their energy content and a test-meal with a variety of foods, including healthy and unhealthy options. We were also interested in investigating
whether any disinhibition effect observed in the laboratory persisted over a 24h period in a free-living environment.

In contrast to our expectations, none of the questionnaires used to measure restraint was able to predict disinhibition in this sample of normal-weight women. In fact, a regulatory eating behaviour was observed, with a higher buffet EI after the LEP compared with after the HEP, when the RRS, the DEBQ and the TFEQ-18R were used to classify restraint. Moreover, no significant main effect of restraint was observed on EI at the buffet lunch. Conflicting results have been previously reported in two studies where participants’ perception of the energy content of the preload was manipulated (Spencer and Fremouw, 1979; Polivy, 1976). When normal-weight males were given a high or low energy pudding, and either correctly or incorrectly informed of its energy content, restrained men, classified according to the RRS, showed a tendency towards counter-regulation (P=0.07), eating more sandwiches after what they believed to be a HEP, regardless of its actual energy content. Unrestrained men ate approximately the same regardless of the condition (Polivy, 1976). It is possible that the narrow time interval between preload and test-meal (15 minutes) had prevented unrestrained men to adequately compensate in response to preloading and that high levels of disinhibition explain the counter-regulatory behaviour observed in restrained men. Similar results were obtained in underweight, normal-weight and overweight females using an ice-cream “taste test” after a 500 kcal preload presented either as a high- or low-energy drink (Spencer and Fremouw, 1979). Highly restrained women, according to the RRS counter-regulated after what they thought was a high-energy drink, while in unrestrained women, ice-cream intake was not affected by the cognitive manipulation (Spencer and Fremouw, 1979). However, both studies were limited by their parallel design.

The absence of disinhibition in the present study in restrained women, defined using pure measures of restraint, such as those derived from the DEBQ and TFEQ-18R, has been consistently reported elsewhere (Lowe and Kleifield, 1988; Ouwens et al., 2003). However, the ability of the RRS to predict disinhibition has been supported by earlier work, (Polivy, 1976; Hibscher and Herman, 1977), although not by more recent studies (Ruderman and Christensen, 1983; Westenhoefer et al., 1994; Ouwens et al., 2003). This inconsistency seem to be related, at least in part, to the fact that different
scales tend to identify specific populations of dieters: whilst the restraint subscale of the TFEQ and DEBQ tends to identify successful dieters, who have a low tendency towards disinhibition, the RRS tend to identify unsuccessful dieters, with a high tendency towards disinhibition and who are, therefore, more prone to counter-regulatory eating behaviour (Laessle et al., 1989a).

Various studies have tried to identify the best questionnaire to predict disinhibition in the laboratory (Jansen et al., 1988; Dritschel et al., 1993; Ridgway and Jeffrey, 1998; van Strien et al., 2000; Ouwens et al., 2003). All have used the taste-test paradigm, involving tasting and rating highly palatable, “diet breaking” food, after a preload/no preload condition. Although some have shown a trend towards disinhibition in restrained participants using the RRS (Jansen et al., 1988), no significant counter-regulatory behaviour has been reported using either the RRS, TFEQ or DEBQ (Dritschel et al., 1993; Ridgway and Jeffrey, 1998). Moreover, even though positive correlations have been reported between some measures of restraint (RRS and DEBQ) and food intake at the test-meal (van Strien et al., 2000), these have not always been replicated (Ouwens et al., 2003). No significant correlations were observed, in the present study, between measures of restraint (RRS, restraint subscales of the DEBQ and TFEQ-18R or flexible and rigid dimensions of restraint) and EI at the buffet lunch or cumulative EI after the HEP or LEP. There are, however, significant differences between the present study and those reported above, namely in the study design, participants’ body weight, type of disinhibitor, type of preload and test-meal and time between preload and test-meal. In order to eliminate any confounding effect of body weight and create a less artificial “eating” environment, only normal-weight women were accepted for this study and the test-meal consisted of an ad libitum buffet containing both healthy and unhealthy options commonly available in everyday life. This allowed the investigation of the impact of restraint not only on total EI, but also on macronutrient intake and food selection. This is the first study to compare different questionnaires designed to measure restraint in their ability to predict disinhibitory eating behaviour at a buffet test-meal, by using a LEP and HEP clearly labelled with their exact energy content, and simultaneously to assess motivation to eat and emotional and cognitive state in response to preloading. In every day life we are rarely given disguised food and we do have an idea, more or less exact, of the energy content of a
meal. For this reason, the use of clearly labelled preloads was thought to represent more accurately a free-living environment.

Although none of the questionnaires used was able to predict disinhibition in this study, a loss in short-term energy compensation, at the buffet meal, was observed with increased levels of restraint, when the RRS, but not the other questionnaires, were used to measure restraint. It was proposed, more than a decade ago, that a history of dieting would be more important in determining susceptibility to disinhibition than current dieting status or restraint level (Lowe, 1993), offering an explanation for the above findings. The RRS puts a great emphasis on weight fluctuation, dieting history and disinhibited eating, therefore, allowing the identification of those more susceptible to counter-regulatory eating behaviour (Herman and Polivy, 1980), as previously discussed. The restraint scale of the DEBQ and TFEQ, on the other hand, measures intention to restrict food intake and control over eating and therefore identify mainly those less prone to disinhibition (Laessle et al., 1989a).

One of the critical assumptions of the restraint theory is that in response to certain events, such as preloading, restrained eaters lose their commitment to dieting, and exhibit a counter-regulatory eating behaviour. Several factors may help to explain the absence of this behaviour in the present study. It is possible that the HEP, clearly labelled with its 607kcal, was not large enough to cause disinhibition of restraint. However, whilst every person has their own dietary boundaries, it is unlikely that this preload was too small to overcome the disinhibition threshold. Support for this comes from the analysis of participants’ cognitive state. Increased feelings of guilt were reported after the HEP compared with after LEP and in restrained compared with unrestrained eaters; although the last became only significant when the DEBQ was used to classify restraint. In this context, it would have been interesting to have included in this study a no preload or placebo control condition consisting of no energy (e.g. water or flavoured water). It is also possible that the use of a buffet as the test-meal, might have allowed restrained eaters to select healthy options, making the disinhibitory effect less apparent. However, when food items and food group choices were analysed, no significant differences were found between restrained and unrestrained women. Although previous studies have reported a higher consumption of healthy food in restrained women, using both, self-reported (Beiseigel and Nickols-Richardson, 2004)
and observational data (Rideout et al., 2004), none of these studies was carried out in a laboratory environment.

The lack of counter-regulatory eating behaviour in restrained women, in the present study, could also have been the result of relatively low median split scores on the different scales, resulting in a restrained group with relatively low levels of restraint. In studies that have used preloading as a disinhibitor, a trend seems to occur, with studies showing counter-regulation presenting, on average, higher RRS median splits (~15) than those showing compensation (~12) (Jansen et al., 1988). In the present study the median split was 13 for the RRS. It is, therefore, possible that the restrained group was not restrained enough, at least according to the RRS, which identifies unsuccessful dieters, more prone to disinhibition in response to preloading. Another possibility is that independently of the actual levels of restraint, restrained women in this study presented with low levels of disinhibition. It has previously been shown that only those who simultaneously present with high levels of restraint and disinhibition counter-regulate in response to preloading (Westenhoefer et al., 1994). Dietary restraint has been shown not to be an homogeneous construct and two independent dimensions have been identified: “rigid control” and “flexible control” (Westenhoefer, 1991). Those exhibiting a “pure” rigid control are more likely to show counter-regulatory eating behaviour, since they tend to set their cognitive diet boundary as a rigid point, which if passed means that further attempts to diet are hopeless, with consequent disinhibition and overeating. Restrained women, in the present study, presented with relatively low levels of “rigid control” (average of 5 in a scale ranging from 0 to 16) compared with “flexible control” (average of 7 in a scale ranging from 0 to 12). Moreover, although uncontrolled eating, an independent measure of disinhibition derived from the TFEQ-18R, was significantly higher in restrained compared with unrestrained women when the DEBQ and the TFEQ-18R were used to classify restraint, the average score in restrained women was of only 20, in a scale ranging from 9 to 36. It seems, therefore, that restrained women in this study presented with relatively low levels of disinhibition which may help to explain the absence of counter-regulation in response to preloading.

It has become a common practice to exclude restrained eaters from appetite studies, particularly those using the “preload-test-meal” paradigm (King and Blundell, 1995; Lluch et al., 1998; Westerterp-Plantenga et al., 1997; Mattes, 1996; Foltin et al.,
1992) based on the assumption that they exhibit an atypical eating behaviour in response to preloading. This has been done somewhat arbitrarily and no consensus exists either on the questionnaire or cut-off points to be used. Although the present investigation failed to show any disinhibition in restrained women and no significant differences were observed in energy compensation, at the buffet test-meal, between groups (despite a higher mean energy compensation in unrestrained eaters) this evidence cannot be used to support or condemn the exclusion of restrained eaters from appetite studies since this study was not powered to look at differences in energy compensation between restrained and unrestrained individuals.

No significant main effect of preload or restraint, or interaction, was found in the AUC for hunger, independent of the questionnaire used to measure restraint. However, restrained women reported feeling more satiated than unrestrained women, despite this becoming only significant when the DEBQ was used to classify restraint. Although the impact of restraint on subjective measures of hunger and fullness has not been extensively studied, the available evidence (Ogden and Wardle, 1991; Chapelot et al., 1995; Drapeau et al., 2005; St-Pierre et al., 2004), extensively discussed in the previous chapter and the findings of Chapter 5, do not support the hypothesis that restrained eaters feel hungrier or less satiated than unrestrained eaters and suggest that restrained individuals may even experience increased fullness, a finding in line with the present study.

To the best of our knowledge, only two studies have looked at the effect of restraint on EI, in response to preloading, outside the laboratory environment. Wardle & Beales (1987) showed that restrained women, according to the DEBQ, did not counter-regulate in the laboratory when presented with ice-cream, after a preload compared with a no preload condition, and neither did the potential disinhibitory effect created by preloading predict eating behaviour during the rest of the day. Neither restrained nor unrestrained eaters were able to reduce their food intake outside the laboratory to compensate for preloading, but also neither group counter-regulated (Wardle and Beales, 1987). Similar results were observed in a mixed sample of normal-weight men and women using the TFEQ to classify restraint (Burton-Freeman, 2005). The present study extended these findings by showing that preloading does not affect cumulative EI
over a 24h period either in restrained or unrestrained women using the RRS, the DEBQ or the TFEQ-18R to measure restrained eating behaviour.

The finding of a significantly higher EI on the 24 hours prior to the LEP comparing to the HEP leg is likely to be the main limitation of this study. However, all the participants had exactly the same breakfast on the morning of each appetite challenge day, which reduces the likelihood of this difference in 24h EI had impacted on EI an the buffet meal or subjective rating of hunger and fullness in response to preloading.

This study emphasizes the fact that counter-regulatory eating behaviour is a phenomenon only observed in a minority of restrained eaters and in very special conditions: when the food presented at the test-meal is good tasting and seen as “dieting-breaking”, when restraint is assessed by the RRS and when high levels of restraint are associated with high levels of disinhibition. This study also offers additional evidence that restraint should be seen as a spectrum and not a dichotomic variable: both groups showed a regulatory eating behaviour in response to preloading, with no significant differences between restrained and unrestrained women in short-term energy compensation, at the buffet meal. Although more research needs to be carried out looking at the impact of restraint on EB in response to acute or chronic exercise, a variable that has been shown to modulate the net impact of exercise on EB (Hill et al., 1995a; Lluch et al., 2000), the results of the present investigation suggest that restraint might have a relatively minor impact on eating behaviour.

In conclusion, none of the psychometric tools used in this study was able to predict disinhibition, in the short-term, when a more natural setting was created by the introduction of an ad libitum buffet test-meal. However, despite the inability of the RRS to predict disinhibition, the loss of compensation observed with increased levels of restraint was better forecasted by this scale. This tool may, therefore, be the best in energy-compensation studies. Outside the laboratory environment neither restrained nor unrestrained participants exhibited a disinhibitory eating behaviour, but they were also not able to compensate for the extra load created by the HEP. Although more studies are needed involving different test-meals, in the absence of “diet breaking” foods, the role of restraint in predicting disinhibition seems to be minor.
Chapter Seven
Chapter 7. General discussion

The four pieces of experimental work described in this thesis were primarily designed to investigate the role of exercise, both in the short- and long-term, and restrained eating behaviour on appetite control in man. This dissertation follows a temporal sequence, where the results of one study prompted further investigation into the complex mechanisms involved in appetite regulation. The limitations, relevance and implications of the present findings will be discussed here.

7.1 Methodological considerations in the measurement of appetite

As extensively discussed in the first chapter of this thesis, eating behaviour is a very complex phenomenon which results from constant physiological and environmental inputs (Blundell, 1991; Blundell and Halford, 1994). Appetite in particular, as an abstract concept used to explain it, embraces a wide range of parameters; both subjective, such as the sensation of hunger, and objective, such as the selection of specific foods (Blundell, 1991; King et al., 1997b), which determine what, how much and when we eat. This makes appetite and human eating behaviour very difficult phenomena to be measured. One of the dilemmas in the field of appetite research is the experimental context, where a compromise has to reached between "precision" and "naturalness" (Hill et al., 1995b). Every effort was made to conduct the studies described in this thesis under controlled conditions in a standardized laboratory environment. Even though this type of approach may lack external validity, a "naturalistic" approach, where eating behaviour is studied in a free-living environment, may make it harder to establish the effect of a particular experimental manipulation, and therefore lack internal validity (Hill et al., 1995b; Stubbs et al., 1998).

In the laboratory environment, the "preload-test-meal" paradigm (Kissileff, 1985) has been consistently used in the area of appetite research to calculate "energy compensation", a measure of short-term appetite control. This paradigm was systematically used throughout this thesis to assess the short-term effect of preloads differing in their energy content and/or cognitive "message" on motivation to eat (using VAS) and subsequent food intake at a test-meal. The large inter-individual variation in
energy compensation, described throughout this thesis, underlines the complexity of the compensatory mechanisms and the involvement of several factors in short-term appetite control. In chapters 3, 4 and 6 of this thesis a mixed food test-meal (buffet lunch) was used, while in chapter 5 the test-meal consisted of a single food (pasta-based lunch). Multi-item test-meals have been criticised by their lack of external validity (Hill et al., 1995b) and it has been suggested that food intake declines as the study progresses due to the loss of the “novelty” effect, something which would not happen with a single food test-meal (Long, 2000). However, no significant difference in energy compensation was observed after two covertly manipulated preloads (241 vs 601 kcal) between a buffet and a pasta-based test-meal (similar to the ones used in this thesis), despite a tendency towards over-consumption with the buffet test-meal (Long, 2000). Overall, an accurate measurement of energy compensation in response to manipulated preloads can be achieved with both single-item and multi-item test-meals, with the advantage that the latter allows the additional measurement of food and macronutrient selection.

EI has been shown to vary during the menstrual cycle, probably as a result of hormonal fluctuations, with the premenstrual phase seen as a time when women are especially vulnerable to over-consumption and food cravings. The time of ovulation coincides with the nadir of food intake and EI has been shown to be higher in the post-ovulatory compared with the pre-ovulatory phase (Cross et al., 2001; Dye and Blundell, 1997). This last trend becomes stronger in women with premenstrual syndrome (PMS) (Cross et al., 2001), but is not observed in those taking oral contraceptives or those with anovulatory cycles (Dye and Blundell, 1997) or highly restrained eating behaviour (Schweiger et al., 1992). These changes in EI seem to parallel changes in metabolic rate (Dye and Blundell, 1997; Bisdee et al., 1989b) and metabolism (substrate utilization) (Bisdee et al., 1989a) across the menstrual cycle. Although CHO cravings have been reported as common in the premenstrual phase, especially in women with PMS (Dye and Blundell, 1997), patterns of macronutrient selection across the menstrual cycle seem to be rather inconsistent (Cross et al., 2001; Dye and Blundell, 1997). A limitation of the studies described in this thesis is, therefore, likely to be the lack of adjustment for the timing of reproductive cycle in women. However, in studies that involve more than one visit adjusting for the phase of menstrual cycle may become a real challenge. In face of the available evidence (described above) and schedule of the study days (two
days apart in Chapters 3 and 6 and one week apart on Chapters 4 and 5) it is thought that the changes in hormonal levels associated with the menstrual cycle, between study days, was likely to be too small to have a significant impact on energy and macronutrient intake.

Finally, any study that relies on self-reported food intake is susceptible to error, and this is likely to be another limitation of the studies described in this dissertation, in particular chapter 3 where a significant proportion of the participants was found to underreport at the end of the study. Errors could have occurred due to misreporting, either intentional or not, or altered eating patterns towards a more “respected” behaviour (Stubbs et al., 1998).

### 7.2 Exercise and appetite control

The initial objective of the present PhD was to elucidate whether the improved short-term appetite response to covert preload energy manipulation observed in active compared with sedentary men in Long and colleagues study (2002) was indeed the result of differences in PA levels and to try to establish the mechanisms behind such differences. This was attempted in Chapter 3 by using a longitudinal design and assessing short-term appetite control at baseline, in sedentary individuals, and after a six-week exercise intervention. The results described in this chapter support previous cross-sectional evidence for a link between PA levels and appetite regulation (Long et al., 2002; van Walleghen et al., 2007), and show, for the first time, that exercise improves short-term appetite control by leading to a more sensitive eating behaviour in response to previous EI, both acutely, at lunch time, and for the next 24 hours. Whilst at baseline, sedentary participants were unable to adjust their subsequent EI in response to preload energy manipulation, after a six-week exercise intervention, EI after the HEP was significantly lower than after the LEP. These findings suggest that the role of exercise on EB extends far beyond its ability merely to increase EE, and includes an indirect effect, modulating food intake towards a more “accurate” eating behaviour in response to previous feeding. Although more studies are needed covering a longer period of time (next 48 hours or one week), the improvement in short-term appetite regulation observed with exercise may help to promote EB in the long-term and a “healthy” body weight in physical active individuals.
Despite the absence of a significant exercise \times output \times gender interaction on buffet EI, secondary analysis showed that only men improved in short-term appetite control, at the buffet meal, in response to exercise. Although this study was not powered to look at male/female differences and more research is clearly needed in this area, the present findings suggest a gender difference in the response to exercise in terms of short-term appetite control. This is in line with previous literature showing a gender pattern in the response to exercise, both acutely, with women up-regulating their EI in response to exercise, in contrast to men (Imbeault et al., 1997; Thompson et al., 1988; Pomerleau et al., 2004), and chronically, in terms of weight loss after an exercise programme, with men losing more weight than women (in absolute terms and also as a percentage of total body weight) (Garrow and Summerbell, 1995; Donnelly et al., 2003; Saris et al., 2003).

The study described in Chapter 3 was not designed to identify the mechanisms behind the improvement in short-term appetite regulation observed with exercise. It was subsequently hypothesised that this improvement could have resulted from changes in the release of post-ingestive satiety signals from the GI tract in the postprandial period. The effect of long-term exercise on the plasma levels of GI hormones involved in appetite control remains, however, largely unknown. At the acute level, the few available studies are limited by the fact that they have not been performed in the context of appetite and have looked, in its majority, only at the impact of exercise on the fasting levels of gut peptides, such as CCK (Bailey et al., 2001), GLP-1 (O'Connor et al., 1995; O'Connor et al., 2006) and PP (Sullivan et al., 1984; Hilsted et al., 1980; O'Connor et al., 1995). This lack of information regarding the impact of exercise on appetite-related hormones prompted the design of the study described in Chapter 4. Here it was shown, for the first time, that an acute bout of moderate intensity exercise, performed in the fed-state, significantly increases the plasma levels of the satiety hormones: PYY, GLP-1 and PP, has no impact on ghrelin, and leads to a temporary reduction in self-reported hunger, suggesting that acute exercise does not trigger compensatory responses, at least at the level of GI hormones involved in appetite regulation, that would lead to an increase in hunger and/or EI in the short-term. PYY plasma levels in response to exercise had never been measured before and although running had been shown to increase GLP-1 plasma levels (O'Connor et al., 1995; O'Connor et al., 2006), these studies were performed in the fasting state, involved high-intensity exercise and were...
carried out in athletes. An increase in PP plasma levels in response to exercise had already been reported, both in fasting (Sullivan et al., 1984; Hilsted et al., 1980) and postprandially (Greenberg et al., 1986), which is in line with the findings of the present investigation.

The mechanism by which acute exercise increases the plasma levels of these satiety gut peptides (PYY, GLP-1 and PP) is not known. However, metabolic and endocrine changes in response to exercise may act as a stimulus for their release. Increased release of catecholamines, as a result of the activation of the sympathetic nervous system, has already been implicated in the increase in PP plasma levels observed with exercise (Gingerich et al., 1979). In addition, although several mechanisms have been proposed to explain “exercise induced anorexia” (Leon et al., 1979; King et al., 1997b; Westerterp-Plantenga et al., 1997), the reason for this phenomenon remains unknown. Evidence was presented and discussed in Chapter 4 for a potential synergistic role of PYY, GLP-1 and PP on this phenomenon. Even though the satiety effects of these hormones are generally only seen at pharmacological levels (Batterham et al., 2003a; Degen et al., 2005), with the exception of GLP-1, where effects have been shown at the top of the physiological level (Flint et al., 1998), there is evidence to suggest that they might have acted additively (Batterham et al., 2003a; Neary et al., 2005) in reducing hunger feelings during exercise. More research is needed to clearly establish the precise role of these gut peptides in the suppression of hunger in response to exercise.

Even though one hour of moderate intensity exercise resulted in a significant increase in absolute EI, at the buffet meal, once the energy expended during exercise had been accounted for, a reduction in REI was observed, with the attainment of a short-term negative EB, which is line with the literature (King et al., 1997b). The extraordinary and completely new findings of this study are that this increase in absolute EI in response to exercise occurred after a transitory increase in satiety peptides (while exercise was performed) and in the absence of any significant difference, between study legs, in hunger sensations, or the plasma levels of any of the appetite-related hormones measured, immediately before the buffet test-meal. These findings reinforce the beneficial role of exercise in creating a negative EB and help to dispel the widespread belief that exercise leads to acute compensatory responses in
some “blood hormones” that would lead to an increase in hunger and food intake in the short-term. The increase in absolute EI in response to acute exercise, observed in this study, is likely to be the result of cognitive factors including attitudes and beliefs associated with exercise, such as “food rewards for exercising” and the belief that “exercise increases appetite” (King, 1999). The findings from these two studies (Chapters 3 and 4) strengthen the concept that exercise has the ability to create a negative EB and reinforces its role in appetite control and weight maintenance. Additional research is required to determine whether similar results occur in overweight/obese individuals and in the longer-term.

Although the efficacy of exercise, alone, in weight loss is relatively low, exercise has been shown to be crucial in preventing weight gain and regain in the long-term (Bensimhon et al., 2006), therefore reinforcing the importance of incorporating exercise in any plan designed to reduce or maintain body weight in the longer term. It can be hypothesized that the increased benefits of exercise on weight maintenance result from an improvement in appetite control, with an increased ability to adjust food intake accordingly, in response to previous EI. The present findings are extremely important in the light of the steady increase in the prevalence of obesity (HSE, 2005) and the reduced levels of PA in the UK (Department of Health, 2004) and bring additional evidence to the need to meet the current national PA targets. The beneficial impact of exercise on appetite regulation, described here, strength the choice of exercise as the first line option for the prevention of both weight gain and regain in the long-term.

### 7.3 Restrained eating behaviour and appetite control

In the present environment where highly palatable energy-dense food is increasingly abundant, with consequent chronic activation of the hedonic appetite system and risk of over-consumption, an increased proportion of the Western population has been actively adopting a restrained eating behaviour (Lowe and Levine, 2005). A substantial amount of evidence has been accumulating over the last three decades suggesting a link between restrained eating behaviour and altered metabolic (Laessle et al., 1989b; Hibscber and Herman, 1977) and endocrine pathways (Keim and Horn, 2004; Pirke et al., 1990; Burton-Freeman, 2005), which would again put restrained individuals at an increased risk for weight gain. This could explain from a
physiological, and not strictly from a psychological (cognitive) point of view, why restrained eaters need to restrict their food intake, in order to maintain their body weight within a social desirable range. Secondary analysis of the data originated from Chapter 4 showed a trend towards lower levels of PYY in the postprandial state in restrained eaters, which could lead to reduced fullness and, potentially, an increased risk for overeating, adding more evidence to the existing findings.

The findings originated from Chapter 4 and the non-existence of any studies looking at the impact of restraint on PYY plasma levels and other appetite-related hormones and metabolites, apart from CCK (Burton-Freeman, 2005), prompted the design of the study described in Chapter 5. In contrast to the findings of Chapter 4, the results of this chapter do not support a link between restraint and abnormal levels of fasting or postprandial TAG or PYY. However, significantly lower fasting insulin plasma levels and lower glucose and insulin plasma levels, in the postprandial state, were observed in restrained individuals, together with a significantly higher fasting and postprandial Si and higher fullness ratings.

The absence of a significant effect of restraint on fasting and postprandial levels of PYY was rather disappointing since this study was specifically powered to detect it, based on pilot data, and a previous study supported a link between restraint and a blunted release of CCK, an hormone with similar satiety properties to PYY, in the postprandial state (Burton-Freeman, 2005). Even though this investigation did not support any significant impact of restraint on PYY plasma levels, it does not invalidate the concept that restraint may be an important factor in determining the release of other appetite-related hormones and more research is needed in this area. This study was also not able to confirm the previous finding of increased levels of fasting TAG in restrained eaters (Laessle et al., 1989b). However, it is possible that this increase in TAG levels had resulted from factors other than restraint itself, namely intermittent food restriction, body weight and disinhibition levels.

The reduced fasting insulin plasma levels in restrained eaters found here is in line with previous research carried out in women (Pirke et al., 1990; Keim and Horn, 2004), and the increased fasting Si had also been reported before, although body weight was a confounder in that study (Keim and Horn, 2004). This, together with the increased postprandial Si observed in the present study, confirms and reinforces earlier
findings and extends them to a mixed sample of men and women. Although the reasons for the increased S_t observed in restrained eaters are not known, body weight and composition, weight fluctuations and PA levels are unlikely to be the main drivers, since no differences were found between groups in these variables. Macronutrient composition of the diet, on the other hand, may be a potential mechanism. The study described in Chapter 5 was not designed to look at differences in macronutrient intake between restrained and unrestrained eaters and the available evidence (Laessle et al., 1989b; Klesges et al., 1992; Lawson et al., 1995; De Castro, 1995; Beiseigel and Nickols-Richardson, 2004; Rideout et al., 2004) is inconclusive in that respect. The mechanisms involved in the better glucose tolerance (lower postprandial plasma levels of glucose and insulin) observed in restrained eaters is also not known, however, lower postprandial noradrenaline in restrained eaters (Pirke et al., 1990) may be a potential mechanism.

Another striking finding of Chapter 5 was increased fullness in restrained eaters. This was rather unexpected since no difference in postprandial PYY plasma levels was observed between the two groups, postprandial glucose and insulin plasma levels were significantly lower and a previous investigation had reported a blunted release of CCK in restrained eaters (Burton-Freeman, 2005). It has been proposed that restrained eaters may be in a state of “perceived deprivation” as a result of restricting the intake of palatable food (eating less than they want instead of less than they need), and, for that reason, experience a constant feeling of hunger (Lowe and Levine, 2005). However, the available evidence (Ogden and Wardle, 1991; Chapelot et al., 1995; Drapeau et al., 2005) and the findings of this investigation do not support the idea that restrained eaters feel hungrier or less satiated than unrestrained eaters and suggest that restrained individuals may even experience increased fullness. Moreover, fasting plasma levels of ghrelin, a peptide with orexigenic properties, likely to be involved in meal initiation (Cummings et al., 2001) was found not to be associated with restrained eating behaviour (St-Pierre et al., 2004).

In spite of this evidence and the existence of one study suggesting that restrained eaters may be more sensitive to the satiety effects of high-energy food (Ogden and Wardle, 1991), no investigation had until now clearly showed increased fullness in restrained eaters irrespective of the preload. Moreover, in the study described in Chapter
6, restrained women reported feeling more satiated than unrestrained women, despite this only becoming statistically significant when the DEBQ was used to classify restraint. In both studies (Chapters 5 and 6), participants were provided with both external and internal cues that regulate feeding behaviour: the preloads carried out a different cognitive message (one vs two mugs of hot chocolate and milkshakes clearly labelled with their exact energy content, respectively) and induced different physiological responses (500 vs 1000 kcal and 246 vs 607 kcal, respectively). Although the original restraint theory had proposed that restrained eaters suffer from a weak sensitivity to physiological cues that regulate food intake and an over-reliance on cognitive cues (Heatherton et al., 1989), experiencing hunger only when very deprived and reaching fullness much later than unrestrained eaters (Ruderman, 1986), this model was shortly dismissed by Ogden & Wardle (1990), who showed that restrained eaters are not less sensitive to internal cues, but do have an increased sensitivity to external cues. The increased fullness observed in restrained eaters in these two studies (Chapter 5 and 6) seems, therefore, to be the result of an increased sensitivity to the cognitive, rather than physiological cues that regulate food intake.

Despite the present findings showing no impact of restraint on PYY plasma levels and increased fullness in restrained eaters, previous research has provided some evidence for metabolic (Tuschl et al., 1990; Westerterp-Plantenga et al., 1992; Keim and Horn, 2004) and endocrine (von Prittwitz et al., 1997; Laessle et al., 2000; Adami et al., 2002; Burton-Freeman, 2005) abnormalities that could put restrained eaters at an increased risk for weight gain and therefore explain their cognitive behaviour. However, as suggested by Montzoros (1997), it is also possible that this pattern is a consequence of the intermittent acute energy restriction characteristic of restrained eaters (Mantzoros, 1997). Unfortunately, the relationship between restraint and obesity is rather complex. Although restrained eating has been shown to be strongly associated with measures of adiposity in normal-weight, but not in obese individuals (Beiseigel and Nickols-Richardson, 2004; de Lauzon-Guillain et al., 2006; Provencher et al., 2003), the effect of dietary restraint on body weight, in the long-term, remains controversial. Some studies show a positive association between dietary restraint at baseline and weight gain one year later in women but not in men (Klesges et al., 1992), while others show that restraint does not promote weight gain over a two-year period (de Lauzon-Guillain et al., 2006). The complexity of the association between restraint and adiposity may be
explained, at least in part, by the fact that restraint is not a homogenous construct. It has been shown that while restraint's rigid construct is positively associated with BMI, restraint's flexible construct is negatively associated with BMI (Provencher et al., 2003). Interestingly, higher values of adiposity at baseline were shown to predict a bigger increase in cognitive restraint two years later, suggesting that restraint may be an adaptive mechanism to fight against weight gain (de Lauzon-Guillain et al., 2006). Pirk and colleagues (1990) suggested that only studies where restrained eaters are asked to change their behaviour to unrestrained, or the other way around, will be able to clearly determine if the metabolic and endocrine abnormalities observed, in some studies, including the altered glucose metabolism observed in the present investigation (Chapter 5), are a cause or a consequence of their behaviour. However, restraint is a very complex cognitive state and, therefore, the feasibility of such studies is questionable.

A pertinent question that arose since the beginning of this PhD relates do the identification of the best questionnaire to be used in appetite studies, particularly those where energy compensation is a primary or secondary outcome, in order to predict disinhibition, with the aim of excluding those participants at risk of counter-regulatory eating behaviour. Counter-regulation has been traditionally associated with restraint (Herman and Mack, 1975; Herman and Polivy, 1980; Polivy, 1976; Woody et al., 1981; Rotenberg and Flood, 2000; Hibscher and Herman, 1977), however, an important number of studies have failed to support this relationship (Wardle and Beales, 1987; Jansen et al., 1988; Lowe and Kleifield, 1988; Ogden and Wardle, 1990; Ogden and Wardle, 1991; Dritschel et al., 1993) and a trend has been identified with only studies using the RRS to measure restraint and ice-cream as the test-meal being able to shown such behaviour in restrained eaters.

The fact that even though several studies have tried to identify the best questionnaire to predict disinhibition in the laboratory (Jansen et al., 1988; Dritschel et al., 1993; Ridgway and Jeffrey, 1998; van Strien et al., 2000; Ouwens et al., 2003), none has produced conclusive results and are all limited by the use of a single, highly palatable food, usually seen as “diet breaking”, as the test-meal, which clearly does not illustrate real life, prompted the study described in Chapter 6. In contrast to the expectations, none of the questionnaires used to measure restraint (RRS, DEBQ and TFEQ-18R) was able to predict disinhibition in normal-weight women, when a more
“natural” setting was used by presenting participants with a buffet test-meal. In fact, a regulatory eating behaviour was observed in both restrained and unrestrained eaters, independently of the questionnaire. Although the absence of disinhibition in restrained women, defined using pure measures of restraint: DEBQ and TFEQ-18R, has been consistently reported elsewhere (Lowe and Kleifield, 1988; Ouwens et al., 2003), the same does not apply for the RRS, with some studies reporting disinhibition in restrained eaters (Polivy, 1976; Hibscher and Herman, 1977) and others not (Ruderman and Christensen, 1983; Westenhoefer et al., 1994; Ouwens et al., 2003). This different performance among questionnaires seems to be related, at least in part, to the fact that different scales identify specific populations of dieters: TFEQ and DEBQ tend to identify successful dieters, with lower tendency towards disinhibition, while the RRS tend to identify unsuccessful dieters, with a high tendency towards disinhibition and who are, therefore, more prone to counter-regulatory eating behaviour (Laassle et al., 1989a).

Counter-regulation was also not observed in the study reported in Chapter 5, using both the DEBQ and the TFEQ-18R as measures of restraint, when a pasta-meal was presented three hours after preloading, simulating a second meal effect. It can, therefore, be hypothesized that in the absence of “diet-breaking” food, counter-regulation is not expected to occur, independently of the questionnaire used to measure restraint. The results of these two studies suggest a minor role for restraint in predicting disinhibition when a more “natural” setting is created. The question as to whether to include or exclude restrained eaters from appetite studies, based on their atypical eating behaviour, deserves further investigation and none of the studies reported here can be used to address such issue. Moreover, it has also been suggested that the level of restraint can modulate the effects of exercise on EB (Hill et al., 1995a) and acute exercise has previously been shown to be more effective in creating a negative EB in restrained compared with unrestrained eaters (Lluch et al., 2000). However, the interaction between restraint and exercise in determining EI in the post-exercise period remains controversial (King et al., 1996; Lluch et al., 1998) and is probably dependent on disinhibition levels.

Although the present findings (Chapters 5 and 6) and previous observations (Polivy, 1976) may support the inclusion of restrained eaters in appetite studies, when
“diet-breaking” foods are not involved, since no counter-regulatory behaviour has been found in these circumstances, restrained eaters may compensate differently, even if they do not counter-regulate. Even though no significant differences were observed between restrained and unrestrained eaters in short-term energy compensation in Chapter 5 and 6 (restrained eaters compensated slightly better than unrestrained eaters in chapter 5, but the opposite in chapter 6) none of these studies was designed to look at such differences. More research is, therefore, needed in this area and also on the impact of restraint on EI in response to exercise, for any firm conclusions to be reached. However, despite the inability of the RRS to predict disinhibition in chapter 6, a significant negative correlation was observed between energy compensation and restraint when using the RRS, but not the other scales, suggesting that the loss of compensation observed with restraint may be better forecasted by this scale and that this tool may be the best option in studies aiming to measure energy compensation.

Overall, counter-regulatory eating behaviour in response to preloading has been shown to be dependent on restraint as much as on disinhibition levels, with only those scoring high on both showing such behaviour (Westenhoefer et al., 1994). It can, therefore, be suggested that disinhibition, as much as restraint levels, need to be accounted for when questioning about the inclusion/exclusion of specific individuals in/from appetite studies. However, the available evidence and the results discussed here suggest a minor role of restraint in predicting disinhibition. This does not mean, nevertheless, that restraint is not an important factor to be taken into account in appetite studies, particularly in those where glucose metabolism is an outcome, since, as shown in Chapter 5 restraint is associated with reduced fasting insulin levels and improved glucose tolerance (lower plasma levels of glucose and insulin in the postprandial state), as well as increased fasting/postprandial SI. The overall evidence points out to restraint being a spectrum and not a dichotomic variable: restrained eaters do not necessarily show a diametrically opposite behaviour to unrestrained, but they may show a slightly or significantly different behaviour depending on the characteristics of the sample (levels of restraint and disinhibition) and the study design (type of preload and test-meal and questionnaire used to measure restraint).
7.4 Conclusions

This thesis has addressed the impact of exercise and restrained eating behaviour on short-term appetite regulation. Exercise has been shown to have beneficial effects on short-term appetite control, by enabling a more sensitive eating behaviour in response to previous EI. Moreover it does not appear to prompt any acute physiological adaptations that would lead to an increase in appetite and/or EI in response to increased EE. These positive effects of exercise in short-term appetite control are likely to extend to the long-term and contribute to a more favourable EB and body weight in physical active individuals. Restrained eating behaviour does not seem to impact on PYY plasma levels, but is associated with increased fullness and is likely to be involved in glucose metabolism. The role of restraint in predicting disinhibition in the laboratory seems, however, to be minor, at least when a less “artificial” setting is created.

7.5 Future work

The four pieces of work described throughout this thesis yielded very interesting results, but have also raised numerous questions that could not be answered in the timeframe available. These questions may form the basis for future research and hopefully elucidate further the role of exercise and restrained eating behaviour in appetite control and metabolism in general. Some examples of future work prompted by the findings of this thesis are described below:

A. To elucidate the optimal intensity and duration of exercise necessary to achieve the greatest improvement in short-term appetite control. Changes in energy compensation would be evaluated, using the “preload-test-meal” paradigm, before and after exercise interventions of different intensities and durations.

B. To determine if the improvement in short-term appetite control reported in normal-weight sedentary volunteers in response to a six-week exercise programme is also observed in overweight/obese individuals and to elucidate further the mechanisms whereby exercise improves short-term appetite regulation by measuring the release of several appetite-related hormones and metabolites, in fasting and postprandially, before and after an exercise intervention.
C. To investigate whether there is a gender difference in short-term appetite control in response to chronic exercise.

D. To determine whether the increase in satiety peptides observed in response to acute exercise in normal-weight volunteers is also observed in overweight/obese individuals together with the associated changes in subjective and objective measures of appetite.

E. To investigate whether Si is a predictor of energy compensation in response to acute exercise and, as a result, of the overall impact of exercise on EB. EI, at a test-meal and for the next 48h, in response to acute exercise and resting, would be investigated in “healthy” normal-weight and overweight individuals with different levels of Si, using a randomised crossover design.

F. To investigate the impact of restraint on the postprandial release of other appetite-related hormones such as GLP-1 and PP.

G. To investigate the impact of restraint on Si using proper measures such as the “hyperinsulinemic euglycemic clamp”.

H. To determine if restraint has a significant impact on energy compensation, even in the absence of counter-regulatory eating behaviour, using different preloads and test-meals. This study would help to establish the need (or not) to exclude highly restrained eaters from appetite studies where energy compensation is an outcome.
Publications
Publications

Full papers


Abstracts


References
References


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Appendices
Appendix I. Restrained Revised Scale

Please read the questions as carefully and honestly as possible and underline or circle the answer that best applies to you.

1. How often are you dieting?
   *Never* (0) / *rarely* (1) / *sometimes* (2) / *often* (3) / *always* (4)

2. What is the maximum amount of weight that you have ever lost within one month?
   *0-4lbs (0-1.8kg)* (0) / *5-9lbs (1.9-4.1kg)* (1) / *10-14lbs (4.2-6.4kg)* (2) / *15-19lbs (6.4-8.6kg)* (3) / *≥20lbs (>8.7kg)* (4)

3. What is your maximum weight gain within a week?
   *0-1lbs (0-0.5kg)* (0) / *1.1-2lbs (0.6-0.9kg)* (1) / *2.1-3lbs (1.0-1.4kg)* (2) / *3.1-5lbs (1.5-2.3kg)* (3) / *≥5.1lbs (≥2.4kg)* (4)

4. In a typical week, how much does your weight fluctuate?
   *0-1lbs (0-0.5kg)* (0) / *1.1-2lbs (0.6-0.9kg)* (1) / *2.1-3lbs (1.0-1.4kg)* (2) / *3.1-5lbs (1.5-2.3kg)* (3) / *≥5.1lbs (≥2.4kg)* (4)

5. Would a weight fluctuation of 5lb (2.3kg) affect the way you live your life?
   *Not at all* (0) / *slightly* (1) / *moderately* (2) / *very much* (3)

6. Do you eat sensibly in front of others and splurge alone?
   *Never* (0) / *rarely* (1) / *often* (2) / *always* (3)

7. Do you give too much time and thought to food?
   *Never* (0) / *rarely* (1) / *often* (2) / *always* (3)

8. Do you have feelings of guilty after overeating?
   *Never* (0) / *rarely* (1) / *often* (2) / *always* (3)

9. How conscious are you of what you are eating?
   *Not at all* (0) / *slightly* (1) / *moderately* (2) / *extremely* (3)

10. How many pounds over your desired weight were you at your maximum weight?
    *0-1 (0-0.5kg)* (0) / *1-5 (0.6-2.3kg)* (1) / *6-10 (2.4-4.5kg)* (2) / *11-20 (4.6-9.1kg)* (3) / *≥21 (>9.2kg)* (4)

**The score (in red) of questions 1 to 10 is added together to obtain the total score.**
Appendix II. Three Factor Eating Questionnaire 18 items revised

Please read the questions as carefully and honestly as possible and underline or circle the answer that best applies to you.

1. When I smell a sizzling steak or a juicy piece of meat, I find it very difficult to keep from eating even if I have just finished a meal.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

2. I deliberately take small helpings as a means of controlling my weight.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

3. When I feel anxious, I find myself eating.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

4. Sometimes when I start eating, I just can't seem to stop.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

5. Being with someone who is eating often makes me hungry enough to eat also.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

6. When I feel blue, I often overeat.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

7. When I see a real delicacy, I often get so hungry that I have to eat right away.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

8. I get so hungry that my stomach often seems like a bottomless pit.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

9. I am always hungry enough to eat at any time.
   *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

10. When I feel lonely, I console myself by eating.
    *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

11. I consciously hold back at meals in order not to gain weight.
    *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

12. I do not eat some foods because they make me fat.
    *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*

13. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.
    *Definitely true (4) / mostly true (3) / mostly false (2) / definitely false (1)*
14. How often do you feel hungry?  
   Only at mealtimes (1) / sometimes between meals (2) / often between meals (3) / almost always (4)  

15. How frequently do you avoid “stocking up” on tempting foods?  
   Almost never (1) / seldom (2) / usually (3) / almost always (4)  

16. How likely are you to consciously eat less than you want?  
   Unlikely (1) / slightly likely (2) / moderately likely (3) / very likely (4)  

17. Do you go on eating binges though you are not hungry?  
   Never (1) / rarely (2) / sometimes (3) / at least one a week (4)  

18. On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never “giving in”), what number would you give yourself?  
   1-2 (1)  
   3-4 (2)  
   5-6 (3)  
   7-8 (4)  

**How to score (scoring system at red):**  
Cognitive Restraint - add the score from questions 2, 11, 12, 15, 16 and 18  
Uncontrolled eating - add the score from questions 1, 4, 5, 7, 8, 9, 13, 14 and 17  
Emotional eating – add the score from questions 3, 6 and 10
Appendix III. Dutch Eating Behaviour Questionnaire

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

<table>
<thead>
<tr>
<th>Question</th>
<th>Never</th>
<th>Seldom</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
<th>Not relevant</th>
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</thead>
<tbody>
<tr>
<td>1. If you have put on weight, do you eat less than you usually do? *</td>
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<td>2. Do you have a desire to eat when you are irritated? *</td>
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<td>3. If food tastes good to you, do you eat more than you usually do? *</td>
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<td>4. Do you try to eat less at meal times than you would like to eat? *</td>
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<td>5. Do you have a desire to eat when you have nothing to do? *</td>
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<td>6. Do you have a desire to eat when you are depressed or discouraged? *</td>
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<td>7. If food smells and looks good, do you eat more than you usually eat? *</td>
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<td>8. How often do you refuse food or drink offered because you are concerned about your weight? *</td>
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<td>9. Do you have a desire to eat when you are feeling lonely? *</td>
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<td>10. If you see or smell something delicious, do you have a desire to eat it? *</td>
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<td>11. Do you watch exactly what you eat? *</td>
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<td>12. Do you have a desire to eat when somebody lets you down? *</td>
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<td>13. If you have something delicious to eat, do you eat it straight away? *</td>
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<td>14. Do you deliberately eat foods that are slimming? *</td>
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<td>15. Do you have a desire to eat when you are cross? *</td>
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<td>16. Do you have a desire to eat when you are approaching something unpleasant to happen? *</td>
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<td>17. If you walk past the baker do you have a desire to buy something delicious? *</td>
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<td>18. When you have eaten too much, do you eat less than usual the following days? *</td>
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<td>19. Do you get a desire to eat when you are anxious, worried or tense? *</td>
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<tr>
<td>20. If you walk past a snack bar or café, do you have a desire to buy something delicious? *</td>
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<td>21. Do you deliberately eat less in order not to become heavier? *</td>
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<td>22. Do you have a desire to eat when things are going against you, or things have gone wrong? *</td>
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<td>23. If you see others eating, do you have also the desire to eat? *</td>
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<td>24. How often do you try not to eat between meals because you are watching your weight? *</td>
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<td>25. Do you have a desire to eat when you are frightened? *</td>
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<td>26. Can you resist eating delicious food? *</td>
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<td>27. How often in the evening do you try not to eat because you are watching your weight? *</td>
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<td>28. Do you have a desire to eat when you are disappointed? *</td>
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<td>29. Do you eat more than usual when you see other eating? *</td>
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<tr>
<td>30. Do you take your weight into account when you eat? *</td>
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<tr>
<td>31. Do you have a desire to eat when you are emotionally upset? *</td>
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<tr>
<td>32. When preparing a meal are you inclined to eat something? *</td>
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<tr>
<td>33. Do you have a desire to eat when you are bored or restless? *</td>
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</table>

Appendix IV. Food preference list

Please indicate which of the food items below you would like to eat at this moment. Do this by placing a cross on the box next to the item or items. Consider each item in turn and independently from the other items – you are not being asked to construct a menu from these foods. What we are interested in, is if you were presented with the item, would you like to eat it now. Therefore, you may mark some of the items, none of the items or all of the items.

<table>
<thead>
<tr>
<th>A medium sized roasted chicken breast</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>A currant bun</td>
<td></td>
</tr>
<tr>
<td>A small pancake (sweet)</td>
<td></td>
</tr>
<tr>
<td>A medium sized peach</td>
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<tr>
<td>A small packet of roasted, salted peanuts</td>
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<tr>
<td>A large grilled cod fillet (no batter)</td>
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<tr>
<td>A large tomato</td>
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<tr>
<td>A small baked potato</td>
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<tr>
<td>A small slice of rich cheesecake</td>
<td></td>
</tr>
<tr>
<td>A small slice of apple pie</td>
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<tr>
<td>A small gammon steak</td>
<td></td>
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<tr>
<td>A medium portion of boiled rice</td>
<td></td>
</tr>
<tr>
<td>A slice of melon</td>
<td></td>
</tr>
<tr>
<td>A plain omelette (2 eggs)</td>
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<tr>
<td>Fruit yoghurt (thick and creamy type, 150g pot)</td>
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<tr>
<td>A large bread roll</td>
<td></td>
</tr>
<tr>
<td>A large portion of prawns</td>
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</tr>
<tr>
<td>A medium chunk of cheddar cheese</td>
<td></td>
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<tr>
<td>A medium sized apple</td>
<td></td>
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<tr>
<td>A plain croissant</td>
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<tr>
<td>A large portion of tuné (canned in brine)</td>
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<tr>
<td>A medium sized bowl of cornflakes with semi-skimmed milk and sugar</td>
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<tr>
<td>A small cream-filled éclair</td>
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<tr>
<td>A large raw carrot (peeled)</td>
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<tr>
<td>A cheese scone</td>
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</tr>
<tr>
<td>Two slices of salami</td>
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</tr>
<tr>
<td>Grilled, lean beef rump steak (5oz)</td>
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</tr>
<tr>
<td>A green salad (no dressing)</td>
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<tr>
<td>A large banana</td>
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<tr>
<td>Three grilled fish fingers (coated in breadcrumbs)</td>
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</tbody>
</table>
Appendix V. Buffet choice questionnaire

As part of this study, a buffet lunch is going to be provided. There are choices for each food group.

1. Sandwiches
Please rank these sandwiches fillings in order of preference (liking) from 1 to 4 (with 1 being the one you most like) and cross out any which you definitely don’t like:
   - Chicken
   - Tuna
   - Cheese
   - Ham
   - Egg
   - Vegetarian option (chicken, ham or turkey flavoured "Quorn Dely")

2. Salad
Please cross out any salad items that you don’t like:
   - Lettuce
   - Cucumber
   - Tomato

3. Crisps
Do you like ready salted crisps? If not please indicate which flavour you prefer.

4. Biscuits
Please rank these biscuits in order of preference (liking) from 1 to 4 (with 1 being the one you most like) and cross out any which you definitely don’t like:
   - Tesco All Butter
   - Tesco All Butter Fruit
   - Tesco Jaffa cake

5. Yoghurt
Please rank these yoghurts flavours in order of preference (liking) from 1 to 4 (with 1 being the one you most like) and cross out any which you definitely don’t like:
   - Strawberry
   - Raspberry
   - Black cherry
   - Apricot
6. **Cakes**

Please rank these cakes in order of preference (liking) from 1 to 5 (with 1 being the one you most like) and cross out any which you definitely don’t like:

- Almond slice
- Lemon slice
- Chocolate mini rolls
- Tesco jam tart (assorted)
- Currant and sultanas slice

7. **Fruit**

Please rank these fruits in order of preference (liking) from 1 to 4 (with 1 being the one you most like) and cross out any which you definitely don’t like:

- Apple
- Clementine/Satsuma
- Grapes
- Banana
Appendix VI. Exercise history

Please indicate each of the following activities/sports, or other if not listed, have you participated in over the last three months.

Use the following key:

**Duration:** in minutes or/and hours

**Frequency:**
- Daily
- Weekly
- Monthly

**Intensity:**
- Low - casual, no breathlessness, sweating or raised heart rate
- Moderate - gently exercise, some breathlessness, sweating and a slightly increase in heart rate to the levels where the pulse can be felt
- High - vigorous exercise, breathlessness, abundant sweating and raised heart rate

<table>
<thead>
<tr>
<th>Type of activity</th>
<th>Duration</th>
<th>Frequency</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jogging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Team sports (volleyball/basketball)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dancing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others, please specify:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>c)</td>
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</tr>
</tbody>
</table>
Appendix VII. Self-certificate medical questionnaire

Please tick all that apply:

☐ I have no prior/present history of coronary heart disease.
☐ I have no prior/present history of Type 1 or Type 2 diabetes.
☐ I have no prior/present history of anemia.
☐ I have no prior/present history of gout.
☐ I have no prior/present history of, or I am currently being treated for, clinical depression and/or other psychological disorders.
☐ I have no prior/present history of eating disorders, including anorexia or bulimia nervosa.
☐ I have no prior/present history of drug or alcohol abuse within the last 2 years.
☐ I am not currently taking any regular medication prescribed by my GP apart from oral contraceptives.

Signed_________________________________________ Date__/__/__
Appendix VIII. Physical Activity Readiness Questionnaire

**PAR-Q & YOU**

*(A Questionnaire for People Aged 15 to 69)*

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

**YES**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>□</td>
<td>□ 1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?</td>
</tr>
<tr>
<td>□</td>
<td>□ 2. Do you feel pain in your chest when you do physical activity?</td>
</tr>
<tr>
<td>□</td>
<td>□ 3. In the past month, have you had chest pain when you were not doing physical activity?</td>
</tr>
<tr>
<td>□</td>
<td>□ 4. Do you lose your balance because of dizziness or do you ever lose consciousness?</td>
</tr>
<tr>
<td>□</td>
<td>□ 5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?</td>
</tr>
<tr>
<td>□</td>
<td>□ 6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?</td>
</tr>
<tr>
<td>□</td>
<td>□ 7. Do you know of any other reason why you should not do physical activity?</td>
</tr>
</tbody>
</table>

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

• You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and listen to his/her advice.

• Find out which community programs are safe and helpful for you.

**NO**

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

• start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.

• take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor

If you started becoming much more physically active before your doctor

**YES to one or more questions**

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

• You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and listen to his/her advice.

• Find out which community programs are safe and helpful for you.

**NO to all questions**

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

• start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.

• take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor

If you started becoming much more physically active before your doctor

**WARNING:**

If your health changes so that you then answer YES to any of the above questions, tell your doctor or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity and/or in doubt after completing this questionnaire, consult your doctor prior to physical activity. No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

**NOTE:** If the PAR-Q is given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

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

NAME ________________________________

SIGNATURE ________________________________

DATE ________________________________

WITNESS ________________________________

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Supported by: Health Canada, Santé Canada

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.
**Appendix IX. Profile of Mood State (POMS) Questionnaire**

The list of words below describes peoples' feelings. Please read them and tick the box which best describes how you have been feeling during the past week, including today.

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</thead>
<tbody>
<tr>
<td>1. Tense</td>
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<tr>
<td>2. Angry</td>
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<tr>
<td>3. Worn out</td>
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<td>4. Unhappy</td>
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<tr>
<td>5. Lively</td>
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<tr>
<td>6. Confused</td>
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<tr>
<td>7. Peeved</td>
<td></td>
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<tr>
<td>8. Sad</td>
<td></td>
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<tr>
<td>9. Active</td>
<td></td>
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<td>10. On edge</td>
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<tr>
<td>11. Grouchy</td>
<td></td>
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<tr>
<td>12. Blue</td>
<td></td>
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<td></td>
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<tr>
<td>13. Energetic</td>
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<td>14. Hopeless</td>
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<td>15. Uneasy</td>
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<tr>
<td>16. Restless</td>
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<td>17. Unable to concentrate</td>
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<td>18. Fatigued</td>
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<tr>
<td>19. Annoyed</td>
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<tr>
<td>20. Discouraged</td>
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<tr>
<td>21. Resentful</td>
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<tr>
<td>22. Nervous</td>
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<tr>
<td>23. Miserable</td>
<td></td>
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<tr>
<td>24. Cheerful</td>
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<tr>
<td>25. Bitter</td>
<td></td>
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<tr>
<td>26. Exhausted</td>
<td></td>
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<tr>
<td>27. Anxious</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>28. Helpless</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>29. Weary</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. Bewildered</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31. Furious</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32. Full of pep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33. Worthless</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>34. Forgetful</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35. Vigorous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36. Uncertain about things</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37. Bushed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please check to make sure you have answered all of the questions
Thank you very much for your time

245
Appendix X. Three-day food diary

A. Instructions

Please write down everything you eat and drink during your day. Give as much detail as you can and if possible use brand names. Don’t forget to note the cooking method and use household measures (mug, small plate, teaspoon…) or packet sizes to describe the amount of food/beverages you eat/drink. You will need to do this during 3 days, including at least one weekend day. At week six of the exercise intervention, your food diary has to include two exercise and one non-exercise days.

B. Example

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Food/drink</th>
<th>Cooking method</th>
<th>Quantity (approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thursday</td>
<td>23/01/2004</td>
<td>Breakfast Tea with semi-skimmed milk Sugar Toast (brown) Jam</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.00 am</td>
<td>1 mug 2 teaspoons 2 medium slices 1 tablespoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Snack banana</td>
<td></td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td>11.15 am</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lunch</td>
<td>Lunch Jacket potato (skin not eaten) Butter Baked beans Lasagne (Sainsbury’s) coke</td>
<td>Baked</td>
<td>1 large 1 tablespoon 3 tablespoons 1 portion (300g) 1 can</td>
</tr>
<tr>
<td></td>
<td>1.20 pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Snack</td>
<td>Lunch Coffee Sugar Kit kat</td>
<td></td>
<td>1 vending machine cup 1 packet 1 packet</td>
</tr>
<tr>
<td></td>
<td>4.00 pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Snack</td>
<td>Dinner Salmon Broccoli Potatoes Butter Orange juice</td>
<td>Grilled Boiled</td>
<td>1 big slice 1 mug 1 medium 1 tablespoon 1 small cup</td>
</tr>
<tr>
<td></td>
<td>5.15 pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dinner</td>
<td>Snack Cornflakes (Tesco – honey and nut bran flakes) Semi-skimmed milk</td>
<td></td>
<td>½ medium bowl ½ medium bowl</td>
</tr>
<tr>
<td></td>
<td>7.30 pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dinner</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix XI. Exercise diary

A. Instructions

Please fill in this diary with all the activities/sports that you perform. You need to indicate the following information:

_Type of activity:_ e.g. cycling, walking, tennis

_Duration:_ in minutes

_Intensity:_ Low - casual, no breathlessness, sweating or raised heart rate.

Moderate - gently exercise, some breathlessness, sweating and a slightly increase in heart rate to the levels where the pulse can be felt.

High - vigorous exercise, breathlessness, abundant sweating and raised heart rate

_Heart rate:_ please state the range you worked within (example - 115-145 bpm)

Please add any additional information or comments that you think may be important.

B. Example of an exercise dairy

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
<th>Duration (minutes)</th>
<th>Intensity</th>
<th>HR range (average)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>cycling</td>
<td>20</td>
<td>moderate</td>
<td>125-140 (132)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>walking</td>
<td>30</td>
<td>low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuesday</td>
<td>walking</td>
<td>30</td>
<td>low</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Wednesday</td>
<td>walking</td>
<td>30</td>
<td>low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thursday</td>
<td>badminton</td>
<td>45</td>
<td>moderate</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Friday</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturday</td>
<td>aerobic class</td>
<td>60</td>
<td>high</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sunday</td>
<td>cycling</td>
<td>45</td>
<td>moderate</td>
<td>115-123 (120)</td>
<td>I felt dizzy</td>
</tr>
</tbody>
</table>
Appendix XII. Pedometer form

You should use the pedometer (Digi-Walker) on the right side of your waist, and record the number of steps you take on each day on the tables provided below. You will need to repeat this during three weeks throughout the study.

You should attach the pedometer at the beginning of the day and take it off when you go to bed (or take a shower). On the next day you just need to press the reset button to return to zero. During weeks 3 and 6 of the exercise programme you should take the pedometer off when you exercise and put it back again when you finish exercising.

Baseline (__/__/__)

<table>
<thead>
<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

Week 3 (__/__/__)

<table>
<thead>
<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
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<tbody>
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</table>

Week 6 (__/__/__)

<table>
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<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
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</tbody>
</table>
Appendix XIII. Visual analogue scales (Chapter 3)

Answer the questions by placing a vertical mark through the line for each question. Mark the line according to how you feel at this moment. Regard both the ends of the lines as indicating the most extreme sensations you have ever felt.

Drowsy | Alert
---|---
Tense | Relaxed
Happy | Sad
Friendly | Angry
Uncertain | Confident
Muddled | Clear-headed
Interested | Bored

I am not hungry at all | I have never been more hungry
Nothing at all | A lot
Not at all full | Extremely full

How hungry do you feel?

How much do you think you can eat?

How full do you feel?

How would you rate the palatability of the milkshake?

Extremely pleasant | Not at all pleasant

(The last questions was presented just immediately after the milkshake)
Appendix XIV. Temporal patterns of hunger/fullness – Chapter 3

Figure i. Subjective ratings of hunger over time, after the HEP and LEP at baseline (1) and end of the study (2). Values represent means ± SEM for all participants (n=25).

Figure ii. Subjective ratings of fullness over time, after the HEP or LEP at baseline (1) and end of the study (2). Values represent means ± SEM for all participants (n=25).
Figure iii. Subjective ratings of hunger over time, after the HEP and LEP at baseline (1) and end of the study (2). Values represent means ± SEM for men (n=11).

Figure iv. Subjective ratings of fullness over time, after the HEP or LEP at baseline (1) and end of the study (2). Values represent means ± SEM for men (n=11).
Figure v. Subjective ratings of hunger over time, after the HEP and LEP at baseline (1) and end of the study (2). Values represent means ± SEM for women (n=14).

Figure vi. Subjective ratings of fullness over time, after the HEP or LEP at baseline (1) and end of the study (2). Values represent means ± SEM for women (n=14).
Appendix XV. Temporal patterns of total energy derived from food preference lists – Chapter 3

Figure vii. Total energy (kcal) over time derived from Food preference lists, after the HEP and LEP at baseline (1) and end of the study (2). Values represent means ± SEM for all participants (n=25).
Figure viii. Total energy (kcal) over time derived from Food preference lists, after the HEP and LEP at baseline (1) and end of the study (2). Values represent means ± SEM for men (n=11).

Figure ix. Total energy (kcal) over time derived from Food preference lists, after the HEP and LEP at baseline (1) and end of the study (2). Values represent means ± SEM for women (n=14).
Appendix XVI. Temporal patterns of hunger/fullness – Chapter 5

Figure x. Subjective ratings of hunger, over time, after the 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters. Values represent means ± SEM for all participants (n=33).

Figure xi. Subjective ratings of fullness over time, after the 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters. Values represent means ± SEM for all participants (n=33).
Figure xii. Subjective ratings of hunger over time, after the 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters. Values represent means ± SEM for men (n=12).

Figure xiii. Subjective ratings of fullness over time, after the 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters. Values represent means ± SEM for men (n=12).
Figure xiv. Subjective ratings of hunger over time, after the 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters. Values represent means ± SEM for women (n=21).

Figure xv. Subjective ratings of fullness over time, after the 500 kcal and 1000 kcal breakfast, in restrained (rest) and unrestrained (unrest) eaters. Values represent means ± SEM for women (n=21).
Appendix XVII. Temporal patterns of hunger/fullness - Chapter 6

Figure xvi. Subjective ratings of hunger, over time, after the HEP and LEP, in restrained (rest) and unrestrained (unrest) eaters according to the RRS. Values represent means ± SEM for all participants (n=12).

Figure xvii. Subjective ratings of hunger over time, after the HEP and LEP, in restrained (rest) and unrestrained (unrest) eaters according to the TFEQ-18R. Values represent means ± SEM for all participants (n=12).
Figure xviii. Subjective ratings of hunger over time, after the HEP and LEP, in restrained (rest) and unrestrained (unrest) eaters according to the DEBQ. Values represent means ± SEM for all participants (n=12).

Figure iv. Subjective ratings of fullness over time, after the HEP and LEP, in restrained (rest) and unrestrained (unrest) eaters according to the RRS. Values represent means ± SEM for all participants (n=12).
Figure v. Subjective ratings of hunger over time, after the HEP and LEP, in restrained (rest) and unrestrained (unrest) eaters according to the TFEQ-18R. Values represent means ± SEM for all participants (n=12).

Figure vi. Subjective ratings of fullness over time, after the HEP and LEP, in restrained (rest) and unrestrained (unrest) eaters according to DEBQ. Values represent means ± SEM for all participants (n=12).
Appendix XVIII.  PYY plasma levels in restrained and unrestrained eaters

Figure VII. Plasma PYY concentrations (pmol/L) over time, after a 500kcal breakfast, in restrained (n=5) and unrestrained (n=7) eaters. Values represent means ± SEM for 12 participants. Repeated measures ANOVA showed a significant effect of time (P<0.0001) and a trend towards a significant effects of restraint (P=0.073).
Appendix XIX. Participants' flowchart (Chapter 5)

133 screened

47 men

- Excluded (13):
  - Overweight - 6
  - History of depression - 3
  - Under medication - 2
  - Elite athlete - 1
  - Weight unstable - 1

33 met the criteria

7 Middle

20 Unrestrained

- No time - 2
- Not needed - 1

6 Unrestrained

86 women

- Excluded (14):
  - Overweight - 5
  - History of anemia - 3
  - Under medication - 1
  - Crohn disease - 1
  - Allergy to wheat - 1
  - History of anorexia nervosa - 1
  - Weight unstable - 1

72 met the criteria

30 Middle

14 restrained

- No time - 4

10 Restrained

28 Unrestrained

- No time - 5
- Not needed - 1

11 Unrestrained
Please underline or circle the answer that best applies to you in the left column.

1. What is your main occupation? .................................................................
2. At work I sit
   never/seldom/sometimes/often/always
3. At work I stand
   never/seldom/sometimes/often/always
4. At work I walk
   never/seldom/sometimes/often/always
5. At work I lift heavy loads
   never/seldom/sometimes/often/always
6. After work I am (physically) tired
   always/sometimes/never
7. At work I sweat
   always/sometimes/never
8. In comparison with others of my own age I think my work is physically much heavier/heavier/as heavy/lighter/much lighter
9. Do you play sport/physical activity? Yes/No
   If yes:
     - Which sport/physical activity do you play most frequently? ..................
     - How many hours a week? <1/1-2/2-3/3-4/4-5>
     - How many months a year? <1/1-3/4-6/7-9>/9
   If you play a second sport/physical activity:
     - Which sport/physical activity do you play most frequently? ..................
     - How many hours a week? <1/1-2/2-3/3-4/4-5>
     - How many months a year? <1/1-3/4-6/7-9>/9
10. In comparison with others of my own age I think my physical activity during leisure time is much more/more/the same/less/much less
11. During leisure time I sweat
    very often/often/sometimes/never
12. During leisure time I play sport
    never/seldom/sometimes/often/always
13. During leisure time I watch television
    never/seldom/sometimes/often/always
14. During leisure time I walk
    never/seldom/sometimes/often/always
15. During leisure time I cycle
    never/seldom/sometimes/often/always
16. How many minutes do you walk and/or cycle per day to and from work, school and shopping?
    <5/5-15/15-30/30-45/45>45
Calculation of the simple sport-score ($I_s$):
(a score of zero is given to people who do not play a sport)
$$I_s = \sum (\text{intensity} \times \text{time} \times \text{proportion})$$
$$= 0.01 \times 4.4 < 8.8 < 12 \times 12$$

Calculation of scores of the indices of physical activity:
Work index = $[I_1 + (6 - I_2) + I_3 + I_4 + I_5 + I_6 - I_7 - I_8 + 8] / 8$
Sport index = $[I_9 + I_{10} - I_{11} - I_{12}] / 4$
Leisure-time index = $[(6 - I_{13}) + I_{14} + I_{15} + I_{16}] / 4$

**Work** - occupational physical activity according to The Netherlands Nutrition Council
1. Low level: clerical work, driving, shopkeeper, teaching, studying, housework, medical practice and all other occupations with a university education
2. Middle level: factory work, plumbing, carpentry and farming
3. High level: dock work, construction work, sport

**Sport**
1. Low level: billiards, sailing, bowling and golf – average energy expenditure (AEE): 0.76 MJ/h
2. Middle level: badminton, cycling, dancing, swimming and tennis – AEE: 1.26 MJ/h
3. High level: boxing, basketball, football, rugby and rowing – AEE: 1.76 MJ/h