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LEPTIN AND ACUTE APPETITE CONTROL

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to
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AS ORIGINAL
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The Appetite Questionnaire used in this thesis
Copyright Statement

I hereby declare that this thesis has been composed by myself, that the work of which has been done by myself except where assistance and collaboration has been acknowledged, that it has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of references.

Fotini Tsouliou
Summary

Objective: Leptin is an adipose-tissue-derived hormone, which regulates (suppresses) appetite in animals. No direct physiological role has previously been identified in humans, except in the case of rare congenital defects. This thesis tested the general hypothesis that another factor - related to physical activity - is necessary for normal leptin function. It investigated the effects of moderate exercise on acute appetite control and on circulating leptin concentrations in obese and lean women. The role of acute physical inactivity on appetite control and on circulating leptin concentrations was also investigated by disrupting exercise training for 7 days in male athletes. The assessment of the relationship between circulating leptin concentrations and appetite/satiety measures and subsequent food intake was used as an indirect indicator of the function of leptin.

Methods: In Study 1 and 2 ten obese (mean age ± SD: 50 ± 8.5 y, mean body mass index (BMI) ± SD: 37 ± 6.5 kg m⁻²) and ten lean women (mean age ± SD: 37 ± 10 years, mean body mass index ± SD: 22 ± 2 kg m⁻²) were submitted randomly to three trials: Moderate physical activity (20 min brisk walking), Snack (58.5 g chocolate-based) and Control (sitting, TV-watching).

In Study 3, ten obese women (mean age ± SD: 50 ± 6 y, mean body mass index (BMI) ± SD: 36 ± 5.5 kg m⁻², mean waist ± SD: 105 ± 13 cm) participated in two separate experimental trials: EXP 1 (Moderate exercise plus α/β adrenergic blocker (labetalol, 100 mg orally) vs Moderate exercise plus placebo (calcium carbonate); EXP 2 (Adrenaline infusion for 20 min (12.5 ng min/kg ideal body weight) vs Saline infusion trial). In both experiments, trials were double blind and performed in random order. In Studies 1, 2, and 3 appetite and satiety were assessed by visual analogue scales and serum leptin, blood glucose and plasma free fatty acids were measured at baseline, pre- and post-intervention and one h post-intervention (i.e., before dinner). A buffet style dinner was provided subsequent to all trials.

In Study 4, eight endurance-trained athletes (mean age ± SD: 28 ± 12 yrs, mean body mass index (BMI) ± SD: 23.6 ± 1.0 kg m⁻²) consumed a 1074 kcal (4.5 MJ) meal (67 % fat, 29 % carbohydrate, 4 % protein) 12 h after overnight fasting, once during
training (Trained condition) and once after seven days of detraining (Detrained condition). Serum leptin, plasma insulin and glucose, and appetite and satiety ratings were measured in the fasting state and at several time points up to 6 h postprandially. The results of this thesis depend heavily on assessment of appetite sensations for which there is no "gold standard" or objective method. The methods developed by Blundell et al were used, employing 10cm visual analogue scales.

Results: In Study 1 and 2, the moderate physical activity and snack intake both produced lower appetite and higher satiety and fullness perceptions, compared to control, following the intervention in obese and lean women. No significant differences were found in subsequent food intake. Serum leptin concentrations did not differ between trials. Serum leptin was not associated with appetite or satiety sensations at any time during the control or the snack trials, but was correlated following moderate physical activity (prospective food consumption $r_s = -0.83, P = 0.003$; hunger $r_s = -0.79, P = 0.007$; desire to eat $r_s = -0.69, P = 0.02$; satiety $r_s = 0.71, P = 0.02$; fullness $r_s = 0.66, P = 0.04$) in obese women. These associations were not influenced by BMI or fat mass. No significant associations were found between serum leptin and appetite-satiety sensations on any trial in lean women but plasma free fatty acid concentrations were significantly associated with appetite and satiety ratings only following the snack intake (prospective food consumption $r = -0.72, P = 0.03$; satiety $r = 0.78, P = 0.007$; fullness $r = 0.71, P = 0.02$).

In EXP 1 of Study 3, blood glucose concentrations were significantly higher ($P < 0.01$) and plasma FFA were significantly lower ($P = 0.039$) following the Moderate exercise plus $\alpha/\beta$ blocker compared to placebo. There was no significant difference on appetite/satiety measures and on subsequent food intake between the two exercise trials. In EXP 2 of Study 3, adrenaline infusion significantly increased energy intake ($P = 0.04$) and carbohydrate intake ($P = 0.01$) at the subsequent meal. Heart rate was significantly increased during the end of the adrenaline infusion compared to saline infusion ($P \leq 0.01$). There was no difference in serum leptin concentrations between trials in either EXP 1 or EXP 2 ($P > 0.05$).
In Study 4, compared with training, serum leptin was greater postprandially in the Detrained condition (Trained 19.85 ± 6.36 ng·ml⁻¹·h, Detrained 26.65 ± 7.85 ng·ml⁻¹·h (AUC) \( P = 0.02 \)). Whole-body insulin sensitivity ISI (gly), based on postprandial glucose and insulin concentrations, was also higher in the Detrained condition (Trained 1.17 ± 0.35; Detrained 0.83 ± 0.18, \( P = 0.003 \)). Fasting and postprandial appetite-satiety ratings did not differ significantly between trials (\( P > 0.05 \)). Appetite and satiety ratings were significantly correlated with serum leptin concentrations (Detrained, \( P < 0.05 \)) 4 h and 6 h postprandially, and with plasma insulin concentrations (Trained, \( P < 0.05 \)) 6 h postprandially, but not at any other time point.

**Conclusions**

Study 1, 2 and 3: Moderate physical activity and snack intake suppress the appetite of obese and lean women acutely. The associations between circulating leptin and appetite-satiety ratings suggest that there is some physiological involvement of leptin in short-term appetite regulation in response to physical activity-induced factors but only in obese women.

The exercise-related factors considered in this thesis as possible mediators of leptin action were catecholamines, fatty acids, glucose and insulin. Adrenaline is unlikely to be the exercise factor responsible for the coupling between leptin and satiety since adrenaline infusion stimulated an increase in subsequent energy intake in obese women. Labetalol decreased circulating FFA and increased glucose concentrations, which confirms at least \( \beta \)-adrenoceptor blockade. Any conclusions with respect to the \( \alpha \)-adrenoceptor blockade should be drawn with caution since labetalol, an \( \alpha/\beta \) blocker, has greater affinity for \( \beta \)- than \( \alpha \)-adrenoceptors. No differences in appetite/satiety sensations were found following exercise with adrenoceptor blockade compared to exercise alone. This indicates that the observed anorexic effect of exercise on appetite in obese women was not mediated by \( \beta \)-adrenoceptors. Noradrenaline is another possible exercise factor that could mediate the coupling between leptin and appetite in obese women since it is known that leptin and noradrenaline (NA) have common hypothalamic targets (e.g. NPY) and their effects are mediated by \( \alpha-1 \) adrenoceptors. Labetalol probably was not a sufficiently strong \( \alpha \)-adrenoceptor blocker to investigate such effects. A study of a more selective \( \alpha_1 \)-
adrenoceptor antagonist might be helpful in the investigation of the interaction between leptin and NA in the regulation of eating.

Study 4: In endurance-trained athletes a short term detraining increases postprandial plasma leptin, induces insulin resistance but has no effect on appetite/satiety ratings.

The results of the present studies implicate leptin, insulin, insulin resistance and noradrenergic factors in the control of eating following exercise and detraining. They strongly support the promotion of physical activity to help regulate appetite and curb excessive food intake.
Chapter One

General Introduction
1.1 General Introduction

Obesity is recognised as a worldwide epidemic, which requires both clinical management and public health preventive measures (WHO, 1998), and there is no sign that the epidemic is abating over the last two decades. In the majority of European countries, where lifestyles and cultures are comparable, the International Task Force estimates that the prevalence of obesity increased by between 10 to 40 per cent from the late 1980s to the late 1990s (Brown, 2000). In England, however, the National Audit Office reports that prevalence has almost tripled since 1980 and will increase further on present trends (NAO, 2001). In 1980, 8% of women and 6% of men were classified as obese in England. By 1998, the prevalence of obesity had nearly trebled to 21% of women and 17% of men. In 2000, NAO predicted that if the average rate of increase in the prevalence of obesity between 1980 and 1998 continued, over one fifth of men and a quarter of women in England will be obese by 2005, and over a quarter of all adults by 2010. This would bring levels of obesity in England up to those experienced now in the United States (NAO, 2001). In fact, by 2001 over 20% of all adults were already obese.

Obesity is a disease with International classification of disease code E.66 (World Health Organisation, 1997) and is characterised as a disease process of excessive accumulation of body fat with multiple organ-specific pathological consequences. For epidemiological purposes, adults ‘obesity’ is now defined by international convention to indicate the state of having a BMI > 30 kg/m², while a BMI of > 25 kg/m² is designated ‘overweight’ and a BMI of < 25 kg/m² is ‘normal’ (WHO, 1995). These BMI cut offs were initially based on life expectancy data from life assurance companies, but they match the overweight-related risks for a range of morbidities.
An alternative and more simple measure of overweight and obesity was required for health promotion purposes, since the BMI is conceptually complex and inaccessible to most of the population (Lean, 2000). Waist circumference is a more recently standardised alternative and it relates to total body fatness and specifically to intra-abdominal fat without the need to adjust for height (Han et al, 1997).

Obesity is caused by interplay between genetic constitution and the environment. Genetic predisposition clearly contributes to individual differences in body weight and fat mass (Barsh et al, 2000), but the rising prevalence of common obesity is a result of the changing environment rather than changes in the biology (Hill et al, 2003). There is debate about the key environmental causes of energy overconsumption and overweight. Is it the increased food availability and consumption of fattening food or the sedentary life style that has triggered the obesity epidemic? The changes in food production, distribution and food availability in modern societies have indeed created a food environment that promotes weight gain. There are significant increases in food availability and fat content in the diet of the average American adult (Putnam et al, 2002). Preliminary data on the nutrient content of the U.S. food supply indicated that per capita availability of total dietary fat jumped 6% between 1999 and 2000, pushing per capita energy (kcal) availability to 3,900 kcal per person per day (USDA’S Center for Nutrition Policy and Promotion, 1999). The main food-based candidates for energy over-consumption are energy-dense foods (usually high in fat), energy-dense drinks (usually high in sugar) and large portion size (Blundell and King, 1996; Prentice, 1998; Rolls, 2000). Perhaps even more important have been the major advances in energy-saving technologies that promoted sedentary behaviour. Diverging trends of decreasing
energy intake and increasing body weight suggest that physical activity may be the main determinant of the rising prevalence of obesity (Prentice and Jebb, 1995). There is little doubt that machines which reduce the energy cost of occupational work and everyday activities, and that provide opportunities for passive recreation have increased the levels of physical inactivity (Swinburn and Egger, 2002). Indeed the World Health Organisation has reported that total energy expenditure is reduced as a result of low physical activity levels in daily living, and declared a fall in spontaneous, work-related physical activity as a principal factor that leads to overweight (WHO, 1998).

Television watching has been used in several epidemiological studies as an indicator of sedentary lifestyle. On average, western children and adults spent more time in sedentary activities, such as watching television than did previous generations (Dietz, 2001; Jebb and Moore, 1999). Several studies in adults and children, conducted in US and in Europe, have reported important associations between the hours of television watching and the prevalence of obesity (Martinez-Gonzalez et al, 1999; Dietz and Gortmaker, 1985; Andersen et al, 1998; Gortmaker et al, 1996). Although physical inactivity is believed to contribute to the rising prevalence of obesity, the reasons of appetite and body weight deregulation residing in a physically inactive compared to an active environment are unknown.
1.2 Exercise and Appetite Regulation

Appetite regulation involves interactions of many psychobiological systems (Blundell et al., 2001), and appetite is only part of the process that governs food consumption. The adipocyte derived hormone leptin is one important part of a complex peripheral and central circuit that probably interacts with neurochemical mediators of feeding to couple appetite and body weight control in animals and humans. The present introduction will discuss current evidence for the role of physical activity and inactivity on appetite regulation and how circulating leptin has been implicated in appetite regulation in humans.

A number of studies show that physical activity is associated with the fine regulation of appetite and body weight regulation in both mice and humans (Mayer and Thomas, 1967). Classic animal studies have demonstrated the importance of exercise in prevention or moderation of weight gain (Mayer, 1953; Mayer et al., 1954). Similarly in humans, with observational studies of mill workers suggested that appetite may only be ‘coupled’ with body weight control when moderate physical activities are undertaken (Mayer et al., 1956). More recent studies indicate that physical inactivity tends to increase food intake (Murgatroyd et al., 1999) and habitual exercisers control better food intake than sedentary individuals (Long et al., 2002). Physical activity is an important adjunct in dietary weight loss programmes and necessary in long-term weight loss maintenance. Several qualitative retrospective studies found a higher percentage of weight maintainers than non-maintainers when they engaged in regular exercise (Ewbank et al., 1995). Prospective studies also found improved weight maintenance when exercise was included in post-weight loss programmes (Williamson, 1996). How physical activity has this
regulatory role for body weight is not clear, but it appears to mediate mechanisms by increasing energy expenditure and regulating (suppressing) appetite (Blundell et al, 2003).

The full extent of interactions between exercise and food intake have not been clearly established. Intervention studies examining the effect of exercise on food intake are difficult to conduct, and have reported conflicting results because of differences in experimental protocols, mode of exercise, intensity and duration of exercise, gender and age of participants (Blundell and King, 2000). However, the majority of studies suggest gender and fat mass as the main indicators of the drive to eat following exercise.

Intervention studies that investigated the acute effects of exercise on energy intake agree that energy intake following exercise in not increasing in obese individuals. Kissileff et al (1990) found that energy intake (from strawberry yogurt) following moderate and strenuous exercise was lower in obese compared to lean women. In another study (Durrant et al, 1982) it was reported that there were no significant differences in acute energy intake following moderate cycling (estimated energy expenditure approximately 100 kcal) between obese and lean individuals. Clinical studies have also reported that obese women did not increase subsequent energy intake following light or moderate exercise (Woo and Pi-Sunyer, 1985; Woo et al, 1982a,b). Recently, one study reported higher food intake following moderate intensity exercise (1h treadmill walking, 60% maximum heart rate) in obese rather than in lean women (George and Morganstein, 2003). In this study food was provided in a non-laboratory setting. Participants selected their lunch among a large
variety of foods in a familiar cafeteria setting, which might have confounded results by stimulating eating (Bellisle, 1999).

In long-term exercise training studies, evidence suggests that subjects increase energy intake to compensate for a high exercise-induced energy expenditure (e.g. athletes during training) (Westerterp, 1998; van Baak, 1999; Tremblay et al, 1985). Some studies have reported gender differences in the feeding response to exercise training. Lean women tend to increase food intake following exercise training but not men (Westerterp et al, 1992; Tremblay et al, 1984; Stubbs et al, 2002a,b). The relationship between exercise and subjective appetite or satiety measures is better described than food intake, and exercise has been reported to decrease appetite sensations in the short-term or not to affect appetite sensations (King et al. 1994; King and Blundell, 1995; Thompson et al, 1988; Hubert et al, 1998; King et al, 1996; Lluch et al, 2000). It seems that there is agreement among studies that physical activity can improve the sensitivity of the appetite control system (Blundell et al, 2003). The mechanisms responsible for the exercise-induced appetite suppression remain unclear.
1.2 Leptin in Appetite Regulation

The hypothalamus has long been recognised to be the site of the feeding regulatory centre, since it was shown that lesions in the medial hypothalamus lead to increased food intake and obesity (Bray et al, 1990). Body weight homeostasis is suggested to be maintained via a series of complex interactions between the hypothalamus and the periphery, notably via a hormone, leptin, which is synthesised and secreted from adipose tissue. In 1994 Zhang et al identified and cloned a gene in the mouse called ob gene which was absent in genetically obese ob/ob mice (Zhang et al, 1994). One year later the ob gene was found to encode a plasma protein (Halaas et al, 1995) named "leptin" (Greek leptos adj. (of a person or animal) lean, ancient greek lépo v.t. to peel). Leptin was proposed to regulate appetite and food intake by acting in the hypothalamus. Administration of leptin either peripherally or centrally reduced food intake and body weight in congenitally leptin deficient ob/ob mice, but not in db/db obese mice which have a mutation in the leptin receptor (Campfield et al, 1995; Halaas et al, 1995; Pelleymounter et al, 1995). The search for obese patients with mutations in the ob gene or the ob-r receptor followed (Clement et al, 1998; Montague et al, 1997; Strobel et al, 1998). Such mutations are very rare and result in morbid obesity in children who are hyperphagic but regain appetite control when treated with recombinant leptin therapy (Farooqui et al, 2002). These findings triggered research for leptin as a putative satiety factor in humans, and for a role in common obesity (without ob-gene or ob-receptors abnormalities).

The hormone leptin is known as key regulator of body weight and food intake in animals but no simple acute regulatory action has been found in humans (Friedman & Halaas, 1998). Studies of recombinant leptin administration showed minimal
effects in obese humans. The majority of the obese population has high circulating leptin concentrations proportional to increased fat mass (Considine et al, 1996). This indicates that leptin production and secretion is normal but high circulating leptin concentrations fail to curtail elevated appetite in obesity.

Leptin circulates at greater levels in obese than lean individuals, but the ratio of cerebrospinal fluid/serum leptin concentrations is greater in lean than in obese individuals (Schwartz et al, 1996). It appeared that the intrinsic sensitivity to leptin is variable and that, in general, obese individuals are leptin-resistant (Friedman, 2003). Hence, several studies investigated the possibility of defective transport of leptin into the brain. The uptake of leptin in the brain was found saturable in vitro and vivo animal studies (Banks et al, 1996; Karonen et al, 1998), and recently a saturable-transport mechanism has been also reported in humans (Koistinen et al, 1998). This evidence suggested that leptin uptake into the brain becomes saturated and limited as adiposity increases, which may explain why high circulating leptin fails to regulate appetite in leptin ‘resistant’ obese individuals (Caro et al, 1996). Thus obesity pathophysiology may include leptin resistance as an additional neuroendocrinological feature. The molecular basis for leptin resistance is not yet fully understood but could lead to new treatments.
1.4 Proposed model for the function of leptin in the hypothalamus

Searching for the role of leptin in energy homeostasis there appear to be two hypotheses. One is that leptin serves as an antiobesity hormone by acting on the brain to inform it about the size of the fat depots. Enlargement of the fat tissue and subsequent increases in leptin production and secretion would signal energy 'affluence' in the brain and lead to decrease in food intake and increase in energy expenditure to avoid obesity (Ahima and Flier 2000; Flier, 1998). Alternatively it is suggested that absence of leptin is a more important signal at low energy intakes to prevent starvation (Jequier, 2002). Leptin has other functions, particularly as a signal of the adequacy of energy stores for reproductive function by influencing a number of target organs in the hypothalamic – pituitary – gonadal axis. Leptin has also been implicated as having a role in hematopoiesis, immune function, angiogenesis and osteogenesis (Lee et al, 2002).

The physiological actions of leptin are mainly mediated by the interaction with neuropeptides, which inhibit food intake and increase energy expenditure. Circulating leptin is transported through the blood-brain barrier, possibly via the short leptin receptor isoform (ObRa) and reaches the hypothalamus where it binds to its long-receptor isoform (ObRb). Following a specific signalling cascade through its hypothalamic receptors (Håkansson et al, 1998), leptin has been proposed to inhibit the action of orexigenic peptides e.g. neuropeptide Y, melanin concentrating hormone, agouti-related peptide or stimulating anorexigenic peptides e.g. a-melanocyte stimulating hormone (a-MSH), corticotrophin releasing hormone (CRH), pro-opiomelanocortin (POMC), cocaine and amphetamine related transcript (CART) (Figure 1.1). By doing so, leptin exerts its effects of decreasing food intake and body
weight, increasing energy expenditure and fat oxidation, thus favoring leaness (Jeanrenaud and Rohner-Jeanrenaud, 2001).

The anorectic activity of leptin in the hypothalamus is suggested to be partly mediated by its inhibition of the noradrenergic system. Studies in rats show that leptin inhibits the noradrenaline (NA) release from neuronal endings in the hypothalamus (Brunetti et al, 1999). Noradrenaline release from presynaptic terminals in the paraventricular nucleus is known to stimulate food intake (Leibowitz and Brown, 1980), probably mediated by α2-adrenoceptors (Wellman et al, 1993). These findings supported that noradrenaline might be also involved in leptin signalling in the hypothalamus (Figure 1.1).
Figure 1.1:

Possible model for the regulation of peripheral leptin concentrations and the action of leptin in the central nervous system. Serum leptin concentration is mainly influenced by body fat content, and by gender. However at any given body weight, there is a large range of serum leptin concentrations. The two major biological functions in humans eating and exercise/physical activity regulate circulating leptin concentrations. Increases in food intake lead to increases in circulating leptin concentrations through increases in circulating insulin concentrations in the short-term and fat stores in the long-term. Increases in exercise/physical activity decrease circulating leptin concentrations possibly through catecholamines stimulation in the short-term or decreases in fat mass in the long-term. Circulating leptin is transported by Ob-Ra receptors through the BBB and into the hypothalamus. Once leptin has entered the hypothalamus it binds to hypothalamic receptors Ob-Rb and regulates the expression of orexigenic and anorexigenic peptides. In this way appetite and food intake are maintained under control.
Figure 1.1

HYPOTHALAMUS

Leptin Ob-R

BBB

Leptin Ob-R

POMC
a-MSH
MC-R
CART
CRH
GLP-1

NPY
AGRP
MCH
Orexins

NA

± Appetite

Food Intake

Insulin
Fat stores

Exercise/
Physical activity

Catecholamines
Fat stores

Leptin

+ 

-
1.5 Leptin response in exercise and feeding studies, and in recombinant leptin administration trials

A plethora of studies have investigated the control of leptin synthesis and secretion and the function of leptin in controlling appetite in humans. Firstly, studies that investigated whether circulating leptin concentration and/or leptin synthesis is acutely affected by energy intake. Secondly, studies that looked at the effects of acute exercise and exercise training on circulating leptin concentration and/or leptin synthesis, and thirdly clinical trials of exogenous leptin administration have explored the use of leptin as an antiobesity drug.

Physically active and habitually trained individuals also tend to match energy intake with long-term physical activity (Maughan et al, 1989), and typically maintain constant low body weight throughout life. Physically active and trained individuals have lower body fat and circulating leptin concentrations (Leal-Cerro et al, 1998; Considine et al, 1996) compared to sedentary controls. In contrast, circulating leptin is high in sedentary and overweight individuals (Considine et al, 1996; Fung et al, 2000) but this does not prevent overeating and weight gain. These observations might indicate that fine coupling between circulating leptin concentrations and centrally mediated anorectic and catabolic effects of leptin is obtained only when physical activity is undertaken.

There are many studies that have investigated the physiological regulation of leptin in relation to short-term and long-term exercise training and following single bouts of exercise. Because of many confounding factors, such as exercise-induced changes in fat mass, the effects of exercise on circulating leptin are not completely clear.
Most studies that investigated the effects of acute exercise on leptin have reported reductions or no changes in leptin concentrations. Only severe and prolonged exercise can decrease plasma leptin concentrations acutely in highly trained men (Duclos et al., 1999; Landt et al., 1997; Leal-Cerro et al., 1998; Zaccaria et al., 2002; Koistinen et al., 1998). A delayed reduction in plasma leptin concentrations has also been found at 48 h after high intensity treadmill exercise (~900 kcal during 1 h of exercise) or endurance running (Essig et al., 2000; Oliver and Miller, 2001) or at 9 h after heavy resistance exercise in lean men (Nindl et al., 2002). Thus in trained subjects the impact of acute exercise on leptin appears to be delayed until after physical activity.

In sedentary male subjects, moderate intensity exercise for 1 h did not acutely affect leptin production and circulating leptin concentrations (Racette et al., 1997; Zafeiridis et al., 2002). Weltman et al. (2000) examined the effects of short duration (30 min) treadmill exercise (at intensities below, at and above the lactate threshold) on serum leptin and reported no leptin changes irrespective of exercise intensity. Kraemer et al. (1999) reported a decrease in leptin after 30 min of exercise at 80% $V_{O_2\text{max}}$ in postmenopausal women but possible diurnal fall in leptin was considered as confounding factor. Fisher et al. (2001) investigated serum leptin in response to 41 min of cycle ergometry at 85% of maximal oxygen consumption and observed no effect on young sedentary men. Acute exercise to exhaustion reduced circulating leptin concentrations at 120 min after exercise in sedentary men (Elias et al., 2000). These results suggested that acute exercise with greater energy expenditure is more influential on circulating leptin concentrations and decreases in plasma leptin following single bouts of exercise are manifested 24-48 h post-exercise.
There are very few data available on the effects of exercise on leptin secretion in obese individuals and these come from exercise training studies. Longer-term training studies have reported reduced serum leptin concentrations induced by fat loss (Okazaki et al, 1999; Perruse et al, 1997; Thong et al, 2000; Kohrt et al, 1996) and others have found reductions independent of the fat loss (Hickey et al, 1997; Pasman et al, 1998; Gutin et al, 1999; Reseland et al, 2001). The decreases in leptin concentrations after exercise are suggested to be related, at least in part, to sympathetic nervous system activity (Sandoval and Davis, 2003). In animals, this exercise effect is mediated by \( \beta_3 \)-adrenergic receptors (Bramlett et al, 1999). The mechanism in humans remain to be determined but catecholamines are found to suppress leptin secretion in cultured human adipocytes possibly through activation of \( \beta_1 \)- and \( \beta_2 \)-adrenergic receptors (Scriba et al, 2000). Circulating catecholamine concentrations that are elevated by the exercise stress (Shoemaker et al, 1998) might explain the exercise-induced suppression in leptin concentrations. The previous studies showed that exercise, possibly through activation of beta-adrenergic receptors, inhibits leptin secretion but no studies have investigated the influence of exercise on the function of leptin. It is not known (it is hypothesised that) whether exercise would influence (enhance) the transport of leptin into the brain and hence activate the effects of leptin on appetite regulation in the hypothalamus.

Several studies have investigated the physiological regulation of leptin and its action in relation to feeding interventions. It is suggested that apart from the status of energy stores, feeding also influences the expression of leptin by the adipose tissue. Serum leptin decreases with fasting for 52 to 72 h, and increases after acute overfeeding (12 h) in normal weight and obese individuals (Kolaczynski et al, 1996;
Boden et al, 1996; Weigle et al, 1996). Single meals do not appear to affect leptin concentrations from 1 h to 4 h postprandially (Clapham et al, 1997; Considine et al, 1996; Dagogo-Jack et al, 1996; Korbonits et al, 1997; Ma et al, 1996; Orban et al, 1999) but may transiently increase leptin at 30 min postprandially (Astrup et al, 1997) or after 4 h postprandially (Dallongeville et al, 1998; Romon et al, 1999). No association has been found between leptin and hunger/or desire to eat ratings acutely after a meal in lean or obese individuals (Heini et al, 1998; Karhunen et al, 1997; Romon et al, 1999). Serum leptin has been associated with appetite ratings only in obese individuals after prolonged weight loss (Keim et al, 1998; Doucet et al, 2000). These results suggested that adipose-derived leptin regulates energy balance in the long-term but does not appear to act as meal-generated satiety signal (Picó et al, 2003).

The regulation of leptin expression by food intake is probably mediated, at least in part, by insulin. Accumulating evidence has proposed insulin as a potent regulator of plasma leptin concentration. First, a correlation between plasma insulin and leptin concentrations has been found in several cross-sectional studies independent of body adiposity. Second, insulin stimulates leptin expression in vivo and in vitro (Rentsch and Chiesi, 1996; Saladin et al, 1995). Third, leptin circulating concentrations have been found to increase during hyperinsulinaemic-euglycaemic clamp experiments (Saad et al, 1998; Malmstrom et al, 1996). Insulin sensitivity is also proposed to regulate circulating leptin concentrations (Haffner et al, 1997). Insulin sensitivity is associated with reduced plasma leptin concentrations independently of body fat mass (Segal et al, 1996, Guldstrand et al, 2003) and controls whether high insulin concentrations will increase the secretion of leptin (Larsson et al, 1996). For
example, in individuals with insulin resistance high insulin concentrations are not associated with increased stimulation of leptin secretion from adipose cells (Saad et al., 1998; Fruehwalt-Schultes et al., 2002; Tuominen et al., 1997).

The clinical trials with leptin replacement therapy in patients with congenital leptin deficiency and with recombinant human leptin administration in common obese adults have suggested that leptin has biological activity in some obese humans. Exogenously administered leptin seems to reduce appetite and food intake at low doses and body fat and weight at maximal dose but only in some obese individuals. In a young girl with severe obesity (42 kg, 3 yr-old) a mutated ob-gene was found. Daily treatment with met-leptin at low doses (0.028 mg/kg lean mass) caused marked appetite and food intake reduction, and body weight loss (16.4 kg weigh loss, 95% of which was body fat). On treatment this girl weighed 32 kg at the age of 7 years (O’Rahilly et al., 2003).

Obese individuals (with no abnormalities associated with congenital leptin deficiency) have increased serum leptin concentrations and decreased leptin sensitivity. Initial trials with recombinant leptin administration aimed to augment circulating leptin concentrations to increase leptin signalling and action in common obese individuals. A double-blind placebo controlled study examined the safety of leptin therapy in 73 obese subjects who self-administered a subcutaneous leptin injection daily for either four or 24 weeks (Heymsfield et al., 1999). A total of 54 lean individuals were in the control group. Throughout the study, lean subjects were maintained on an eucaloric diet, while obese subjects consumed a 500-kcal deficient diet. The results of this trial indicated that daily administration of recombinant
methionyl human leptin induced modest dose-related weight loss in some obese subjects with elevated endogenous serum leptin concentrations but not all subjects, with a large degree of variability in the amount of weight lost by individual subjects.

Reductions in fasting hunger ratings and in generalised hunger as measured by the three factor eating questionnaire were found after 20 mg subcutaneous treatment with recombinant human leptin in obese men (Westerterp-Plantenga et al, 2001). Despite these reductions in the appetite profile, changes in body composition or body weight loss compared to placebo treatment were not found. Westerterp-Plantenga et al (2000) suggested that recombinant leptin treatment has central rather peripheral biological activity in obese individuals. These findings indicate that only a subset of obese people respond to leptin therapy with a significant amount of weight loss, but the majority appear to be centrally resistant (Caro et al, 1996) to the action of increased plasma leptin concentrations or peripherally administered leptin. Therapeutic approaches to deliver leptin effectively into the brain through exogenous increases in circulating leptin concentrations are problematic (Mantzoros and Flier, 2000). Probably the therapeutic approach should target the transport of leptin concentrations through the blood brain barrier, which is suggested to be defective in obese individuals.
1.6 Research Questions that arise from the literature and hypotheses to be tested

For many years the relationship between physical activity-induced energy expenditure and energy intake has been the centre of interest through research in energy balance (Blundell et al, 2003). The energy balance is achieved when energy intake meets energy expenditure and especially physical activity related energy expenditure. Physical activity is recognised as an important component of an obesity treatment regimen. Physical activity alone is not particularly effective in producing substantial weight loss, but it is effective in the prevention of weight gain (Rissanen et al, 1991, Hill and Melanson, 1999). Observational, cross-sectional and longitudinal studies show that subjects with high levels of physical activity have lower body fat and abdominal fat and are less likely to gain total and abdominal fat than those with low levels of physical activity (Astrup, 2001; Ross and Janssen, 2001). A number of mechanisms linking exercise to successful weight control have been proposed (Tremblay et al, 1999). For example exercise increases energy expenditure. The regular physical activity promotes metabolic adaptations, for example, the increase in resting metabolic rate and the sympathetic nervous system activity, that facilitate the regulation of energy and fat balance. Physical activity may also influence the complex regulation of appetite and food intake, including possible effects on insulin sensitivity, gastrointestinal hormonal release, behavioural responses and neurobiological hormones.
Summarising, the feeding interventions that were reviewed in this chapter indicated that circulating leptin is not importantly involved in acute appetite regulation through postprandial metabolic processes. The results of the clinical trials with recombinant human leptin administration indicated that leptin has biological activity in at least some obese individuals who appear to retain leptin 'sensitivity'. The studies that investigated the effects of exercise on leptin concentrations reported that serum leptin concentrations decrease following moderate to high-intensity exercise and prolonged exercise training. The responses and adaptations of circulating leptin to exercise may have important implications because exercise is known to regulate body weight by promoting weight loss and maintenance, and by improving appetite control in obesity. However the concomitant effects of exercise, if any, on appetite and circulating leptin concentrations have not been documented in previous studies.

It is not clear what causes this feeling of increased satiety or suppressed hunger after exercise in some obese and lean individuals. The assessment of possible associations between ratings of appetite-satiety and biochemical measures known as hunger or satiety signals could provide useful information about the mechanisms of appetite regulation. Previous studies have not combined measurements of the drive to eat and biochemical measurements, which indirectly assess the sensitivity of the appetite control system. Therefore the present studies were designed to investigate the effects of exercise on the drive to eat, on subsequent food intake and on serum leptin concentrations. Whether there is a relationship between the drive to eat and serum leptin concentrations after acute exercise was also investigated as a means of assessing leptin sensitivity.
Chapter Two

General Methods
2. General Methods

This chapter describes the general methodologies chosen, developed and used throughout this thesis. The thesis comprises of 4 main experimental studies (Studies 1, 2, 3 and 4). Methods specific to each study are described in the relevant chapters.

2.1 Subjects and study approval

All experiments described in this thesis involved human volunteers recruited by local advertisement and word of mouth. The subjects were obese (Study 1 and 3) and normal weight (Study 2) women, and well-trained male athletes (Study 4). All experiments were approved by the Glasgow Royal Infirmary Research Ethics Committee. The nature and purpose of each experiment were explained verbally and in writing to each subject prior to each experiment. It was emphasised that subjects should only participate in the experiment if they were willing to follow all instructions. Subjects were also made aware that they could withdraw from the study at any point without being required to provide an explanation. All subjects provided written informed consent prior to taking part in an experiment. The results of studies 1 and 3 in obese subjects were discussed with the subjects to explain the relevance to their weight problems.

2.2 Experimental design and preferred methods (from literature)

The exercise and dietary interventions described in Studies 1 and 2 followed a Latin-square block, cross over design. In Study 3 the exercise interventions preceded the infusion interventions and all four trials were double blind, placebo-controlled randomised trials. In Study 4 the trained condition preceded the detraining
intervention. Subjects also acted as their own controls in all experiments.

2.3 The measurement of the drive to eat and of subsequent food consumption

There is no “gold standard” or objective way to measure appetite or satiety. The technique used to evaluate the drive to eat was visual analogue scales (VAS) for self-report ratings of hunger, desire to eat, prospective food consumption, satiety and fullness (Flint et al, 2000). This is one of the two most common methods for assessing feelings of hunger and satiety and has been validated in previous feeding studies (Stubbs et al, 2000). The feelings of desire to eat and prospective food consumption are considered as the early phase of the drive to eat. They can be triggered by eating-related stimuli (e.g. view of food, smell of food etc.) and can motivate eating whilst still being satiated. Feelings of appetite and desire to eat lead people to search for food while hunger is considered as the heightened feeling of the drive to eat. Hunger is associated with physical symptoms and dominates behaviour patterns in quests for food. When hunger is dominant, then actual eating will start. The feelings of satiety and fullness follow the food intake and both should signal termination of a meal. Fullness is related to gastrointestinal feelings that will stop eating and satiety is related to a mental perception of ‘fullness’ that will signal ‘stop eating’.

It is recognised that the terms appetite and hunger may not be interpreted identically by different individuals within the general public. There is no definition given for these terms in the VAS questionnaires and this might have some influence in
reducing the discrimination between these terms. Future questionnaires could amplify the distinctions between appetite, desire to eat and hunger and add brief definitions/explanations of these terms not included in the present appetite questionnaires.

The visual analogue scales are horizontal lines (often 100 or 150 mm long), unbroken and unmarked except for word anchors at either end that describe extremes of experience. The subject is instructed to mark the scale with a vertical line at a point that most accurately reflects the intensity of the feeling at the moment in time. The distance to that mark from the negative end (e.g. not at all hungry) is measured, thus having a score of 0-100 millimetres. It is useful that subjects are instructed to avoid marking out of either end. Subjects could also be advised that marking exactly at the end of the visual analogue scale could represent extreme conditions of hunger or satiety i.e., like being hungry of starvation or satiated of overfeeding. In Studies 1, 2, 3 participants were offered a buffet type dinner at the end of each trial. Buffet-style meals are usually used since they provide a bigger variety of foods than a single meal and allow food choice (Hill and Rogers, 1998). Prior to investigation participants were asked about food likes and dislikes. In this way, compliance with the experimental meal(s) was ensured. The amount of food consumed was measured by weighing the food items before and after eating. Each subject's selection from the buffet dinner was analysed for energy intake and macronutrient content using a computerised version of McCance and Widdowson's food composition tables (Holland et al, 1991).
2.4 Blood sampling and analytical procedures

Arterialised-venous blood samples were obtained at rest in all experiments; this method has been validated by Forster et al (1972). This involved heating one hand with diagram and placing either a 20 G or 18 G venous cannula in a superficial vein on the dorsal surface of the heated hand. In some subjects when sampling from the hand proved difficult, a superficial vein in the forearm was used. Subjects were comfortably seated with their forearm immersed in water at 42-44° C for at least 10 min before a resting sample was obtained. The cannula was kept patent by infusing a small volume of isotonic saline between samples. Blood was drawn into dry syringes and dispensed into tubes containing K3EDTA and into tubes containing coagulation factors. Duplicate aliquots (400 µl) of whole blood from the K3EDTA tube were rapidly deproteinised in 800 µl of ice-cold 0.3 mol/l perchloric acid; following centrifugation the supernatant was used for the measurement of glucose (Maughan, 1982). An aliquot of whole blood was dispensed equally into two eppendorf tubes and spun for 3 min and the plasma supernatant was separated and stored at -20° C and later used for the measurement of FFA (colorimetric method, Boehringer Mannheim Biochemica, London, UK).

The blood in tubes with coagulation factors was allowed to coagulate and then centrifuged; the serum collected was used for the measurement of serum leptin. The assay used for leptin analysis was a RIA for total leptin in serum based on a locally prepared antibody that is now readily available from Diagnostics Scotland (product code no. T270). The minimum detection limit (analyte concentration at an intra-assay coefficient of variation (CV) of 22%) was 0.9 ng/ml and the working range (analyte
concentration range with intra-assay CV < 10%) was 2.5-50 ng/ml (McConway et al., 2000).

2.5 Statistical Analysis

Information of data collected was examined by calculating numbers from the data (statistics). Data from all experiments are expressed as the mean or median, and the measures of dispersion were presented as standard deviation and range. The summary extracted from the data were presented in the form of tables and graphs to convey information. Statistical analysis of Studies 1 and 2 was carried out using Kruskal-Wallis tests followed by Wilcoxon signed rank tests. Statistical analysis of Study 3 was carried out using two factor ANOVA for repeated measures followed by Student's t-test for paired data where necessary. Statistical analysis of Study 4 was carried out using two factor ANOVA for repeated measures followed by Tukey post-hoc test. T-test for correlated data was used for the additional comparison of summary measures of postprandial responses of biochemical and appetite variables (time averaged areas under response vs time curves (AUC)). Serum leptin concentrations (Study 4) were log_{10} transformed prior to statistical analysis.

Correlation analysis in Studies 1, 2, 3 was carried out using Spearman's rank correlation coefficient ($r_s$) when at least one of the variates did not have normal distribution, and Pearson's correlation coefficient ($r$) (Study 4) when each of the variates had a normal distribution. Statistical significance was declared when $P \leq 0.05$. 

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Chapter Three

(Study 1)

Effects of moderate physical activity or a snack intake on hunger/satiety measures, subsequent food intake and serum leptin in obese women

This Chapter presents a study in essentially identical form to its publication in International Journal of Obesity
3.1 Introduction: Research Questions to be addressed and hypotheses to be tested

Previous studies have used several eating or diet and exercise interventions in an attempt to induce changes in circulating leptin concentrations to investigate the link between serum leptin and appetite regulation (Astrup et al, 1997; Boden et al, 1996; Caixas et al, 2002; Dallongeville et al, 1998; Doucet et al, 2000; Evans et al, 2001, Heini et al, 1998; Joannic et al, 1998; Karhunen et al, 1997; Keim et al, 1998; Kolaczynski et al, 1996; Raben and Astrup, 2000; Romon et al, 1999; Weigle et al, 1996). Circulating leptin has been considered as a regulator of energy balance during prolonged and severe energy deficits (e.g. weight loss, fasting) where, presumably, signals increased appetite and food intake for adaptation to food deprivation. Acute exercise interventions have involved high-intensity exercise and examined only the effects of exercise on leptin synthesis and/or secretion in normal weight or overweight men (Duclos et al, 1999; Landt et al, 1997; Leal-Cerro et al, 1998; Perusse et al, 1997; Racette et al, 1997).

The purpose of the present study was to investigate the effects of more moderate physical activity and eating interventions, similar to those encountered in normal living, on short-term appetite sensations. This was achieved by investigating the effect of moderate physical activity in the form of brisk walking and a modest snack on appetite sensations and on subsequent food intake in obese women. The association between serum leptin concentration and appetite sensations after moderate physical activity and snack was also investigated.
3.2 Research methods and procedures

3.2.1 Subjects
Ten obese but otherwise healthy women (Table 3.1) gave their written informed consent to take part in the study, which was approved by the Glasgow Royal Infirmary Research Ethics Committee. Of the ten women, five were pre- and five were postmenopausal. All subjects were in good physical and mental health, non-smokers, not on any medication known to affect appetite, not known to be anaemic or hyperlipidemic and not on a special diet.

3.2.2 Experimental design and protocol
Subjects were first familiarised with the appetite questionnaire (Flint et al, 2000) and kept food and physical activity records for two days preceding the first experimental trial and up to arrival at the laboratory. These food and activity patterns were replicated before subsequent trials. Household measures (i.e., glasses, cupfuls, tablespoons, slices, etc.) were used to quantify food and drink consumption.

Subjects took part in three experimental trials: Moderate physical activity, Snack and Control. The order of the three trials was randomised across subjects in a counterbalanced Latin–square design. There was an interval of at least two days between trials, and all trials were performed within 2 weeks for each subject. The study design is represented diagrammatically in Figure 3.1. On each of the three study days, subjects visited the laboratory approximately 2.5 h after having consumed a standard lunch. Upon arrival at the laboratory, body mass and height were recorded and percentage body fat and fat free mass were measured using a Bodystat-1500 Bioimpedance analyser (Bodystat Ltd., Isle of Man) (Kushner et al,
1986). The Bioimpedance analyser uses pairs of electrodes attached to the left hand and left foot of the subject. A current of 800 microamps at a frequency of 50 MHz is passed between the outer electrodes, and the voltage drop is measured at the proximal electrodes, from which the resistance of the tissues is calculated. The measured value for impedance is entered into a regression equation, together with anthropometric data such as weight, height, age and gender. The bioelectrical impedance analysis (BIA) provides a reliable assessment of total body water under most conditions in healthy individuals and those with mild-to-moderate obesity. The BIA is not reliable in severe obesity (35 kg.m\(^{-2}\) \(\leq\) BMI \(\leq\) 45 kg.m\(^{-2}\)), or as a method of estimating the composition of tissues gained or lost during weight change. Despite the limitations, studies have found that BIA gives better estimates of body fat than those based on skinfolds or weight-height indices (Heitman et al, 1990).

Following this, subjects rested in a seated position for 10 min, and a baseline, venous blood sample (-60 min) was then taken. The cannula was kept patent by a slow (ca. 0.5 ml.min\(^{-1}\)) infusion of isotonic saline. Serial blood samples (10 ml) were drawn at 0, 30 and 90 min. Subjects remained seated and relaxed for at least 10 min prior to each blood sample. A set of self-rating 100-mm visual analogue scales for hunger, desire to eat, prospective food consumption, satiety and fullness (Flint et al, 2000) was completed after each blood sample. Within-subject comparisons are suggested to provide the best use of visual analogue scales, eliminating the inter-subject variation in appetite response (Stubbs et al, 2000).

Throughout each trial, subjects were seated in a comfortable environment and watched food-related videotapes for the first hour. For each trial there was a set of
videotapes demonstrating recipes of appetizing foods. Food-related videotapes were intended to direct participants’ attention towards food and eating, to stimulate a familiar form of home entertainment which might distract subjects and reduce eating restraint (Bellisle and Dalix, 2001). Subjects were required to remain seated for 30 min (Control trial) or were served a snack (58.5g chocolate-based snack: 1189 kJ (284 kcal), 36.0 g carbohydrate, 13.6 g fat, 4.6 g protein) and asked to consume it within 20 min while remaining seated (Snack trial) or were asked to walk at a brisk pace for 20 min (Moderate physical activity trial). The television was switched off for 30 min during each intervention. Following each intervention, subjects continued to watch food-related videotapes for another 1 h. Subjects were then served a buffet-type dinner comprising 10 food items. At dinner, subjects were asked to eat as much as they wanted within 1 h. Subjects ate alone and non-supervised during the buffet-dinner because the number of people present at a meal has been established to influence the amount eaten in a meal (de Castro, 2000). All food items were weighed before eating, and the leftovers were weighed again at the end of the dinner. Each subject’s selection from the buffet dinner was analysed for energy intake and macronutrient content using a computerised version of McCance and Widdowson’s food composition tables (Holland et al, 1991). Water was provided upon request at the first trial and subjects were asked to replicate the amount drunk during the following two trials. Prior to the study subjects were asked about food likes and dislikes to define the snack and the buffet meal, which all subjects would like.

The brisk walking was performed indoors under supervision in the Clinical Investigation Unit of the Department of Human Nutrition. Heart rate was measured (Polar Sport Tester, Polar Electro OY, Kempele, Finland) and ratings of perceived
exertion (RPE) (Borg, 1982) recorded separately for breathlessness and leg exertion at 5 min intervals during the exercise. Subjects were instructed to maintain a level of exertion of approximately 13 on the RPE scale (i.e., corresponding to 'somewhat hard'). Heart rate at rest and at the end of the moderate physical activity intervention was 80 ± 6 and 123 ± 18 b·min⁻¹, respectively (mean ± SD). Subjective perceived exertion was somewhat hard (14 ± 2) at the end of the moderate physical activity.

3.2.3 Blood treatment and analyses
Venous blood was collected into K₃EDTA vacutainers for the measurement of blood glucose, plasma free fatty acids (FFA) and into clot activator vacutainers for serum leptin measurement. Duplicate aliquots (400 µl) of whole blood from the K₃EDTA tube were rapidly deproteinised in 800 µl of ice-cold 0.3 mol·l⁻¹ perchloric acid; following centrifugation the supernatant was used for the measurement of glucose (Maughan, 1982). The remaining plasma supernatant was separated and stored at -20°C and later used for the measurement of FFA (colorimetric method, Boehringer Mannheim Biochemica, London, UK). Blood collected into the clot activator vacutainer was allowed to clot for 10 min. Following centrifugation, the serum was stored at -70°C and subsequently analysed for leptin by radioimmunoassay (McConway et al, 2000).

3.2.4 Statistical Analysis
Data are expressed as mean ± SD or median (range) as appropriate following a test for normality of distribution. Data describing serum leptin concentrations and appetite-satiety ratings were not normally distributed, so all comparisons of
responses to the three interventions were made using non-parametric tests. The Kruskal-Wallis test was performed to determine at which time points there were treatment effects. Post-hoc analysis by the Wilcoxon-signed rank test was performed to determine treatment difference at each time point and effects over time within each treatment. Correlation analysis between serum leptin and appetite measures (for each time point separately) and adiposity indices was carried out using the Spearman rank correlation coefficient ($r_s$). Statistical significance was taken as $P < 0.05$.

3.3 Results

3.3.1 Effects on self-reported appetite-satiety measures and subsequent dietary intake

Profiles of hunger, desire to eat, prospective food consumption, fullness and satiety throughout each trial are shown in Figure 3.2. The Moderate physical activity and Snack interventions both induced significantly higher perceptions of satiety and fullness compared to Control; ratings were significantly higher compared to Control immediately after the Moderate physical activity ($P = 0.01$ satiety; $P = 0.02$ fullness) and Snack intervention (30 min) ($P = 0.01$ satiety; $P = 0.01$ fullness). Only in the Moderate physical activity trial was satiety still significantly higher 1 h after the intervention (90 min) compared to Control ($P = 0.02$). Significant suppression of hunger was found immediately after the Snack intervention (30 min) compared to Control ($P = 0.01$) and Moderate physical activity ($P = 0.03$). Desire to eat and prospective food consumption were significantly lower immediately after the Snack intervention (30 min) compared to Control ($P = 0.01$ desire to eat; $P = 0.01$ prospective food consumption) but only desire to eat was still suppressed 1 h after
the Snack intervention (90 min) \((P = 0.01)\). Desire to eat and prospective food consumption were also significantly lower immediately after the Moderate physical activity intervention (30 min) compared to Control \((P = 0.03\) desire to eat; \(P = 0.009\) prospective food consumption).

Self-selected food intake at dinner did not differ significantly between trials (2860 (2134-4234 kJ) Moderate physical activity, 2751 (2268-3108 kJ) Snack, 3032 (2134-5733 kJ) Control; Protein 67.1 (38.4-79.9) g Moderate physical activity, 54.9 (41.3-70.8) g Snack, 59.7 (35.3-96.2) g Control; Carbohydrate 59.7 (49.8-103.8) g Moderate physical activity, 65.3 (34.7-75.6) g Snack, 76.9 (45.0-138.6) g Control; Fat 19.6 (6.5-32.7) g Moderate physical activity, 23.9 (13.2-30.3) g Snack, 25.7 (12.6-51.4) g Control), median (range).

### 3.3.2 Effects on biochemical measures

Serum leptin, blood glucose and plasma FFA concentrations during the three trial conditions are shown in Table 3.2. There was no significant effect of any intervention or effect over time on serum leptin concentrations \((P > 0.05)\). Significant differences between trials were found in blood glucose and plasma FFA concentrations after the Moderate physical activity and Snack interventions. Snack intake induced significantly higher glucose concentrations immediately after the Snack intervention (30 min) compared to Control or Moderate physical activity trial \((P = 0.009)\). One h after the Snack intervention (90 min), glucose concentrations were still higher in the Snack trial than in the Control \((P = 0.02)\) or Moderate physical activity trial \((P = 0.02)\), whereas plasma FFA concentrations were significantly lower in the Snack trial compared to the Control and the Moderate
physical activity trial \((P = 0.009)\). The Moderate physical activity intervention induced higher plasma FFA concentrations immediately after intervention (30 min) compared to both the Control and the Snack trial \((P = 0.009)\). Significant time effect for glucose and FFA concentrations were found in the Moderate physical activity and Snack trials.

No significant associations were demonstrated between serum leptin and blood glucose or plasma FFA concentrations at any time point in the three trials \((P > 0.05)\). Baseline serum leptin concentrations correlated significantly with body mass index \((\text{BMI} \text{ (kg} \cdot \text{m}^{-2})\) and fat mass \((\text{FM (kg)}\) in all trials \((\text{BMI} r_s = 0.73, P = 0.02, \text{FM} r_s = 0.88, P = 0.001)\) Moderate physical activity; \text{BMI} r_s = 0.69, P = 0.02, \text{FM} r_s = 0.85, P = 0.002)\) Snack; \text{BMI} r_s = 0.78, P = 0.008, \text{FM} r_s = 0.90, P < 0.001 Control).

### 3.3.3 Correlations between biochemical measures and self-reported appetite-satiety measures

No significant correlations were found between serum leptin and appetite or satiety ratings at any time in the Control or the Snack trial. Only in the Moderate physical activity trial was serum leptin concentration significantly correlated with prospective food consumption immediately after intervention (30 min) \((r_s = -0.83, P = 0.003)\). Additionally, 1 h after the moderate physical activity intervention (90 min) serum leptin concentrations were significantly correlated with appetite or satiety ratings (hunger \(r_s = -0.79, P = 0.007\); desire to eat \(r_s = -0.69, P = 0.02\); satiety \(r_s = 0.71, P = 0.02\); fullness \(r_s = 0.66, P = 0.04\) \((\text{Figure 3.3})\). The associations between leptin and appetite-satiety ratings found immediately after and 1 h after the moderate physical activity intervention remained significant when circulating leptin concentrations
were adjusted for adiposity by dividing by body mass index (30 min hunger $r_s = -0.75$, $P = 0.01$; desire to eat $r_s = -0.75$, $P = 0.01$; prospective food consumption $r_s = -0.86$, $P = 0.002$; 90 min hunger $r_s = -0.74$, $P = 0.01$; satiety $r_s = 0.67$, $P = 0.03$; fullness $r_s = 0.66$, $P = 0.04$). The associations also remained significant when serum leptin concentrations were adjusted for fat mass (30 min hunger $r_s = -0.71$, $P = 0.02$; desire to eat $r_s = -0.71$, $P = 0.02$, prospective food consumption $r_s = -0.84$, $P = 0.002$; 90 min hunger $r_s = -0.74$, $P = 0.01$; satiety $r_s = 0.67$, $P = 0.03$; fullness $r_s = 0.66$, $P = 0.04$).

### 3.4 Discussion

The study used brisk walking and a chocolate-based snack, in an attempt to replicate typical physical activity and eating behaviours, to investigate the effects on appetite and on associations between serum leptin and appetite. Associations between circulating leptin and suppressed appetite or elevated satiety were found following 20 min of moderate physical activity (walking about 1 km), but not at any time point during the snack or the control conditions.

In other studies of leptin and appetite, circulating leptin concentrations have been associated with appetite or fullness perceptions, but only in fasting obese or post-obese individuals and during weight loss or maintenance produced by diet or diet and aerobic exercise (Doucet et al, 2000; Heini et al, 1998; Keim et al, 1998). These observations support the view that leptin regulates appetite centrally only after sustained fat loss to re-establish fat homeostasis in fat tissue (Caro et al, 1996a). In contrast, during the process of weight gain, high serum leptin concentrations are closely related to body fat (Considine et al, 1996), but are not usually coupled with
appetite suppression in obese individuals. Therefore, serum leptin has not been considered to play a role in short-term appetite processes, or there is possibly some form of 'resistance' to short-term central actions of leptin in human obesity. However, the present study indicates that circulating leptin may indeed be involved in short-term appetite regulation in obese individuals but only after physical activity. Therefore, physical activity-induced factor(s) may be responsible for the observed 'coupling' of leptin to appetite.

The moderate physical activity employed in the present study, as expected, did not affect circulating leptin concentrations. Only extreme exercise (2 to 3.5 h marathon running, 2 h of strenuous cycling) is known to decrease plasma leptin concentrations (Duclos et al, 1999; Landt et al, 1997; Leal-Cerro et al, 1998). It is likely that physical activity could influence leptin transport into the brain which could explain the 'coupling' of leptin to appetite found after a bout of moderate-intensity physical activity.

Some studies have suggested impaired leptin transport across the blood brain barrier in animals (Banks et al, 1999) and probably in humans (Caro et al, 1996b) residing in an 'obesigenic' environment (i.e., increased food intake and/or physical inactivity). This reduced transport of leptin into the brain is proposed as a possible mechanism for leptin 'resistance' in obesity. An exercise effect on leptin transport into the brain has not been investigated in humans but animal findings indicate enhanced leptin transport into the brain mediated by elevated circulating adrenaline concentrations (Banks, 2001).
Plasma catecholamines were not measured in the present study. However, increased plasma FFA concentrations were found after the moderate physical activity (average FFA 1.2 mmol\textsuperscript{-1}L\textsuperscript{1}), which is indicative of adrenaline-stimulated lipolysis (Cryer, 1993). Catecholamines have been recognised as important modulators of leptin production and secretion (Carulli et al., 1999; Couillard et al., 2002) but whether they could regulate leptin uptake into the brain in humans is unknown. If catecholamines are responsible for the ‘coupling’ of circulating leptin to satiety following moderate physical activity, then this begins to unravel a mechanism by which physical activity-induced factors may influence appetite by enhancing leptin transport into the brain. The role of exercise as an effective strategy to prevent or attenuate the development of leptin resistance is supported by recent findings in rats. Steinberg et al. (2004) found that endurance training reverses the development of skeletal muscle leptin resistance induced by high-fat diet in rats. Leptin has been suggested to have a particular function in the hunger drive under starvation conditions (Flier, 1998), but may have a more extended physiological role. It is possible that individuals predisposed to obesity may need greater physical activity than others, in order for serum leptin to be transported into brain efficiently and curtail appetite.

Circulating catecholamines concentrations are also increased after carbohydrate-rich meals in parallel with serum leptin concentrations (Raben and Astrup, 2000; Raben et al. 1994; Raben et al., 1997), and a positive association has been found between the elevated circulating leptin and catecholamines concentrations after a carbohydrate rich diet. Food intake, and predominantly carbohydrate intake stimulates leptin secretion (Astrup et al., 1997; Caixas et al., 2002; Dallongeville et al., 1998; Doucet et al., 2000; Raben and Astrup 2000; Romon et al., 1999) but no association has been
found between the increased serum leptin concentrations and the heightened postprandial satiety in the short-term (up to 9 h postprandially) in lean or post-obese individuals (Raben and Astrup 2000; Romon et al, 1999).

In the context of weight management for obesity, physical activity or exercise alone are better linked with weight maintenance than with enhanced weight reduction (Cowburn et al, 1997; Wing and Hill, 2001). The present results indicated very consistently that obese individuals who engage in 20 min of moderate physical activity during the course of the day could improve acute appetite control and avoid the caloric burden of snacking. The numbers in the current study were small increasing the chance of type 2 errors but there was no previous source of bias, which might confound these results. Baseline measures of appetite and satiety sensations and of biochemical variables were no different between subjects in the three trial conditions. This indicates subjects’ adherence to instructions to standardise diet and physical activity for two days prior to each study day. The consumption of a modest snack (1189 kJ) produced lower feelings of appetite and higher satiety-fullness perceptions, as expected, but did not decrease subsequent food intake. Similarly, a short bout of brisk walking equivalent to approximately 502 kJ energy cost, increased satiety-fullness perceptions transiently, and most importantly did not increase the subsequent food intake. Moderate-intensity physical activities can be adopted by obese individuals to promote satiety and are more likely to be continued than high-intensity physical activities (Pollock, 1988).
The present study assessed appetite and satiety in relation to snacking or moderate physical activity in the afternoon and evening. Most previous research has been conducted with the morning fasting state as baseline, but it is in the afternoon or evening that most obese individuals tend to report higher food intake (Andersson and Rossner, 1996). Snack intake did not decrease the subsequent food intake, and serum leptin concentrations in accordance with previous studies (Heini et al, 1998; Joannic et al, 1998; Romon et al, 1999) were not associated with post-snack satiety ratings. Hence, circulating leptin concentrations do not appear to be primary regulators of short-term satiety following a meal. The observations that moderate physical activity can suppress appetite without increasing subsequent food intake, supports the view that the apparent urge to eat, experienced by obese women, may be a misinterpreted signal of boredom whilst physically inactive. However, studies in normal weight individuals are needed to explore this possibility. The present results suggest that moderate physical activity could be used to prolong meal-induced satiety and suppress the drive to eat during the early post-prandial phase, i.e., the period of ‘readiness to eat’. ‘Readiness to eat’ appears to be resumed soon after meal cessation, when there is still relative satiation and before appetite has developed.
3.5 New Research Questions arising from this study

These results therefore support a specific role for moderate exercise in linking circulating leptin to appetite regulation in obese women. Whether there is a coupling between leptin and appetite regulation following moderate exercise in lean women is one of the new research questions raised from this study:

1. What is the effect of moderate exercise or a mild snack on appetite sensations, subsequent food intake and serum leptin in lean women?

2. Is there a coupling between circulating leptin and appetite control following exercise or snacking in lean women?
Table 3.1 Subject characteristics, n = 10

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96 ± 18</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161 ± 5</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>37 ± 6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>45 ± 10</td>
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<tr>
<td>Fat mass (%)</td>
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<td>Fat free mass (kg)</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Fat free mass (%)</td>
<td>53 ± 4</td>
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</tbody>
</table>

Values are mean ± SD.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Moderate exercise</th>
<th>Snack</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy intake (kcal)</strong></td>
<td>724 (509-1369)</td>
<td>683 (509-1011)</td>
<td>657 (541-742)</td>
</tr>
<tr>
<td><strong>(kJ)</strong></td>
<td>2751 (2268-3108)</td>
<td>2860 (2134-4234)</td>
<td>2751 (2268-3108)</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>49 ± 17</td>
<td>45 ± 16</td>
<td>39 ± 12</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>84 ± 40</td>
<td>75 ± 31</td>
<td>66 ± 24</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>22 ± 12</td>
<td>23 ± 11</td>
<td>23 ± 13</td>
</tr>
</tbody>
</table>

Values are median (range)

No significant differences were found between the three trial conditions
Table 3.3 Serum leptin, blood glucose and plasma free fatty acids (FFA) concentrations during the Control, Moderate physical activity and Snack trials

<table>
<thead>
<tr>
<th></th>
<th>Baseline (-60 min)</th>
<th>Pre intervention (0 min)</th>
<th>Post intervention (30 min)</th>
<th>1 h post-intervention (90 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng ml⁻¹)</td>
<td>45.4 (31.0 - 123.0)</td>
<td>48.1 (30.5 - 122.4)</td>
<td>51.3 (26.2 - 131.6)</td>
<td>55.9 (27.4 - 130.6)</td>
</tr>
<tr>
<td>Blood glucose (mmol l⁻¹)</td>
<td>4.9 (4.1 - 5.9)</td>
<td>4.7 (4.1 - 5.9)</td>
<td>4.7 (4.3 - 5.3)</td>
<td>4.6 (4.4 - 5.3)</td>
</tr>
<tr>
<td>Plasma FFA (mmol l⁻¹)</td>
<td>0.5 (0.2 - 1.0)</td>
<td>0.6 (0.1 - 1.0)</td>
<td>0.8 (0.2 - 1.4) **</td>
<td>0.8 (0.4 - 1.3) **</td>
</tr>
<tr>
<td><strong>Moderate activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng ml⁻¹)</td>
<td>54.8 (29.1 - 91.4)</td>
<td>56.7 (26.9 - 119.2)</td>
<td>58.7 (33.7 - 121.0)</td>
<td>56.1 (26.0 - 133.0)</td>
</tr>
<tr>
<td>Blood glucose (mmol l⁻¹)</td>
<td>5.2 (3.9 - 6.3)</td>
<td>4.7 (4.1 - 6.1)</td>
<td>4.7 (3.9 - 5.5)</td>
<td>4.9 (3.8 - 5.2)</td>
</tr>
<tr>
<td>Plasma FFA (mmol l⁻¹)</td>
<td>0.5 (0.1 - 0.8)</td>
<td>0.6 (0.3 - 1.1) §</td>
<td>1.3 (0.5 - 1.9) *↑,§</td>
<td>0.9 (0.4 - 1.2) §</td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng ml⁻¹)</td>
<td>44.1 (26.4 - 147.4)</td>
<td>47.6 (22.4 - 136.0)</td>
<td>48.6 (23.2 - 112.0)</td>
<td>54.4 (27.4 - 100.2)</td>
</tr>
<tr>
<td>Blood glucose (mmol l⁻¹)</td>
<td>5.2 (4.6 - 6.0)</td>
<td>4.9 (4.3 - 5.4)</td>
<td></td>
<td>6.0 (4.7 - 8.0) *↑,</td>
</tr>
<tr>
<td>Plasma FFA (mmol l⁻¹)</td>
<td>0.5 (0.2 - 0.9)</td>
<td>0.6 (0.1 - 0.9)</td>
<td>0.7 (0.3 - 1.0)</td>
<td>0.2 (0.1 - 0.4) *↑,</td>
</tr>
</tbody>
</table>

Values are median (range). Medians within row with different superscript symbols are significantly different between trials (* P < 0.01: Moderate physical activity vs Control; † P < 0.05: Snack vs Moderate physical activity; ‡ P < 0.05: Snack vs Control), (Kruskal-Wallis test followed by Wilcoxon-signed rank test). Medians within row with superscript symbols §||** are significantly different from baseline within Moderate physical activity (§ P < 0.01), Snack (|| P < 0.05) or Control (** P < 0.01) trial, (Wilcoxon-signed rank test).
Figure 3.1: Schematic representation of the study design.
Figure 3.2: Median profiles of self-reported appetite-satiety ratings under the Moderate physical activity (■), Snack (▲) and Control (●) trials; Data were analysed using Kruskal-Wallis test followed by Wilcoxon-signed rank test to determine the differences in ratings between trials. *, †, ‡ indicate significant differences between trials, $P < 0.05$ (*: Moderate physical activity vs Control; †: Snack vs Moderate physical activity; ‡: Snack vs Control); § || ** are significantly different from baseline within the Moderate physical activity (§ $P < 0.05$), the Snack (|| $P < 0.05$) or the Control (** $P \leq 0.01$) trial. The range has been excluded for clarity reasons.
Figure 3.2

- Hunger (mm)
- Desire to eat (mm)
- Prospective Food Consumption (mm)
- Fullness (mm)
- Satiety (mm)

- Moderate physical activity
- Snack
- Control

Time (min)
Figure 3.3: Spearman rank associations between ranked serum leptin concentrations (ng ml\(^{-1}\)) and ranked appetite-satiety measures (recordings on a 0-100-mm scale) in the physical activity trial 1 h after the moderate physical activity intervention (hunger \(r_s = -0.79\ P = 0.007\); desire to eat \(r_s = -0.69\ P = 0.02\); satiety \(r_s = 0.71\ P = 0.02\); fullness \(r_s = 0.66\ P = 0.04\).
Chapter Four
(Study 2)

Effects of moderate exercise or a snack intake on hunger/satiety measures, subsequent food intake and serum leptin in lean women

This Chapter presents results of a study in essentially the same form as has been prepared for submission to Appetite
4.1 Introduction: Research Questions to be addressed and hypotheses to be tested

In Chapter 3 (Study 1) an inverse relationship between serum leptin concentrations and satiety or appetite ratings was found acutely following moderate exercise in obese women. These results were the first to report leptin involvement in the disordered short-term appetite regulation of obese individuals. The results led to a new research question. What is the relationship between leptin and appetite after exercise in lean women? Is serum leptin coupled with appetite control, by moderate exercise, in lean women too? The present study investigated the influence of moderate exercise on the association between serum leptin and appetite in lean women. In this way leptin sensitivity after moderate exercise was indirectly assessed in lean women.

4.2 Research methods and procedures

4.2.1 Subjects

Ten lean women (Table 4.1) gave their written informed consent to take part in the study, which was approved by the Glasgow Royal Infirmary Research Ethics Committee. Subjects were recruited by advertisement, which required lean, never-obese women in good physical and mental health, non-smokers, not on any medication known to affect appetite, not known to be anaemic or hyperlipidemic, and not on a special diet or exercising regularly (more than 2 times per week).
4.2.2 Experimental design and procedures

The same study design and experimental procedures were used, as previously in obese women (Chapter 1, Study 1). Subjects took part in three experimental trials: Moderate exercise, Snack and Control. The order of the three trials was randomised across subjects in a counterbalanced, Latin–square design. There was an interval of at least two days between trials, and all trials were performed within 2 weeks for each subject.

On each of the three study days, subjects visited the laboratory approximately 2.5 hrs after having consumed a standard lunch. Upon arrival at the laboratory, body mass, waist and height were recorded. Body fat was measured using an air displacement plethysmograph, the BOD POD (McCrory et al, 1995). Plethysmography determines body volume based upon the pressure/volume relationship. Boyles’ law explains this relationship at isothermic conditions. \( PV = k \) where \( k \) is the proportionality constant. The BOD POD is a single ‘egg’ shaped unit consisting of two chambers; a testing chamber where the subject sits and a reference chamber where the breathing circuit, pressure transducers, and electronics. The testing procedure involves several steps. First, calibration was conducted prior to subject entry into the BOD POD. After the calibration was completed and procedures fully explained to the subject the relevant clothing scheme and swimcap (worn to minimize isothermal air trapped within the hair) were donned. The subject entered the BOD POD for two trials for approximately 45 seconds each. During this stage the subject’s raw body volume (\( V_{b_{raw}} \)) was determined with the testing chamber door being opened between trials. If both volumes were within 150 ml then the two trials were averaged. However, if the
trials were not within 150 ml a third trial was performed and the two trials that were the closest were averaged.

Throughout each trial, serial blood samples (10 ml) and subjective appetite and satiety sensations (Flint et al., 2000) were measured as previously described (Study 1, Chapter 3). In the moderate exercise trial, heart rate (Polar Sport Tester, Polar Electro OY, Kempele, Finland) and ratings of perceived exertion (RPE) (Borg, 1982) were recorded. Heart rate at rest and at the end of the moderate exercise intervention was 76.5 ± 9 and 135.6 ± 12 b/min⁻¹, respectively (mean ± SD). Subjective perceived exertion was somewhat hard (13 ± 2) at the end of the moderate exercise. Following each trial, subjects were served a buffet-type dinner. Each subject's selection from the buffet dinner was analysed for energy intake and macronutrient content using a computerised version of McCance and Widdowson's food composition tables (Holland et al., 1991).

### 4.2.3 Blood treatment and analyses

Venous blood was collected into K₃EDTA vacutainers for the measurement of blood glucose, plasma free fatty acids (FFA) and into clot activator vacutainers for serum leptin measurement. Duplicate aliquots (400 μl) of whole blood from the K₃EDTA tube were rapidly deproteinised in 800 μl of ice-cold 0.3 mol/l perchloric acid; following centrifugation the supernatant was used for the measurement of glucose (Maughan, 1982). The remaining plasma supernatant was separated and stored at -20°C and later used for the measurement of FFA (colorimetric method, Boehringer Mannheim Biochemica, London, UK). Blood collected into the clot activator
vacutainer was allowed to clot for 10 min. Following centrifugation, the serum was stored at -70°C and subsequently analysed for leptin by radioimmunoassay (McConway et al, 2000).

4.2.4 Statistical analysis

Data are expressed as mean ± SD or median (range) as appropriate following a test for normality of distribution. Statistical analysis of the data was carried out using Kruskal-Wallis Wilcoxon signed rank test. Correlation analysis between serum leptin, body composition measurements and appetite perceptions (for each time point separately) was carried out using the Spearman rank-order correlation coefficient ($r_s$). Statistical significance was taken as $P < 0.05$.

4.3 Results

4.3.1 Effects on appetite and satiety ratings in lean women

Profiles of hunger, desire to eat, prospective food consumption, fullness and satiety throughout each trial are shown in Figure 4.1. Snack intake induced significantly higher perceptions of satiety and fullness compared to Control and Moderate exercise trials, ratings were significantly higher immediately after the Snack intervention (30min) ($P = 0.03$ satiety; $P = 0.01$ fullness) compared to Control, and 1 hr after the Snack intervention (90 min) compared to Control ($P = 0.01$ satiety; $P = 0.04$ fullness) and Moderate exercise ($P = 0.007$ satiety; $P = 0.02$ fullness). Desire to eat was significantly lower 1 hr after the snack intervention (at 90min) compared to Control ($p = 0.03$ desire to eat). Moderate exercise similarly induced significantly higher fullness perception immediately after the intervention (30 min) ($p = 0.03$ fullness) compared to Control. There were no significant differences in hunger or
prospective food consumption ratings at any time point between the three trials. Self-selected energy and macronutrient intake at dinner did not differ significantly between trials (Table 4.2).

4.3.2 Effects on biochemical measures

There was no significant effect of intervention on serum leptin concentrations and no significant time-by-condition interaction ($P > 0.05$). There were significant differences in blood glucose and plasma FFA after the interventions. Snack intake induced significantly higher glucose concentrations immediately post-intervention (30 min) compared to Control ($P = 0.01$) or Moderate exercise trial ($P = 0.01$). One hr post-intervention glucose concentrations were still higher in the Snack trial than in the Control ($P = 0.01$) or Moderate exercise trial ($P = 0.01$), whereas plasma FFA concentrations were significantly lower in the Snack trial compared to the Control ($P = 0.005$) and the Moderate exercise trial ($P = 0.005$). Moderate exercise induced higher plasma FFA concentrations compared to the Control ($P = 0.005$) and the Snack trial ($P = 0.005$) immediately post-intervention (30 min) (Table 4.3).

No significant correlations were found between serum leptin and blood glucose or plasma FFA concentrations at any time point in the three trials ($P > 0.05$). Baseline serum leptin concentrations correlated significantly with body mass index (BMI (kg.m$^{-2}$), fat mass (FM (kg)) and percent fat mass (FM (%) in all trials (BMI $r_s = 0.74$, $P = 0.01$; FM $r_s = 0.84$, $P = 0.002$, FM (%) $r_s = 0.81$, $P = 0.005$).
4.3.3 Correlations between biochemical measures and self-reported appetite-satiety ratings in lean women

Hunger, desire to eat and fullness ratings were significant correlated with blood glucose concentrations only pre-intervention in the Moderate exercise trial. Fullness correlated positively \( (r_s = 0.75, \ p = 0.03) \), and hunger and desire to eat correlated negatively with blood glucose concentrations immediately pre-intervention (0 min) (hunger \( r_s = -0.76, \ p = 0.03 \); desire to eat \( r_s = -0.75, \ p = 0.03 \)). Significant correlations were found between plasma FFA concentrations and hunger-prospective food consumption or satiety-fullness sensations 1 hr post-intervention (90 min) only in the Snack trial (prospective food consumption \( r_s = -0.72, \ p = 0.03 \); satiety \( r_s = 0.78, \ p = 0.007 \); fullness \( r_s = 0.71, \ p = 0.02 \)) (Figure 4.2). No significant correlations were found between serum leptin concentrations and appetite-satiety ratings at any time in the three trials in lean women.

4.4 Discussion

In chapter 1, it was found that circulating leptin concentrations are coupled with “controlled” appetite after moderate exercise in obese women. The present study (Study 2) aimed to find out what is the relationship between serum leptin and appetite control after moderate exercise in lean women. Therefore exercise in the form of brisk walking and snack intake were used in lean women identically to those tests in obese women, and the effects on appetite/satiety ratings, subsequent food intake and serum leptin were investigated.
Similar results of decreased desire to eat after moderate increases in daily physical activity (~100 kcal/day) have been reported in both obese and lean women (Durrant et al, 1982). A short-lived (15 min) suppression of appetite has been clearly demonstrated after acute exercise (cycling 60-77% VO2 max, 27-120min) in lean men (King et al, 1994; King and Blundell, 1995; Thompson et al, 1988) but not in women (Hubert et al, 1998; King et al, 1996; Lluch et al, 2000). However the present study was conducted in non-regularly exercising women who reported increased fullness after moderate exercise, whereas regular exercisers (at least 3 times per week) have been previously tested (Hubert et al, 1998; King et al, 1996; Lluch et al, 2000). This difference in habitual exercise state may explain why exercise did not influence subjective appetite in these previous studies.

Snack intake increased satiety and decreased hunger feelings in lean women, which is probably related to elevated postprandial blood glucose concentrations. Mayer (1955) postulated that ‘short-term articulation between energy needs and energy intake was under glucostatic control’. Postprandially, plasma FFA concentrations were lower and blood glucose concentrations were higher in lean women. Associations between plasma free fatty acid concentrations and hunger suppression or elevated satiety were found one hr following the snack intake, but not throughout the control or exercise conditions in lean women. This biological profile is indicative of suppressed hunger and not eating (Himaya et al, 1997), possibly through the increased postprandial glucose metabolism. After a carbohydrate and fat-containing meal, carbohydrate oxidation prevails over fatty acid oxidation and stimulates lipogenesis. Thus, ingested fat is less readily oxidised and preferentially stored (Flatt et al, 1985; Jequier, 1994). The transient increase in blood glucose is thought to be
the signal of affluent available glucose, which is detected by central glucoreceptive elements that induce satiety (Bray, 2000). However it is interesting that plasma FFA but not blood glucose concentrations were associated with the increases in postprandial satiety/fullness perceptions. The snack intake 1189 kJ (284 kcal) did not affect subsequent food intake. These results agree with previous findings. Rolls et al (1991) found that neither a high carbohydrate nor a high fat snack (both 1.46 MJ) consumed 180 min before a meal reduced subsequent food intake. Porrini et al (1997) obtained similar results with a less energetic high protein or high fat snack (0.65, 0.79 MJ respectively) consumed 160 min before a test meal. Marmonier et al (2000) found that a nutritionally balanced snack (1 MJ) consumed in a satiated state did not affect subsequent food intake in an ad libitum lunch. No significant differences were found in energy intake between the three trial conditions. However it should be noted that the sample power could be calculated retrospectively after this study has been undertaken in lean women, and non significant differences in average energy intake (i.e. 594 kcal Snack, 670 kcal Moderate Exercise, 724 kcal Control) might be significant with more subjects.

No association was found between circulating leptin and hunger or satiety sensations following snack intake or moderate exercise in lean women. This would be in keeping with previous studies, which have reported no association between circulating leptin concentrations and postprandial satiety in lean or obese individuals (Joannic et al, 1998; Heini et al, 1998). Serum leptin concentrations were not associated with higher fullness following exercise in lean women. The present Study 2 suggests that moderate exercise can induce increased fullness in lean women.
acutely. This effect of exercise on appetite control is not associated with circulating leptin concentrations in lean women.

4.5 New Research Questions arising from this Study

The present Study 2 indicates similarities in the behavioural appetite control following snacking or moderate exercise between lean and obese women, but differences in the biological control of appetite between between lean and obese women. Leptin does not appear to be involved in acute appetite control after exercise in lean women. It is possible that lean individuals do not need exercise-related factors for leptin to function and regulate appetite because they have normal leptin secretion and transport (Schwartz et al, 1996). Alternatively different transport pathways of leptin might exist between lean and obese individuals. This study thus raises some new research questions:

1. Is the transport of leptin different between obese and lean individuals; is the blood brain barrier the predominant transport site of leptin in obese compared to lean individuals?
Table 4.1 Subject characteristics, n = 10

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37 ± 10</td>
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<tr>
<td>Weight (kg)</td>
<td>57 ± 6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 ± 6</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>FM (%)</td>
<td>27 ± 7</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>42 ± 4</td>
</tr>
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</table>

Values are mean ± SD
Table 4.2 Self-selected nutrient intake of lean women at dinner after the trial conditions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Moderate exercise</th>
<th>Snack</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal)</td>
<td>724 ± 286</td>
<td>670 ± 244</td>
<td>594 ± 208</td>
</tr>
<tr>
<td></td>
<td>(kJ)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3031 ± 1197</td>
<td>2805 ± 1021</td>
<td>2487 ± 871</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>49 ± 17</td>
<td>45 ± 16</td>
<td>39 ± 12</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>84 ± 40</td>
<td>75 ± 31</td>
<td>66 ± 24</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>22 ± 12</td>
<td>23 ± 11</td>
<td>23 ± 13</td>
</tr>
</tbody>
</table>

Values are mean ± SD

No significant differences were found between the three trial conditions
Table 4.3 Serum leptin, blood glucose and plasma free fatty acids (FFA) concentrations during the Control, Moderate exercise and Snack trials

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Moderate exercise</th>
<th>Snack</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (-60 min)</td>
<td>Pre intervention (0 min)</td>
<td>Post intervention (30 min)</td>
</tr>
<tr>
<td>Serum leptin (ng.ml⁻¹)</td>
<td>7.05 (3-31)</td>
<td>7.1 (3.6-35.2)</td>
<td>7.1 (3.4-39.8)</td>
</tr>
<tr>
<td>Blood glucose (mmol.l⁻¹)</td>
<td>4.61 (4-5.42)</td>
<td>4.27 (3.85-4.8)</td>
<td>4.23 (3.96-5)</td>
</tr>
<tr>
<td>Plasma FFA (mmol.l⁻¹)</td>
<td>0.44 (0.05-1.41)</td>
<td>0.69 (0.05-1.37)</td>
<td>0.78 (0.1-1.26)</td>
</tr>
<tr>
<td>Serum leptin (ng.ml⁻¹)</td>
<td>8.1 (1.8-16.8)</td>
<td>7.9 (1.4-17.2)</td>
<td>8.1 (1.8-20)</td>
</tr>
<tr>
<td>Blood glucose (mmol.l⁻¹)</td>
<td>4.55 (3.97-6.38)</td>
<td>4.23 (3.78-4.86)§</td>
<td>4.56 (4.17-4.71) *</td>
</tr>
<tr>
<td>Plasma FFA (mmol.l⁻¹)</td>
<td>0.34 (0.03-0.93)</td>
<td>0.51 (0.08-1.07)</td>
<td>1.46 (0.15-2.54) * † §</td>
</tr>
<tr>
<td>Serum leptin (ng.ml⁻¹)</td>
<td>7.1 (2.5-25)</td>
<td>7.5 (2-28.4)</td>
<td>8.1 (2.1-26.8)</td>
</tr>
<tr>
<td>Blood glucose (mmol.l⁻¹)</td>
<td>4.47 (3.66-5.84)</td>
<td>4.29 (3.78-4.86)</td>
<td>6.22 (4.63-6.68) † † ‼</td>
</tr>
<tr>
<td>Plasma FFA (mmol.l⁻¹)</td>
<td>0.29 (0.06-1.08)</td>
<td>0.59 (0.11-0.95)</td>
<td>0.61 (0.07-1.13)</td>
</tr>
</tbody>
</table>

Values are median (range). Medians within row with different superscript symbols are significantly different between trials (* P < 0.01: Moderate physical activity vs Control; † P < 0.05: Snack vs Moderate physical activity; ‡ P < 0.05: Snack vs Control), (Kruskal-Wallis test followed by Wilcoxon-signed rank test). Medians within row with superscript symbols §∥** are significantly different from baseline within Moderate physical activity (§ P < 0.01), Snack (∥ P < 0.05) or Control (** P < 0.01) trial, (Wilcoxon-signed rank test).
Figure 4.1: Profiles of self-reported appetite ratings under the Control (○), Moderate exercise (□) and Snack (△) trial. Medians followed by letters a, b, c indicate significant differences between trials (a: Snack vs Control, b: Moderate exercise vs Control, c: Snack vs Moderate exercise). Medians followed by symbols *, †, # are significantly different from baseline within the Control (*), the Moderate-intensity activity (†) or the Snack (#) trial, $P < 0.05$; Ranges have been excluded for clarity reasons.
Figure 4.1

Hunger (mm)

Desire (mm)

Prospective food consumption (mm)

Fullness (mm)

Satiety (mm)

-60 0 30 90

Time (min)

Moderate exercise
Snack
Control
Figure 4.2: Associations between plasma FFA concentrations (mmol.l⁻¹) and fullness-satiety/prospective food consumption measures (on a 0-100-mm scale, ranked) 1 hr post-intervention in the snack trial.

- \( r_s = 0.78, P = 0.007 \)
- \( r_s = 0.71, P = 0.02 \)
- \( r_s = -0.72, P = 0.03 \)
Chapter Five
(Study 3)

Effects of circulating adrenaline concentrations and of moderate exercise plus \(\alpha/\beta\) adrenergic blockade on serum leptin, appetite/satiety measures and food intake in obese women – two pilot studies

This Chapter presents a study in essentially identical form as has been prepared for submission to International Journal of Obesity
5.1 Introduction: Research Questions to be addressed and hypotheses to be tested

The sympathoadrenal system plays an important role in the regulation of total energy balance by affecting both energy intake and energy expenditure (Del Rio, 2000). It is functionally separated into the sympathetic nervous system (SNS) and adrenal medulla. The SNS is the major regulator of fat mobilisation from adipose tissue to provide energy homeostasis in the body (Rayner, 2001). Sympathetic β-adrenergic stimulation is known to evoke an increase in energy expenditure (i.e. thermogenesis) under basal fasting conditions (Blaak et al, 1993). It has also been shown that the SNS is responsible for the facultative component of the thermic effect of acute food intake in humans (Astrup et al, 1989). The adrenal medulla, via catecholamines, contributes to the regulation of food intake by influencing leptin homeostasis. Several human and animal studies indicate that catecholamines stimulate adipose signalling to the brain to increase food intake. This is suggested to be mediated through β-adrenergic stimulation, which inhibits leptin mRNA production and leptin secretion (Trayhurn et al, 1998, Carruli et al, 1999).

Adipose tissue is a heterogenous metabolic organ and several differences in the rate of lipolysis have been observed among various fat depots. For example, visceral adipose tissue from subjects not selected for age or body weight has a higher lipolytic activity than subcutaneous adipose tissue due to a combination of increased β-adrenoceptor-mediated catecholamine-induced lipolysis and reduced antilipolytic action of insulin in the visceral fat depot. Studies have shown alterations in the lipolytic action in various pathological conditions (e.g. obesity and metabolic syndrome) but minor regional differences in adipocyte lipolytic response are found in
normal weight-healthy conditions (Hoffstedt et al, 1997). Resistance to catecholamine-stimulated lipolysis in subcutaneous adipocytes, due to reduced $\beta_2$-adrenoceptor function, has been found in obese females (Reynisdottir et al, 1994), in subjects with hypertriglyceridemia (Arner et al, 1993), and in men with the metabolic syndrome (Reynisdottir et al, 1994). Lipolytic subcutaneous catecholamine resistance in obese males due to an increased $\alpha_2$ adrenoceptor response has also been reported (Mauriege et al, 1991). In contrast the lipolytic function in omental fat cells seems to be enhanced in subjects with upper-body obesity and the metabolic syndrome, mainly due to an increased $\beta_3$ adrenoceptor function (Lonnqvist et al, 1995, Hoffstedt et al, 1996).

In Chapter 3 (Study 1) an association between serum leptin and appetite suppression was found in obese individuals, but only following an acute bout of moderate-intensity exercise. Sedentary and obese individuals have lower exercise capacity and elevated cardiac response during moderate exercise compared to normal weight controls (Salvadori et al, 2003). The rapid increase in catecholamines that normally accompanies such a response may be responsible for the coupling of leptin and appetite. This raises the question whether increased circulating adrenaline concentrations could suppress appetite and food intake by enhancing leptin transport into the brain. Moreover, adrenoceptor blockade is known to promote weight gain (Buemann et al, 1992). Part of this weight gain mechanism could mimic mechanisms that abolish the effect of exercise in promoting satiety by ‘uncoupling’ the effects of circulating leptin.
The present chapter 5 includes the results of two experiments that were designed to infuse adrenaline (Webber et al., 1994; Walsh et al., 1998), to raise circulating concentrations to those typically seen during moderate exercise (Gustafson et al., 1990) (EXP 1) and on another occasion to administer labetalol, a combined α- and β-adrenoceptor blocker (Conner, 1983), before moderate exercise (EXP 2). In this way, the effects of moderate exercise performed during α/β adrenoceptor blockade, and of increased circulating adrenaline concentrations by exogenous intravenous administration on appetite/satiety measures and on subsequent food intake were investigated in obese women. Associations between serum leptin and appetite/satiety sensations were also investigated.

5.2 Research methods and procedures

5.2.1 Subjects
Ten obese but otherwise healthy women (Table 5.1) gave their written informed consent to take part in the study, which was approved by the Glasgow Royal Infirmary Research Ethics Committee. All subjects were in good physical and mental health with systolic blood pressure ≤ 140 and diastolic blood pressure ≤ 90 mmHg, non-smokers, not on any medication known to affect appetite, not known to be anaemic or hyperlipidemic and not on a special diet. In EXP 2 (Adrenaline infusion vs. Saline infusion) one subject did not fulfil the inclusion criteria thus results are from nine subjects.
5.2.2 Experimental design and procedures

Subjects kept food and physical activity records for two days preceding the first experimental trial and up to arrival at the laboratory. These food and activity patterns were replicated before all subsequent trials. Household measures (i.e., glasses, cupfuls, tablespoons, slices, etc.) were used to quantify food and fluid consumption.

Subjects visited the laboratory on four occasions to participate in four separate acute interventions; EXP 1: Moderate-intensity exercise plus α- and β- adrenoceptor blocker vs. Moderate-intensity exercise plus placebo, and EXP 2: Adrenaline infusion vs. Saline infusion. All trials were double-blinded controlled trials. The order of the trials for each experiment (EXP 1 & 2) was randomised separately. There was an interval of at least seven days between trials. The study design is represented diagrammatically in Figure 5.1. On each experiment, subjects visited the laboratory approximately 5 h after having consumed a standard lunch. Upon arrival at the laboratory body mass and body composition were measured (Lean et al., 1996). Arterialised-venous blood samples (McLoughlin et al., 1992) was collected from a 18 G indwelling catheter placed by percutaneous puncture into a vein on the dorsum of a heated hand and a baseline sample (-60 min) was taken. Serial blood samples (10 ml) were then drawn at 0, 20 and 80 min. Following each blood sample, subjects completed a set of self-rating 100-mm visual analogue scales for hunger, desire to eat, prospective food consumption, satiety and fullness.

Throughout each trial, subjects were seated in a comfortable environment and watched food-related videotapes for the first hour. Food-related videotapes were intended to direct participants attention towards food and eating, to stimulate a
familiar form of home entertainment which might reduce anxiety and eating restraint of subjects (Bellisle and Dalix, 2001). After the first hour, subjects took part in one of the following interventions on each of the four study days:

EXP 1 (Moderate-intensity exercise plus α- and β- adrenoceptor blocker vs Moderate-intensity exercise plus placebo): Prior to each of the two exercise trials, i.e. moderate exercise plus alpha- and beta-adrenoceptor blocker and moderate exercise plus placebo, subjects were given either 100 mg labetalol (a combined α- and β-blocker) or an equivalent amount of inert ‘placebo’ (calcium carbonate) 60 min before performing exercise. The dosage of 100 mg labetalol ensures that alpha and beta-adrenoceptor blockade is obtained (Richards et al, 1974) without influencing the exercise-induced changes in heart rate. Subjects were required to walk on a motorised treadmill at a moderate pace for 20 minutes.

EXP 2 (Adrenaline infusion vs Saline infusion): a single dose of either adrenaline hydrochloride (i.e., a 1:10,000) diluted in normal saline solution or normal saline, was infused intravenously at a rate of 12.5 ng min/kg ideal body weight, via a pump for 20 min (Webber et al, 1994), to yield a plasma level not exceeding 1 nmol/L. This dosage ensures that the plasma adrenaline will not exceed the level typically measured following moderate-intensity exercise (Gustafson et al, 1990). The video was switched off for 20 min during each intervention.

Following each intervention, subjects continued to watch food-related videotapes for another 1 h. Subjects were then served a buffet-type dinner comprising 10 food items. At dinner, subjects were asked to eat as much as they wanted within 1 h. Each
subject's selection from the buffet dinner was analysed for energy intake and macronutrient content using a computerised version of McCance and Widdowson's food composition tables (Holland et al., 1991).

For both experiments, Rating of Perceived Exertion (separately, for breathlessness and leg exertion) (Borg, 1982) and Heart rate (Polar Sport Tester, Polar Electro Oy, Finland) were recorded every 10 min during the moderate exercise and the infusion interventions. For EXP 1, expired gas was collected in Douglas bags for 5 min at rest, and thereafter 1 min collections were obtained every 10 min during the moderate exercise and the infusion interventions. Expired gases were analysed within 5 min of collection for \([O_2]\) (Servomex 570A, East Sussex, UK) and \([CO_2]\) (Servomex 1400 B4, East Sussex, UK), volume (dry gas meter, Harvard Apparatus Ltd., Hertfordshire, UK) and temperature (C6600 10-Channel Microprocessor, Comark, Hertfordshire, UK). Barometric pressure was measured using a standard mercury barometer. Oxygen uptake (\(\dot{V}O_2\)), carbon dioxide production (\(\dot{V}CO_2\)) and respiratory exchange ratio (RER) were subsequently determined and, consequently, the percentages of fuel oxidation were determined.

5.2.3 Blood treatment and analyses

Venous blood was collected into K$_3$EDTA vacutainers for the measurement of blood glucose, plasma free fatty acids (FFA) and into clot activator vacutainers for serum leptin measurement. Duplicate aliquots (400 μl) of whole blood from the K$_3$EDTA tube were rapidly deproteinised in 800 μl of ice-cold 0.3 mol\text{-}l$^{-1}$ perchloric acid; following centrifugation the supernatant was used for the measurement of glucose.
(Maughan, 1982). Plasma supernatant was separated and plasma (500 µl) was mixed with 50 µl EGTA-glutathione and stored at -70° C for subsequent determination of adrenaline and noradrenaline. The remaining plasma was stored at -20° C and later used for the measurement of FFA (colorimetric method, Boehringer Mannheim Biochemica, London, UK). Blood collected into the clot activator vacutainer was allowed to clot for 10 min. Following centrifugation, the serum was stored at -70° C and subsequently analysed for leptin by radioimmunoassay.

5.2.4 Statistical analysis

Data are expressed as mean ± SD. Statistical analysis of the data was carried out using two-way ANOVA for repeated measures followed by paired Student t-test. Correlation analysis between serum leptin concentrations and appetite measures (for each time point separately) and adiposity indices was carried out using the Pearson correlation coefficient (r). Statistical significance was taken as P ≤ 0.05.

5.3 Results

5.3.1 EXP 1: Moderate exercise plus α/β adrenergic blocker vs Moderate exercise plus placebo

5.3.1.1 Effects on self-reported appetite-satiety measures and subsequent dietary intake: Profiles of hunger, desire to eat, prospective food consumption, fullness and satiety throughout each trial are shown in Figure 5.2. There were no significant differences on appetite/satiety measures between trials, but it is interesting that satiety tended to be lower, even if not significant, after the exercise plus blocker compared to exercise with placebo. There was an increase in hunger/desire to
eat/prospective food consumption measures over time on the Moderate exercise plus placebo trial \( (P < 0.05) \), and in desire to eat on the Moderate exercise plus blocker trial, which stopped transiently immediately after exercise. On the Moderate exercise plus blocker trial, no differences were found over time in hunger, prospective food consumption or fullness ratings. Self-selected food intake at dinner did not differ significantly between trials (990 ± 245 kcal Moderate exercise (α/β blocker), 903 ± 259 kcal Moderate exercise (Placebo); Protein 53.5 ± 14.0 Moderate exercise (α/β blocker), 56.2 ± 17.7 g Moderate exercise (placebo); Carbohydrate 113.0 ± 23.2 g Moderate exercise (α/β blocker), 106.4 ± 52.5 g Moderate exercise (placebo); Fat 37.4 ± 14.8 g Moderate exercise (α/β blocker), 32.9 ± 10.2 g Moderate exercise (placebo), mean ± SD).

5.3.1.2 Effects on biochemical measures

Blood glucose, plasma FFA concentrations and serum leptin, during the two trials are shown in Table 5.2. There was no significant difference on serum leptin concentrations between the two moderate exercise trials but there was an increase over time. Serum leptin concentrations were significantly increased immediately after moderate exercise (20 min) compared to baseline (-60 min), and not at any other time-points, in both trials \( (P = 0.02 \) Moderate exercise plus α/β blocker, \( P = 0.007 \) Moderate exercise plus placebo). No significant associations were found at any time-point between serum leptin and appetite/satiety measures after the moderate exercise \( (P > 0.05) \). This study therefore failed to replicate the phenomenon observed in Chapter 3.
Baseline serum leptin concentrations correlated significantly with body mass index (BMI (kg·m\(^{-2}\)), fat mass (FM (kg) and waist (cm) (BMI \(r = 0.85, P = 0.002\), FM \(r = 0.63, P = 0.05\), Waist \(r = 0.71, P = 0.02\)).

Significant differences were found in blood glucose and plasma FFA between the two Moderate exercise trials. Blood glucose concentrations were significantly higher and plasma FFA were significantly lower for 1 h after the Moderate exercise plus \(\alpha/\beta\) blocker intervention compared to Exercise plus placebo (Table 5.2).

5.3.2 EXP 2: Adrenaline infusion vs. Saline infusion

5.3.2.1 Effects on self-reported appetite-satiety measures and subsequent dietary intake:

Profiles of hunger, desire to eat, prospective food consumption, fullness and satiety throughout each trial are shown in Figure 5.3. There was no significant difference on appetite/satiety measures between adrenaline infusion and saline infusion trials but it is interesting that satiety was higher immediately after the adrenaline infusion compared to saline infusion. There was a progressive increase in appetite measures and a decrease in satiety/fullness over time on both trials which stopped transiently immediately after the adrenaline infusion (\(P < 0.05\)).

Self-selected energy intake and carbohydrate intake was significantly greater following the adrenaline infusion compared to saline infusion (1146 ± 259 kcal adrenaline infusion, 1082 ± 263 kcal saline infusion (\(P = 0.04\)); Protein 65.2 ±18.4 g adrenaline infusion, 62.6 ± 14.7 g saline infusion; Carbohydrate 128.1 ± 37.2 g
adrenaline infusion, 114.6 ± 40.3 g saline infusion \( (P = 0.01) \); Fat 44.1 ± 13.5 g adrenaline infusion, 43.2 ± 13.4 g saline infusion), mean ± SD) (Figure 5.4).

5.3.2.2 Effects on biochemical measures

Blood glucose, plasma FFA and serum leptin concentrations during the two infusion trials are shown in Table 5.3. There was no significant difference on serum leptin concentrations and blood glucose concentrations between the adrenaline and the saline infusions or over time, throughout the trials \( (P > 0.05) \). Plasma FFA concentrations were significantly higher immediately after the adrenaline infusion compared to saline infusion (Table 5.3).

No significant associations were found between serum leptin concentrations and appetite-satiety measures at any time point in the two trials \( (P > 0.05) \). Thus again this study failed to replicate the phenomenon observed in Chapter 2. However, the power to detect significant associations could be calculated retrospectively, with the data obtained from this study.

Baseline serum leptin concentrations correlated significantly with body mass index (BMI \( \text{kg} \cdot \text{m}^{-2} \)), fat mass (FM (%)) and waist in both trials (BMI \( r = 0.79, P = 0.01 \), FM \( r = 0.69, P = 0.04 \), Waist \( r = 0.78, P = 0.01 \)).

5.3.2.3 Cardiopulmonary variables and fuel oxidation rates

For both experiments, rating of perceived exertion (RPE) (separately, for breathlessness and leg exertion) (Borg, 1982) and heart rate (Polar Sport Tester, Polar Electro Oy, Finland) were recorded every 10 min during moderate exercise or
adrenaline-saline infusion. Heart rate was increased significantly at the end of both exercise trials \((P < 0.001)\) in EXP 1 (Table 5.4). In EXP 2, heart rate was significantly increased during the adrenaline infusion compared to saline infusion (at 15 min \(P = 0.01\), at 20 min \(P < 0.001\)) (Table 5.5). In EXP 1 oxygen uptake \((\dot{V}O_2)\), carbon dioxide production \((\dot{V}CO_2)\) and respiratory exchange ratio (RER) were not different between trials (Table 5.6).

5.4 Discussion

The present study was designed in an attempt to replicate and explain, the coupling between leptin and suppressed appetite that was previously (Chapter 3) found after exercise in obese women. Evidence in rats suggested that raised adrenaline and noradrenaline concentrations might enhance leptin uptake into the brain (Banks, 2001). We therefore proposed that elevated adrenaline concentrations induced by exercise might be responsible for the correlation between serum leptin and suppressed appetite that was found after exercise in obese women, and that an \(\alpha/\beta\) adrenoceptor blockade would eliminate this effect of exercise. Considering that our sample size is similar to other adrenaline infusion studies (Webber and Macdonald 1993; Webber et al, 1994) statistical power is expected to be sufficient to distinguish differences between the two study arms in terms of cardio-respiratory and metabolic effects.

Adrenaline was infused intravenously (12.5 ng per kg IBW per minute) to raise circulating adrenaline concentrations at levels typically found during moderate-intensity exercise in obese women (Gustafson et al, 1990). Heart rate was significantly increased towards the end of the adrenaline infusion in the present
study, which is in agreement with previous studies (Walsh et al, 1998). Plasma FFA also reached concentrations of 0.95 nmol·ml⁻¹, which is a level known to indicate adrenaline-stimulated lipolysis (Webber et al, 1994). These results indicate that plasma adrenaline concentrations were above 0.6 nmol·ml⁻¹ (approximately 0.8 nmol·ml⁻¹ during 20 min of 12.5 ng per kg IBW per minute infusion), which affects heart rate and stimulates lipolysis (Clutter et al, 1980; Webber et al, 1994). The plasma catecholamine concentrations in the present study will be measured to confirm this but assays have been delayed until late 2004.

In the present study adrenaline infusion for 20 min significantly increased subsequent energy intake and carbohydrate intake in obese women. Previous studies of adrenaline infusion have found decreased circulating leptin concentrations after 60 min of infusion (0.010 µg/kg fat free mass/min) and speculated that this is associated with increased caloric intake (Couillard et al, 2002). Others have found that β₁- and β₂- adrenergic stimulation (adrenaline or insoproterenol infusion) suppresses the synthesis of leptin (ob) mRNA gene in obese men (Carruli et al, 1999; Ricci & Fried, 1999), which then results in decreased circulating leptin levels.

A possible explanation for the results of the present study is that the adrenaline infusion in the present study stimulated beta-adrenergic receptors known to reduce the ob-gene expression in adipocytes and this overwhelmed any effect on leptin transport and sensitivity. If a decrease in ob-gene expression occurred after the present adrenaline infusion and was ‘sensed’ by central orexigenic mechanisms, then this could explain the increased caloric intake following the adrenaline infusion in obese women. We did not detect any significant decreases in serum leptin.
concentrations following the adrenaline infusion, but this may be due to the large range of leptin response to adrenaline that is observed in human obesity (Couillard et al, 2002). In our obese subjects, four women (i.e. subjects 4, 7, 8, and 9) appear to have reduced leptin concentrations immediately after the infusion and five (i.e. subjects 1, 2, 3, 6, 10) did not respond to the adrenaline infusion (Figure 5.5).

Another possible explanation for the increased energy intake after adrenaline infusion could lie in a relationship between raised adrenaline/noradrenaline concentrations and hunger-related hormones e.g. increased ghrelin concentrations, which stimulate eating (Shiiya et al, 2002). A positive significant association has been found between plasma ghrelin and adrenaline concentrations in chronic heart failure patients (Nagaya et al, 2001), which indicates an interaction between circulating ghrelin and catecholamines.

The study of exercise with the α/β adrenergic blockade was a first attempt to indentify whether adrenergic stimulation through exercise is involved in appetite control. The combined α/β blocker labetalol was used in an attempt to abolish the effect of exercise on appetite supression and the correlation between leptin and appetite in obese women (Study 1, Chapter 3). It was hypothesised that combined adrenergic blockade will impair appetite control after exercise compared to placebo. Subjects ate a little less (by a mean of 87 kcal) after the moderate exercise with placebo (average kcal 903) compared to moderate exercise with α/β adrenergic blockade (average kcal 990) but this did not reach statistical significance ($P = 0.3$). Labetalol blocks both $\beta_1$ and $\beta_2$ adrenoceptors, and also $\alpha_1$ adrenoceptors (Wallin and O'Neill, 1983) but with greater degree of β blockade than that of α-blockade (ratio of
effective β/α blockade ~3:1 after oral dosing). Studies in rats and hyperthyroid patients have suggested that α-adrenoceptor antagonists, but not β-antagonists, block hypothalamic noradrenergic-induced feeding (Ritter and Epstein, 1975; Pijl et al, 2001). Thus the potential effects of catecholamines on hypothalamic food intake regulation appear to be mediated by α-adrenoceptors.

The present results of the blood glucose and plasma FFA concentrations confirm that 100 mg oral intake of labetalol 1 h before moderate exercise blocked the β-adrenoceptor-related lipolytic response to exercise. In the present study labetalol at 100 mg resulted in lower plasma FFA immediately after moderate exercise compared to placebo possibly by blocking the β-receptor mediated lipolysis in obese women. Previous studies have also found reduced arterial plasma concentration of FFA during exercise and post-exercise (Hartling et al, 1980). The α/β adrenergic blockade with labetalol also induced a significant increase in blood glucose concentrations after exercise compared to placebo. In other studies, labetalol has been found to prevent exercise-induced hypoglycaemia. Christensen et al (1978) reported that blood glucose concentration tended to increase after i.v. labetalol in hypertensive subjects when standing and during supine exercise. They also observed higher plasma noradrenaline concentration after labetalol when standing and during supine exercise and higher plasma adrenaline only in the standing position. Hartling et al (1980) also found higher arterial blood glucose concentration after labetalol in healthy men during dynamic forearm exercise.

With regard to α- blockade labetalol inhibits the increase in BP induced by phenylephrine and noradrenaline while leaving reflex reductions in cardiac output
and heart rate unaffected (Richards and Prichard, 1979). In the present study labetalol at 100 mg did not produce significant differences in exercise heart rate increases 1 h after the oral dosing compared to placebo in our subjects. This is probably because labetalol has α-adrenoceptors blockade properties. Similar findings have been reported by others. Richards et al (1974) have found that in healthy males labetalol at doses of 100, 200 and 400 mg did not significantly alter resting heart rate compared to placebo, and significantly reduced exercise-induced increases in heart rate only at 2 or 3 hrs after oral dosing. Richards et al (1974, 1977) have shown that oral doses of labetalol 100, 200 and 400 mg had no significant effect on peak expiratory flow rate at rest or after exercise in healthy males.

The present findings suggest that elevated adrenaline concentrations by exogenous intravenous administration do not mimic the effects of moderate exercise on appetite/satiety sensations and subsequent food intake in obese women. In contrast, a higher subsequent food intake is found. An α/β adrenergic blockade during moderate-intensity exercise did not influence appetite/satiety sensations and subsequent food intake following exercise in obese women. The changes in blood glucose and plasma FFA suggest that the 100 mg of α/β adrenergic blocker were sufficient to induce β-adrenergic blockade.
5.5 New Research Questions arising from this Study

These results therefore do not support a specific role for adrenaline in linking physical activity to appetite regulation via enhanced leptin transport. On the other hand, the results do not completely exclude catecholamines as mediators. The possibility remains that noradrenaline is the physiologically active compound, and studies might more appropriately use pure \( \alpha \)-adrenoreceptor blockade to try to block its effects. This study thus raises new research questions:

1. What is the effect of noradrenaline infusion on appetite/satiety ratings and subsequent food intake in obese women?

2. What is the effect of moderate exercise performed with an \( \alpha \)-adrenergic receptor antagonist on appetite/satiety ratings and food intake in obese women?
Table 5.1 Subject characteristics, n = 10

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.3 ± 6.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>90.2 ± 16.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.0 ± 12.8</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>36.1 ± 5.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104.8 ± 12.8</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>115.2 ± 9.7</td>
</tr>
<tr>
<td>Fat mass (%) predicted by waist</td>
<td>47.7 ± 5.4</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>129.6 ± 7.2</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>89.2 ± 4.1</td>
</tr>
</tbody>
</table>

Values are mean ± SD
Table 5.2 Serum leptin, blood glucose and plasma free fatty acids (FFA) concentrations during the Exercise plus α/β blocker and the Exercise plus placebo trials, n = 10

<table>
<thead>
<tr>
<th></th>
<th>Baseline (-60 min)</th>
<th>Pre intervention (0 min)</th>
<th>Post intervention (20 min)</th>
<th>1 h post-intervention (80 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise plus α/β blocker</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng ml(^{-1}))</td>
<td>62.7 ± 22.9</td>
<td>63.4 ± 23.2</td>
<td>68.9 ± 23.8(^{§})</td>
<td>65.2 ± 24.8</td>
</tr>
<tr>
<td>Blood glucose (mmol l(^{-1}))</td>
<td>4.6 ± 0.5</td>
<td>4.8 ± 0.3</td>
<td>4.9 ± 0.2(^{*})</td>
<td>4.9 ± 0.2(^{*})</td>
</tr>
<tr>
<td>Plasma FFA (mmol l(^{-1}))</td>
<td>0.69 ± 0.3</td>
<td>0.59 ± 0.18</td>
<td>0.49 ± 0.15(^{*})(^{§})</td>
<td>0.61 ± 0.16(^{*})</td>
</tr>
<tr>
<td><strong>Exercise plus placebo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng ml(^{-1}))</td>
<td>62.3 ± 22.1</td>
<td>65.7 ± 26.5</td>
<td>73.0 ± 26.7(^{</td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mmol l(^{-1}))</td>
<td>4.9 ± 0.5</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.3</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Plasma FFA (mmol l(^{-1}))</td>
<td>0.67 ± 0.34</td>
<td>0.68 ± 0.22</td>
<td>0.8 ± 0.26(^{*})</td>
<td>0.78 ± 0.24(^{*})</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Means within row with superscript symbols * are significantly different between Exercise plus α/β blocker and Exercise plus placebo (glucose 20 min \(P = 0.008\), 90 min \(P < 0.001\); FFA 20 min \(P = 0.002\), 90 min \(P = 0.009\)). Means within row with superscript symbols §, || are significantly different from baseline and pre-intervention within Exercise plus α/β blocker (§ \(P < 0.01\)) or Exercise plus placebo (|| \(P < 0.05\)) (Paired t-test).
Table 5.3 Serum leptin, blood glucose and plasma free fatty acids (FFA) concentrations during the Adrenaline infusion and the Saline infusion trials, n = 9

<table>
<thead>
<tr>
<th></th>
<th>Baseline (-60 min)</th>
<th>Pre intervention (0 min)</th>
<th>Post intervention (20 min)</th>
<th>1 h post-intervention (80 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adrenaline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng ml⁻¹)</td>
<td>60.9 ± 21.9</td>
<td>60.1 ± 23.4</td>
<td>61.9 ± 24.3</td>
<td>64.2 ± 29.7</td>
</tr>
<tr>
<td>Blood glucose (mmol l⁻¹)</td>
<td>4.6 ± 0.7</td>
<td>4.6 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Plasma FFA (mmol l⁻¹)</td>
<td>0.76 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.95 ± 0.4 * §</td>
<td>0.75 ± 0.2</td>
</tr>
<tr>
<td><strong>Saline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng ml⁻¹)</td>
<td>62.3 ± 23.9</td>
<td>62.3 ± 23.9</td>
<td>61.7 ± 26.6</td>
<td>64.6 ± 24.1</td>
</tr>
<tr>
<td>Blood glucose (mmol l⁻¹)</td>
<td>4.9 ± 0.6</td>
<td>4.8 ± 0.3</td>
<td>4.7 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Plasma FFA (mmol l⁻¹)</td>
<td>0.63 ± 0.3</td>
<td>0.61 ± 0.3</td>
<td>0.65 ± 0.3</td>
<td>0.66 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Adrenaline effect P = 0.03, time effect P < 0.001 ANOVA; The superscript symbol * indicates significant differences between trials (* P = 0.04: Adrenaline infusion vs Saline infusion, Paired t-test). Means within row with superscript symbols §, || are significantly different from baseline and pre-intervention within Exercise plus α/β blocker (§ P < 0.01) or Exercise plus placebo (|| P < 0.05) (Paired t-test).
Table 5.4 Heart rate, perceived breathlessness and leg-tiredness during the Exercise plus α/β blocker and the Exercise plus placebo interventions, n = 10

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise plus α/β blocker</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats.min⁻¹)</td>
<td>121.2 ± 18.6</td>
<td>129.3 ± 20.4</td>
<td>128.0 ± 16.59</td>
<td>129.4 ± 22.4</td>
</tr>
<tr>
<td>Perceived breathlessness rating (0-20)</td>
<td>10.0 ± 1.5</td>
<td>11.5 ± 1.3</td>
<td>11.6 ± 1.9</td>
<td>12.9 ± 1.6</td>
</tr>
<tr>
<td>Perceived leg-tiredness ratings (0-20)</td>
<td>10.5 ± 1.2</td>
<td>12.0 ± 1.4</td>
<td>12.8 ± 1.9</td>
<td>13.0 ± 1.7</td>
</tr>
<tr>
<td><strong>Exercise plus placebo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats.min⁻¹)</td>
<td>122.7 ± 19.4</td>
<td>133.2 ± 20.5</td>
<td>132.3 ± 17.5</td>
<td>135.3 ± 20.3</td>
</tr>
<tr>
<td>Perceived breathlessness rating (0-20)</td>
<td>10.0 ± 1.5</td>
<td>11.5 ± 1.3</td>
<td>12.2 ± 0.9</td>
<td>12.6 ± 1.2</td>
</tr>
<tr>
<td>Perceived leg-tiredness ratings (0-20)</td>
<td>10.6 ± 1.5</td>
<td>12.1 ± 1.7</td>
<td>13.2 ± 1.4</td>
<td>13.6 ± 2.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD. 2-way ANOVA did not detect differences with \( P < 0.05 \)
Table 5.5 Heart rate, perceived breathlessness and leg-tiredness among nine subjects during the 20 min Adrenaline and the Saline infusions, n = 9

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adrenaline infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats.min(^{-1}))</td>
<td>72.2 ± 11.2</td>
<td>73.6 ± 13.8</td>
<td>77.4 ± 10.1 *</td>
<td>77.9 ± 10.1 *</td>
</tr>
<tr>
<td>Perceived breathlessness rating (0-20)</td>
<td>7.6 ± 1.6</td>
<td>8.1 ± 1.6</td>
<td>7.7 ± 1.5</td>
<td>7.7 ± 1.5</td>
</tr>
<tr>
<td>Perceived leg-tiredness ratings (0-20)</td>
<td>7.5 ± 1.3</td>
<td>7.9 ± 1.7</td>
<td>7.7 ± 1.41</td>
<td>7.7 ± 1.4</td>
</tr>
<tr>
<td><strong>Saline infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats.min(^{-1}))</td>
<td>71.7 ± 9.8</td>
<td>71.4 ± 12.0</td>
<td>70.8 ± 11.5</td>
<td>71.4 ± 10.4</td>
</tr>
<tr>
<td>Perceived breathlessness rating (0-20)</td>
<td>7.9 ± 1.6</td>
<td>7.8 ± 1.6</td>
<td>7.7 ± 1.5</td>
<td>7.7 ± 1.5</td>
</tr>
<tr>
<td>Perceived leg-tiredness ratings (0-20)</td>
<td>8.7 ± 2.1</td>
<td>8.7 ± 1.9</td>
<td>8.7 ± 1.9</td>
<td>8.7 ± 1.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The superscript symbol * indicates significant differences between trials (* P < 0.01: Adrenaline infusion vs Saline infusion, Paired t-test). ANOVA treatment × time effect, P = 0.05.
Table 5.6 Cardiopulmonary variables for each of the two 20 min exercise trials in EXP 1, n = 10

<table>
<thead>
<tr>
<th>Exercise Time (min)</th>
<th>8:30 - 9:30 min</th>
<th>18:30 - 19:30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise plus α/β blocker</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO$_2$ (lt/min)</td>
<td>1.3 ± 0.5</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>VCO$_2$ (lt/min)</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>RER</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.03</td>
</tr>
<tr>
<td>%Fat oxidised</td>
<td>65.1 ± 13.8</td>
<td>67.4 ± 12.4</td>
</tr>
<tr>
<td>%CHO oxidised</td>
<td>34.9 ± 13.8</td>
<td>32.6 ± 12.4</td>
</tr>
<tr>
<td><strong>Exercise plus placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO$_2$ (lt/min)</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>VCO$_2$ (lt/min)</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>RER</td>
<td>0.8 ± 0.03</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>%Fat oxidised</td>
<td>62.4 ± 12.1</td>
<td>78.6 ± 29.7</td>
</tr>
<tr>
<td>%CHO oxidised</td>
<td>37.1 ± 12.1</td>
<td>21.4 ± 29.7</td>
</tr>
</tbody>
</table>

Values are mean ± SD. No significant differences were found.
Figure 5.1: Schematic representation of the study design.

EXP 1: Adrenaline vs. Saline infusion for 20 min

EXP 2: Moderate exercise plus alpha/beta adrenoceptor blockade vs. placebo for 20 min
Figure 5.2: Mean ± SD profiles of self-reported appetite-satiety ratings during the Moderate exercise plus α/β blocker (■) and Moderate exercise plus placebo (●) trials; Data were analysed using 2 way ANOVA followed by paired t-test to determine the over time differences in ratings within trials: §§ are significantly different from baseline within the Moderate exercise plus α/β blocker (§) or the Moderate exercise plus placebo (||) trial, $P < 0.05$. 
Figure 5.2

- Hunger (mm)
- Desire to Eat (mm)
- Prospective Food Consumption (mm)
- Fullness (mm)
- Satisfaction (mm)

- Moderate exercise plus blocker
- Moderate exercise plus placebo

Time (min)
Figure 5.3: Mean ± SD profiles of self-reported appetite-satiety ratings under the Adrenaline infusion (■) and Saline infusion (▲); Data were analysed using 2 way ANOVA followed by paired t-test to determine the differences in ratings within trials §‖ are significantly different from baseline within the Adrenaline infusion (§) or the Control (‖) trial, $P < 0.05$. 
Figure 5.4: Energy intake after infusion of adrenaline or saline. Data were analysed by Paired t-test: * $P = 0.04$, adrenaline infusion vs saline infusion.
Figure 5.5: Profiles of serum leptin concentrations (ng/ml) during the Adrenaline and Saline infusion trials (from 17:00 pm to 20:20 pm), n = 9

- □ Adrenaline infusion
- ▲ Saline infusion
Effects of short-term detraining on serum leptin and appetite/satiety measures in trained men

This study presents a study in a form that is essentially the same as a manuscript, which has been prepared for submission to International Journal of Obesity
6.1 Introduction: Research Questions to be addressed and hypotheses to be tested

Combined behavioural and metabolic data have suggested that increased physical activity is necessary for accurate control of appetite and body weight. Indeed physically active and habitually trained individuals tend to match energy intake with energy expenditure (Maughan et al, 1989), and often maintain constant low body weight throughout life. Increases in physical activity are strongly associated with lower levels of obesity (Lean, 2000), and regular exercise is positively related with long-term weight maintenance after initial weight loss (Fogelholm et al, 2000). Several studies that observed or manipulated physical activity have also suggested that inactivity may result in appetite and body weight deregulation (Mayer and Thomas, 1967; Murgatroyd et al, 1999; Long et al, 2002; Epstein, 2002). These observations suggest that long-term adiposity signals, such as serum leptin, finely regulate appetite and energy intake during a trained physical state, thus assisting in the maintenance of low body weight and adiposity of trained people.

Trained individuals have increased “leptin sensitivity” i.e. low circulating leptin concentrations which seem effective to regulate appetite (Leal Cerro et al, 1998; Considine et al, 1996) and also increased insulin sensitivity compared to sedentary and obese individuals (Mujika and Padilla, 2000). Insulin sensitivity appears to be a factor besides obesity that regulates circulating leptin concentrations (Haffner et al, 1997). The interrelationship between leptin and insulin sensitivity is demonstrated by positive associations between impaired insulin sensitivity and elevated leptin concentrations independent of adiposity and fat distribution (Zimmet et al, 1998). The previous Studies 1 (Chapter 3), 2 (Chapter 4) and 3 (Chapter 5) investigated the
appetite control and the function of circulating leptin concentrations in relation to exercise-related factors in obese and lean women. The findings (Study 1, Chapter 3) that exercise-related factors mediated a coupling between serum leptin and controlled appetite following moderate-exercise in obese individuals triggered the question whether removal of the exercise stimulus would influence appetite control, circulating leptin concentrations and the function of circulating leptin concentrations.

In the present study, the aim was to investigate how short-term detraining (for 7 days) would influence serum leptin concentrations in endurance-trained men. The relationship between leptin, insulin and appetite profile in the trained and the detrained condition was examined. Some other aspects of this study are also presented elsewhere (Gill et al, 2003). Insulin data are presented here and discussed only in the context of leptin.

6.2 Research methods and procedures
6.2.1 Subjects
Eight endurance-trained men (Table 6.1) gave their written informed consent to participate in the study, which was approved by the North Glasgow NHS Trust Ethics Committee. Three were distance runners, two were triathletes, two were swimmers and one was a cyclist. They had been training regularly for between four and ten years and typically performed four to eight hours of endurance training per week. Six subjects competed regularly at regional or national level, the other two participants were recreational athletes. All subjects were healthy non-smokers. None were on any special diet or on medication known to affect appetite.
6.2.2 Study Design

All subjects were studied in the fasting state and after consuming a meal 1074 kcal (4.5 MJ) on two different occasions, during training (Trained) and after seven days of detraining (Detrained). The subjects were asked to undertake their normal training routine on the week prior to the first visit (Trained), ensuring that they trained on the day before the test, and to keep a training diary during this week. For 7 days before the second visit (Detrained), they refrained from training (gentle walking over short distances was permitted). Subjects kept weighed food records for two days preceding the first test and these food patterns were replicated exactly two days before the second test. The subjects were also asked to refrain from alcohol for the two days before each test.

On each of the two study days (Trained or Detrained), subjects visited the laboratory at 08:30 h approximately 12 h after their last meal. A venous cannula was then inserted and, after an interval of 10 min, a baseline-fasting blood sample was obtained. Subjects then consumed a 1074 kcal (4.5 MJ) breakfast (fat 67 % of energy, carbohydrate 29 % of energy, protein 4 % of energy) within 15 min. Serial blood samples were then drawn at 30, 60, 90, 120, 240 and 360 min after the meal consumption. Following each blood sample, subjects completed a set of self-rating 100-mm visual analogue scales for hunger, desire to eat, prospective food consumption, satiety and fullness (Flint et al, 2000). Subjects rested throughout day and consumed only water. This was provided ad libitum during the test in the trained condition and water intake was replicated in the detrained state.
6.2.3 Anthropometry

Body mass, height and waist were determined using standard techniques (WHO, 1995). Percentage of body fat (% body fat) was estimated from skin-fold thicknesses over the biceps, triceps, sub scapular, and superailiac areas (Durnin and Womersley, 1974). The skinfolds method is very convenient method in estimating fat in people of reasonably normal build, provided that the measurements are made by a trained observer and an error of about 3% of body weight (i.e. 2 kg in an average subject) is acceptable. Three measurements are made at each site (e.g. biceps, triceps, subscapular, and suprailiac), and if the span of readings was greater than 2mm, more readings are taken until a set of three consecutive readings agreeing to 2mm is obtained. The averages of the three readings at each site are calculated, and the sum of these values is entered into the table given by Durnin and Womersley (1974) in the column appropriate to the age and sex of the subjects. The percentage of body fat related to the sum of the four skinfold values is then estimated.

6.2.4 Measurements of the metabolic profile and analyses

Venous blood was collected into EDTA, lithium heparin and into plain serum vacutainers for the measurement of plasma non-esterified fatty acids (NEFA) and glucose, plasma insulin and serum leptin, respectively, and placed immediately on ice. The plasma from the EDTA tube was separated and stored at -70° C. Glucose concentration was determined in EDTA plasma by enzymatic colorimetric methods using commercially available kits (Roche Diagnostics Gmbh, Mannheim, Germany and Wako Chemicals USA, Inc., VA, USA). Insulin was analysed in lithium heparin plasma by an in-house immunoradiometric (IRMA) assay using a radiolabelled mouse monoclonal anti-insulin and solid phase guinea pig anti-insulin (both
antibodies supplied by Scottish Antibody Production Unit) (Dorrian C, PhD thesis). Blood collected into the plain serum vacutainer was allowed to clot for 10 min. Following centrifugation, the serum was stored at -70° C and subsequently analysed for leptin by radioimmunoassay (Mc Conway et al, 2000).

6.2.5 Calculations
Whole-body insulin sensitivity with regard to insulin effect on glycemia (ISI (gly)) was calculated as follows: 

\[ \text{ISI (gly)} = \frac{2}{(|\text{INS} \times \text{GLY}| + 1)} \]

where INS and GLY are insulin and glucose area under the curve, respectively, over 6 h after meal ingestion expressed relative to the average values of the group of subjects (Belfiore et al, 2001).

6.2.6 Statistical Analysis
Results are shown as mean ± SE unless otherwise stated. Postprandial responses were compared by two-way ANOVA for repeated measures followed by the Tukey post-hoc test to find the significant differences. T-test for correlated data was used for the additional comparison of summary measures of postprandial responses of leptin, insulin, glucose and appetite ratings (time averaged areas under response vs time curves (AUC)). Whole-body insulin sensitivity was compared by t-test for correlated data. Relationships between variables at each time point and between AUC were tested with the Pearson’s correlations (r). Serum leptin concentrations were \( \log_{10} \) transformed prior to statistical analysis. Statistical significance is considered as \( P < 0.05 \).
6.3 Results

6.3.1 Effects on serum leptin, plasma glucose and insulin concentrations

Data comparing serum leptin concentration are presented in Figure 6.1. Fasting serum leptin was not significantly different (ANOVA, \( P = 0.06 \)) between trials, but was greater postprandially in the detrained condition compared with training (ANOVA, \( P = 0.04 \); Trained 19.85 ± 6.36 ng·ml\(^{-1}\)·h, Detrained 26.65 ± 7.85 ng·ml\(^{-1}\)·h (AUC) \( P = 0.02 \))

Fasting glucose concentrations were not significantly different between trials (Trained, 4.98 ± 0.12 mmol.l\(^{-1}\); Detrained, 5.00 ± 0.08 mmol.l\(^{-1}\)) and nor were glucose responses to the meal (AUC) (Trained, 31.20 ± 1.99 mmol·l\(^{-1}\)·h; Detrained 31.07 ± 1.76 mmol·l\(^{-1}\)·h). Plasma insulin concentration was higher in the detrained trial, both in the fasting state (Trained 4.1 ± 0.04 μU·ml\(^{-1}\); Detrained 5.7 ± 0.6 μU·ml\(^{-1}\), ANOVA, \( P = 0.03 \)) and postprandially (Trained 74.0 ± 6.4 μU·ml\(^{-1}\); Detrained 102.1 ± 7.7 μU·ml\(^{-1}\) (AUC), \( P = 0.002 \)). Whole-body insulin sensitivity ISI (gly), based on postprandial glucose and insulin concentrations, was higher in the Detrained condition (Trained 1.17 ± 0.35; Detrained 0.83 ± 0.18, \( P = 0.003 \)) (Table 6.2).
6.3.2 Effects on appetite and satiety ratings

Significant changes in hunger, desire to eat and prospective food consumption ratings, and in satiety and fullness ratings were found with time during both the Trained and the Detrained condition (Figure 6.2). No significant differences were found in appetite and satiety ratings at any time-point between the two trials. No significant differences were found in the areas under the curve (AUC) of the appetite and satiety ratings.

6.3.3 Correlations between self-reported appetite-satiety measures and plasma insulin and serum leptin concentrations in the trained and the detrained conditions

Significant associations were found between plasma insulin and appetite/satiety ratings in the Trained condition, and between serum leptin and appetite/satiety ratings in the Detrained condition. Plasma insulin concentrations, in the Trained condition, were significantly correlated with appetite and satiety ratings 6 h postprandially (360 min) (hunger $r = -0.73$, $P = 0.04$; desire to eat $r = -0.88$, $P = 0.004$; prospective food consumption $r = -0.92$, $P = 0.001$; satiety $r = 0.92$, $P = 0.011$; fullness $r = 0.87$, $P = 0.005$) (Figure 6.3).

In the Detrained condition serum leptin concentrations were significantly correlated with appetite and satiety ratings 4 h postprandially (hunger $r = -0.79$, $P = 0.02$; prospective food consumption $r = -0.73$, $P = 0.04$) and 6 h postprandially (360 min) (desire to eat $r = -0.91$, $P = 0.002$; prospective food consumption $r = -0.82$, $P = 0.007$; satiety $r = 0.75$, $P = 0.03$; fullness $r = 0.78$, $P = 0.02$) (Figure 6.4).
6.4 Discussion

Exercise-trained individuals have low circulating leptin concentrations and heightened insulin sensitivity. Insulin sensitivity plays a key role in the regulation of leptin, however, it was not known how a detraining-induced decline in insulin sensitivity would affect serum leptin concentrations in trained individuals. In the present study, short-term detraining increased serum leptin concentrations throughout the 6 h postprandial study period in endurance-trained men without detectable changes in fat mass. Previous studies have not found significant fat mass changes after even much longer (3 weeks) detraining (LaForgia et al, 1999). In our subjects the increase in leptin is probably related to detraining-induced factors other than changes in fat mass. Gutin et al (1999) investigated the effects of long-term (4 months) physical detraining in obese children and also found increased serum leptin concentrations (adjusted for the increase in fat mass). Leptin concentrations can indeed vary disproportionately to changes in fat mass. For example, circulating leptin concentrations decrease during prolonged fasting (52 h) and increase during overfeeding (12 h) without marked changes in fat mass (Boden et al, 1996; Weigle et al, 1996). Exercise training also decreases serum leptin concentrations independently of fat mass in obese men and children (Pasman et al, 1998; Gutin et al, 1999). These observations and our findings suggest that leptin is not only a marker of adiposity but also indicates the net flux of energy, probably the glucose flux, through adipocytes (Considine et al, 2000).

Among other biological candidates glucose, insulin and catecholamines are proposed as determinants of leptin production and secretion (Dagogo-Jack, 2001). In the present study, the fasting and postprandial plasma insulin concentrations were
increased but glucose did not change after short-term detraining. Insulin sensitivity was impaired by short-term detraining in endurance-trained men, which is in accordance with findings of previous studies (five to six detraining days) (Mikines et al, 1989; Vukovich et al, 1996). Previous studies have shown that high insulin concentrations stimulate leptin secretion (Saad et al, 1998). The increased insulin concentrations found after detraining could explain the present increase in leptin in our men. Fruehwald-Schultes et al (2002), however, found that experimentally induced insulin resistance reduces the stimulatory effect of insulin on leptin secretion in lean individuals. Our results indicate that in the case of a ‘naturally’ induced insulin resistance, e.g. detraining-induced insulin resistance, the effect of insulin on leptin secretion is not counteracted by insulin resistance in trained men.

Recent studies have found that glucose uptake and metabolism is suggested to be the primary regulator of leptin secretion by human adipocytes rather than insulin per se (Wellhoener et al, 2000). It is known that during training circulating glucose is mainly taken up by the skeletal muscle (Malkova et al, 2000). If during detraining adipose tissue becomes the predominant site of glucose disposal and metabolism, this may have led to an increase in serum leptin concentrations in these trained men. This effect of glucose on leptin secretion is suggested to be mediated through the hexosamine biosynthesis in adipose tissue (Considine et al, 2000). This speculation is also supported by our detraining-induced insulin resistance, which is another metabolic outcome of the hexosamine biosynthetic pathway (McClain and Crook, 1996).
In the present study, fasting and 6 h postprandial appetite-satiety ratings were not influenced after seven days of detraining. The present results, however, suggest an association between serum leptin and the late postprandial drive to eat (i.e. 6 h postprandially) in detrained men, wherein minimal postprandial satiety is reached and maximal hunger is developed. Serum leptin concentrations have been previously associated only with fasting appetite ratings in obese individuals possibly due to weight loss (Keim et al, 1998; Heini et al, 1998; Doucet et al, 2000). There is no previous evidence of an association between postprandial leptin and early postprandial satiety or hunger (i.e. 4 to 9h postprandially) (Joannic et al, 1998; Karhunen et al, 1997; Romon et al, 1999). It has been suggested that the association between circulating leptin concentrations and hunger and satiety may develop in the later postprandial state (Romon et al, 1999). It seems possible that the detraining-induced increase in serum leptin concentrations stimulated the present coupling between serum leptin and postprandial appetite/satiety sensations in these trained men.

In the trained condition plasma insulin was associated with appetite ratings. This is in agreement with previous studies that reported an anorexic effect of insulin in the immediate postprandial period (Heini et al, 1998; Raben et al, 1994). The shift in the association between trained and detrained conditions could be possibly stimulated by detraining-induced factor(s). It has been found that improved insulin sensitivity in parallel with decreased circulating leptin levels are related to decreased appetite in obese rats (Wang et al, 2001). It is unknown whether this implies the same condition in man and if a reversed condition of increased leptin concentrations and/or decreased insulin sensitivity would affect appetite regulation in trained men.
6.5 New Research Questions arising from this Study

These results suggest that physical inactivity, for example detraining, increases the secretion of leptin in the short-term. This increase is accompanied by impaired insulin sensitivity, which probably suggests common exercise-related pathways in the regulation of leptin and insulin secretion. Interestingly in the trained state plasma insulin was associated with appetite/satiety ratings during an acute postprandial profile, but serum leptin overtook this role in the detrained state. The questions remain about the effects of long-term physical inactivity on the biological and behavioural control of appetite and food intake. This study therefore raises the following new research question:

1. What is the effect of long-term detraining on serum leptin concentrations, insulin sensitivity and energy balance in trained and sedentary individuals?
<table>
<thead>
<tr>
<th>Subject characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28 ± 12</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.7 ± 17</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176 ± 0.06</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>79.3 ± 4.1</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>23.6 ± 1.0</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td>39.6 ± 9.4</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>17.0 ± 3.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
Table 6.2 Plasma insulin, glucose and ISI(gly) (whole-body insulin sensitivity) in the trained and detrained condition, n = 8.

<table>
<thead>
<tr>
<th>Time scale</th>
<th>0 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trained</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin (μU·ml⁻¹)</td>
<td>4.1 ± 0.4 *</td>
<td>40.3 ± 5.1 *</td>
<td>21.1 ± 4.2</td>
<td>13.4 ± 1.2 *</td>
<td>5.9 ± 1.0 *</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>Plasma Glucose (mmol·ml⁻¹)</td>
<td>4.98 ± 0.12</td>
<td>5.86 ± 0.36</td>
<td>4.83 ± 0.27</td>
<td>5.44 ± 0.20</td>
<td>5.13 ± 0.08</td>
<td>5.13 ± 0.09</td>
</tr>
<tr>
<td>ISI(gly)</td>
<td>1.17 ± 0.35 *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Detrained</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin (nmol·ml⁻¹)</td>
<td>5.7 ± 0.6</td>
<td>56.1 ± 5.3</td>
<td>30.3 ± 4.2</td>
<td>19.1 ± 2.5</td>
<td>7.6 ± 0.9</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Plasma Glucose (nmol·ml⁻¹)</td>
<td>5.0 ± 0.08</td>
<td>5.91 ± 0.25</td>
<td>4.86 ± 0.36</td>
<td>5.22 ± 0.19</td>
<td>5.13 ± 0.07</td>
<td>5.10 ± 0.07</td>
</tr>
<tr>
<td>ISI(gly)</td>
<td>0.83 ± 0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SEM. The symbol * indicates significant differences between trained and detrained condition, P < 0.05.
Figure 6.1: Profiles of serum leptin concentrations under the Trained (□) and the Detrained (■) conditions; * indicate significant differences between trials (* $P < 0.05$). Values are mean ± SEM, $n = 8$. 
Figure 6.2: Profiles of self-reported appetite-satiety ratings under the Trained (○) and the Detrained (■) conditions. Data were analysed by Students' t-test to determine the changes in ratings over time within trials. §|| are significantly different from baseline within the Trained (§ $P < 0.05$) or the Detrained (|| $P < 0.05$) conditions. Values are mean ± SEM, $n = 8$
Figure 6.3: Associations between insulin plasma concentrations (μU·ml⁻¹) and appetite-satiety measures (on a 0-100-mm visual analogue scale) in the trained condition at 6 h postprandially (prospective food consumption \( r = -0.91 \ P = 0.001 \); desire to eat \( r = -0.88 \ P = 0.004 \); satiety \( r = 0.92 \ P = 0.001 \); fullness \( r = 0.87 \ P = 0.005 \). No significant associations were found between insulin plasma concentrations and appetite-satiety measures in the detrained condition.
Figure 6.3

Plasma insulin (mU/ml)

- Consumption (mm)
- Desire to Eat (mm)
- Hunger (mm)
- Salivation (mm)

- Trained
- Detrained
Figure 6.4: Associations between $\log_{10}$ serum leptin concentrations (ng·ml$^{-1}$) and appetite-satiety measures (on a 0-100-mm visual analogue scale) in the detrained condition at 6 h postprandially (prospective food consumption ($r = -0.82 \ P = 0.007$); desire to eat ($r = -0.91 \ P = 0.002$); satiety ($r = 0.75 \ P = 0.03$); fullness ($r = 0.78 \ P = 0.02$). No significant associations were found between $\log_{10}$ serum leptin concentrations and appetite-satiety measures in the trained condition.
Chapter Seven

General Discussion
7.1 Discussion

At the onset of this work, leptin was already well established to have a critical role in appetite regulation in animal models of obesity and in humans with congenital leptin deficiency but its role amongst common obese humans was not clear. The increased concentrations of circulating leptin in overweight/obese subjects suggested a resistance to the physiological effects of the hormone. Elevated circulating leptin concentrations are independent risk factors for coronary heart disease compared to BMI and are usually accompanied by a concomitant increase in circulating insulin and lipid concentrations (Wallace et al., 2001). Subjects with the metabolic syndrome have also raised serum leptin concentrations despite the increased central body fat (waist-hip ratio > 0.90 in men, waist-hip ratio > 0.85 in women) (Bonora et al., 2003). High circulating leptin concentrations thus appear to indicate not only leptin resistance but also increased risk for obesity-associated metabolic abnormalities. The leptin resistance in overweight and obese individuals possibly limits the therapeutic use of leptin in the treatment or prevention of obesity and associated diseases. New approaches are therefore needed to overcome the leptin resistance and elucidate the use of leptin in the battle against obesity.

Physical inactivity triggers or aggravates the onset of several pathologies related to sedentary life-style, e.g. obesity, diabetes, heart disease and metabolic syndrome, but physical activity prevents many of these diseases. It is important to understand how physical activity and inactivity influence appetite and body weight regulation and whether circulating leptin is involved in the related mechanisms. Therefore, the primary objectives of the Studies 1, 2 and 3 were to determine the effects of moderate exercise on acute appetite control and on circulating leptin concentrations.
in obese and lean women. Study 4 aimed to investigate the role of acute physical inactivity on appetite control and on circulating leptin concentrations by disrupting exercise training for 7 days in male athletes. The assessment of the relationship between circulating leptin concentrations and appetite/satiety measures and subsequent food intake was used as an indirect indicator of the function of leptin. If circulating leptin concentrations are found to associate with appetite control then this might indicate improved leptin sensitivity and effective function of leptin in controlling appetite.

The acute effects of moderate exercise and eating interventions on the drive to eat and subsequent food intake were investigated in obese (Study 1, Chapter 3) and lean women (Study 2, Chapter 4). The effects on some biochemical measures known to be involved in appetite control, e.g. blood glucose, plasma FFA and serum leptin concentrations were also investigated. The results in obese women in Study 1 (Chapter 3), showed correlations between serum leptin concentrations and appetite/satiety sensations following moderate-exercise, which raised the possibility of an acute 'coupling' between circulating leptin and appetite, mediated by exercise-induced factors. One plausible explanation is that catecholamines released during exercise may be responsible for inducing this 'coupling' between circulating leptin and appetite suppression since it is known that they facilitate leptin transport into the brain. The increase in plasma FFA concentrations following exercise in obese women indicated catecholamine-stimulated lipolysis. Animal findings indeed indicate that raised adrenaline concentrations enhance leptin transport across the blood brain barrier (Banks et al, 2001). Once in the brain, increased leptin concentrations may alter the balance between orexigenic and anorexigenic...
neuropeptides in favour of decreased appetite. The role of catecholamines in this process therefore merits further investigation.

In Study 2 (Chapter 4) associations between serum leptin concentrations and appetite control were not found in lean women so a primary role could not be demonstrated for leptin in the short-term appetite control in lean women. The present results suggest differences in the relationship between appetite regulation and exercise in obese and lean individuals. Signals other than circulating leptin may mediate exercise-induced satiety in lean individuals. Alternatively exercise-induced factors necessary to facilitate leptin transport into the brain in obese individuals (Banks et al., 2001) may differ or not be needed in lean individuals, i.e. the threshold for coupling leptin to appetite is different between obese and lean individuals.

It is suggested that leptin transport into the brain differs between normal weight/healthy and obese/pathological conditions. Absence of sensitive transport mechanism for leptin at the blood brain barrier has been observed in normal weight rats (Zlokovic et al., 2000). Alternatively, it has been suggested that the choroid plexus was suggested to regulate leptin entry into the cerebrospinal fluid in normal weight conditions. Whether the non-coupling between serum leptin and satiety after exercise in lean women indicates different regulation of leptin transport in lean and obese individuals is uncertain. If leptin transport is lower across the blood brain barrier than through the choroid plexus in normal serum leptin concentrations (e.g. normal weight individuals), this could explain the non-coupling of leptin to satiety in lean women as adrenaline-induced effects on leptin transport have been found at the blood brain barrier but not at the choroid plexus (Banks et al., 2001). Alternatively, it
is possible that factors, which promote brain leptin transport and central action, are
only necessary in obese individuals to curtail appetite. It is known that leptin
transport into the brain is efficient in lean individuals (Caro et al, 1996). The low
concentrations of circulating insulin and leptin appear to inhibit efficiently
hypothalamic NPY expression in lean but not in obese rats (Schwartz et al, 1991).
Hence, it is likely that a transport-enhancing effect of adrenaline/noradrenaline on
leptin is only required when serum leptin concentrations are high, i.e. in obesity.

In the Studies 1 and 2 in obese and lean women respectively, the exercise-induced
satiety resembled the satiety of a snack but most importantly avoided the extra
calories of snacking. Overall the present observations reported in lean and in obese
women suggest moderate exercise as an efficient route to appetite and body weight
control in individuals who wish to avoid weight gain or to maintain weight loss after
slimming. Moderate exercise is indeed recommended as a snack ‘substitute’ in
dietetic practices for weight management. Obese individuals on dietary weight loss
programmes commonly find it difficult to control their hunger and frequently resort
to snacking. This is when moderate exercise could be used effectively to induce
satiety.

The age and body fat differences between the two subject groups (middle-aged obese
compared to younger lean women) may be factors that contribute to the difference in
leptin response to exercise between obese and lean women. The women in the Study
2 (Chapter 4) were lean and young compared to middle-aged and obese women in
Study 1 (Chapter 3). It is known that adiposity increases with age (Schwartz et al,
1990) and that circulating leptin concentrations dissociate with body fat in older
humans (Moller et al, 1998). Moreover, findings in rats suggested that the inhibitory function of leptin on food intake is decreased with age through suppression of hypothalamic NPY (Scarpace et al, 2002). It is also suggested that human obesity is associated with leptin resistance, which becomes more pronounced with progressive degrees of obesity and aging (Considine et al, 1996; Scarpace et al, 2002). For example, diet induced obese (DIO) rats are a good surrogate for human obesity and develop defective leptin transport only as they age and became obese (Levin et al, 2004). It is likely that the exercise-induced factors that appear to mediate the function of leptin in suppressing appetite are effective only in obese and middle-aged individuals who are prone to leptin resistance.

Study 3 (Chapter 5) was designed to elucidate whether circulating adrenaline at concentrations seen during moderate exercise (Gustafson et al, 1990) is responsible for the exercise-induced coupling of leptin to appetite/satiety found in obese women (Study 1, Chapter 3). Adrenaline concentrations raised by exogenous intravenous administration (12.5 ng min/kg ideal body weight) to concentrations seen during moderate exercise, do not appear to mimic the effects of moderate exercise on appetite suppression in obese women. On the contrary, the adrenaline infusion increased subsequent energy intake. Adrenaline infusion lasted 20 min to mimic a bout of light exercise and was administered 1 hr before the meal. Adrenaline infusion may have increased energy intake by inhibiting leptin gene expression (Carulli et al, 1999), although no decrease in circulating leptin concentrations was detected.
In contrast to the above, administration of labetalol, a combined α/β adrenergic blocker before moderate exercise (Study 3, Chapter 5) aimed to abolish any effects of the catecholamines in mediating the ‘coupling’ between serum leptin and appetite in obese women (Study 1, Chapter 3). Labetalol was chosen as a safe and well understood α/β blocker, however, it has greater affinity for β- than α-adrenoceptors. For this reason, any conclusions with respect to the α-adrenoceptor blockade should be drawn with caution. Labetalol decreased circulating FFA and increased glucose concentrations, which indicate inhibition of catecholamine-stimulated lipolysis and confirm the primarily β-adrenoceptor blockade. There is no simple way to know if α-blockade was completed. No differences in appetite/satiety sensations were found following exercise with adrenoceptor blockade compared to exercise alone. This indicates that the observed anorexic effect of exercise on appetite in obese women was not mediated by β-adrenoceptors. Indeed, a number of studies attribute the anorexigenic effect to α-adrenoceptors in the brain (Ritter and Epstein, 1975; Pijl et al, 2001). It is this effect that a popular class of antiobesity drugs exploit to reduce eating behaviour (e.g. sibutramine) by blocking noradrenaline (NA) reuptake through activation of brain α₁-adrenoceptor receptors (Lean, 2001).

In Study 4, seven consecutive days of detraining decreased insulin sensitivity and increased circulating leptin concentrations in endurance-trained men. There is evidence that circulating leptin is regulated by exercise factors but the exact mechanism(s) have not been fully understood. A 'nutrient sensing pathway' (the hexosamine biosynthetic pathway), which regulates leptin gene expression, has been proposed as a possible mechanism by which an exercise-induced energy deficit may
decrease serum leptin concentrations (Hulver and Houmard, 2003). Similarly, the
detraining-induced increases in leptin concentrations may be due to alterations in
nutrient availability or nutrient flux at the level of the adipocytes. Catecholamines,
beta-agonists, and agents that increase cellular levels of cAMP all acutely reduce
leptin mRNA (Trayhurn et al., 1995). Recent findings suggested that fatty acids
might mediate the inhibitory effects of catecholamines, e.g. NA on insulin-stimulated
leptin secretion. In vivo studies using isolated white adipocytes and human
preadipocytes have shown the intracellular increase in fatty acids, generated by
activated lipolysis, to result in an inhibition of insulin-stimulated leptin secretion
(Cammisotto et al., 2003, Arai et al., 2002, Van Harmelen et al., 2002). An elevated
circulating catecholamine concentration, typical of the trained state, suppresses
circulating leptin concentrations (Fritsche et al., 1998; Couillard et al., 2002). Activation of lipolysis in this manner could initiate a signalling cascade that
suppresses leptin mRNA. Therefore, factors related to CNS alterations could be
responsible for the increase in leptin concentrations. It is interesting to note that
detraining results in a decrease in catecholamine release (Shoemaker et al., 1998) and
this may have resulted in the increase in serum leptin concentrations observed in
trained men. Alternatively, the increase in serum leptin concentrations, without
weight gain in trained men, implies an induction of leptin resistance. This could be a
result of reduced transport into the brain.

The association between circulating leptin and insulin concentrations with
postprandial appetite/satiety was investigated to obtain information about the role of
these hormones on the short-term drive to eat in trained individuals. Interestingly,
leptin was ‘coupled’ with hunger in the detrained condition while insulin was
coupled with raised hunger and decreased satiety in the trained condition. These associations demonstrated in the present study may suggest different regulators of short-term postprandial appetite and satiety during training (e.g. insulin) and after detraining (e.g. leptin). Serum leptin concentrations appear to play a role in the postprandial appetite/satiety when the action of insulin has been reduced, for example after detraining in trained men. Circulating leptin concentrations have not been associated with postprandial drive to eat in previous studies, nevertheless the present coupling supports a biological function of leptin in appetite regulation in detrained conditions. It is possible that in lean trained individuals there is a threshold of detraining above which leptin and insulin exchange roles in appetite regulation. This is when leptin increases and insulin resistance develops. Further studies are required to investigate how the inactivity-induced increase in serum leptin concentrations may respond to longer detraining and how it may relate to possible concomitant changes in adipose tissue and energy intake in trained and non-trained individuals.

7.2 Conclusions from the present thesis

The present series of studies aimed to elucidate the role of leptin in acute appetite regulation, particularly in relation to physical activity and inactivity factors. The general conclusions and new research questions below are generated from this thesis.

7.2.1 Conclusions from Study 1 and 2

The effects of moderate exercise on the drive to eat and subsequent food intake were investigated and compared to snacking and sitting (control) interventions. Consumption of a chocolate-based snack suppressed appetite transiently but did not
decrease subsequent food intake in both obese and lean women. Moderate exercise in
the form of brisk walking induced higher satiety and fullness sensations acutely in
obese and lean women. These results support the use of moderate exercise for people
that often fail in controlling appetite, for example overweight and obese individuals.

The associations between leptin and suppressed appetite/elevated satiety support a
biological role for leptin in appetite regulation, but only in obese women, which is
probably mediated through moderate exercise. These observations extend our
understanding for the role of moderate physical activity in appetite regulation and
obesity prevention. Physical activity may have a permissive role, allowing effective
signalling from a high leptin concentration to curtail appetite. This could explain,
firstly the paradoxical finding of deregulation of appetite during chronic inactivity
(Mayer and Thomas, 1967). Secondly, it provides a mechanism for why inactivity
(i.e., watching television, often in the afternoon and/or evening) is having such
pervasive effects on appetite and body weight regulation (Dietz, 2001), possibly by
an 'uncoupling' of circulating leptin to appetite control. Additionally, the present
findings may offer an explanation for the disappointing results in clinical trials with
recombinant human leptin administration, which alone, provides little benefit in
obesity treatment (Hukshorn et al, 2000). Physical activity trials in conjunction with
recombinant leptin administration might be a new approach to investigate the
potential therapeutic role of leptin in obesity and related diseases.

The exercise-induce appetite suppression has been documented in animal and human
studies but the related-mechanisms remain unclear. Studies in rats reported that
treadmill exercise suppressed the expression of neuropeptide Y in the hypothalamus
(Shin et al, 2003). Shin et al (2003) also suggested that exercise-intensity is an important factor in modulating hypothalamic NPY expression with low-intensity exercise having a more potent suppressive effect on NPY expression than high-intensity exercise. These findings indicate that exercise-induced appetite suppression could be mediated through the effects of exercise on hypothalamic neuropeptides. Future studies should investigate the effects of different modes of exercise on appetite regulation by leptin. It is unclear from the present results whether coupling between leptin and appetite can also be mediated by higher-intensity exercise.

The results of Studies 1 and 2 indicate possible similarities and differences in the acute appetite control in lean and obese individuals. The drive to eat and the reciprocal feelings of satiety and fullness are similar in lean and obese women after a short bout of moderate exercise or eating. However, differences may occur in the biological regulation of appetite. High serum leptin concentrations may regulate short-term appetite in obese individuals but exercise is possibly required to facilitate this action of leptin. Additional studies are required to investigate if these differences are related to distinct physiologic responses to appetite in obese and lean individuals.

7.2.2 Conclusions from Study 3

Adrenaline is unlikely to be the exercise factor responsible for the coupling between leptin and satiety since adrenaline infusion stimulated an increase in subsequent energy intake in obese women. Noradrenaline is also known to increase leptin uptake in rats (Banks, 2001). The plasma threshold concentration, however, for cardiovascular and metabolic changes is approximately 10 times higher for noradrenaline than for adrenaline (Walsh et al, 1998). Thus, to mimic physiological
effects of exercise using noradrenaline infusion, would pose a potential risk to the subjects. For the above reasons, adrenaline alone was chosen for the present study. It is known that leptin and noradrenaline (NA) have common hypothalamic targets (e.g. NPY) (Wellman, 2000) and their effects are mediated by \( \alpha_1 \) adrenoceptors. Exogenous NA can elicit or reduce eating, depending on the site of infusion (paraventricular nucleus) and the relative balance of postsynaptic \( \alpha_2 \)-adrenoceptors (stimulate eating) and \( \alpha_1 \)-adrenoceptors (suppress eating). Recently, \( \alpha_1 \)-adrenoceptors were found to enhance the transport of leptin through the blood brain barrier in mice (Banks, 2001), which extends the anorexigenic action of \( \alpha_1 \)-adrenoceptors. Labetalol probably was not a sufficiently strong \( \alpha \)-adrenoceptor blocker to investigate such effects. Probably a more selective \( \alpha_1 \)-adrenoceptor antagonist could aid the investigation of the interaction between leptin and NA in the regulation of eating.

7.2.3 Conclusions from Study 4

The results from the detraining study are amongst the first reports of an ob protein increase with physical inactivity. This is in agreement with Blanc et al (2000) who reported an increase in leptin levels after physical inactivity induced by 7 days bed rest. Future studies are required to elucidate the effects of physical inactivity on the regulation of leptin and on factors related to hexosamine synthesis in normal weight, obese and trained individuals. The results in trained individuals (Study 4, Chapter 6) suggest that exercise detraining promotes hyperleptinemia and associated metabolic risk factors for coronary heart disease (i.e. hyperinsulinemia, insulin resistance). The elevated leptin level commonly found in obese people may reflect chronic detraining.
Overall summary of the findings

Fifty years ago, Mayer *et al* (1956) speculated that the mechanisms controlling energy balance are accurate in individuals with higher levels of physical activity, but in sedentary individuals there is a threshold of physical activity below which these mechanisms become imprecise and result in overweight. The exact mechanisms controlling energy balance are still unknown but the findings of the present thesis implicate leptin, insulin, insulin resistance and noradrenergic factors in the control of appetite. An attempt to synthesise the findings of this thesis, together with existing evidence, is made in Figure 7.1, as a progression from the model shown in Figure 1.1.
7.3 New Research Questions that this thesis has identified

The present thesis identified several new research questions and some future research questions are also proposed.

1. Chapter 3 (Study 1): *What is the effect of moderate exercise and a mild snack on appetite/satiety ratings, subsequent food intake and serum leptin in obese women*?

Moderate physical activity and snack intake suppress the appetite of obese women acutely. The associations between circulating leptin and appetite-satiety ratings suggest leptin involvement in short-term appetite regulation in response to physical activity-induced factors.

These results suggested a new approach to search the potential therapeutic role of recombinant leptin administration in the treatment of obesity:

*What is the effect of pegylated recombinant human leptin (PEG-OB) on appetite control and body weight in conjunction with a moderate physical activity programme in obese individuals*?

2. Chapter 4 (Study 2): *What is the effect of moderate exercise and a mild snack on appetite/satiety ratings, subsequent food intake and serum leptin in lean women? Is there a coupling between serum leptin and appetite control following physical activity in lean women*?

Moderate exercise and snack intake suppress the appetite of lean women acutely. Serum leptin is not associated with acute appetite regulation after exercise or eating in lean women whereas plasma free fatty acids might have a role on postprandial satiety in lean women. The acute physiological regulation of appetite appears to differ between lean and obese women.
Emerging from the results in Chapter 3 (Study 1) and Chapter 4 (Study 2), some new research questions are proposed:

1: 'Is the transport pathway of leptin different between obese and normal weight individuals; is the blood brain barrier the predominant controlling site for the entry of leptin into the brain in obese compared to normal weight individuals?'

3. Chapter 5 (EXP 1): 'What is the effect of moderate exercise performed with a combined α/β adrenoceptor antagonist on appetite/satiety ratings, subsequent food intake and serum leptin in obese women?'

Combined α/β adrenergic blockade with labetalol during moderate exercise did not alter appetite control following exercise compared to placebo. It appears that α/β adrenergic stimulation through exercise is not responsible for the increased satiety reported previously in obese women.

Chapter 5 (EXP 2): 'What is the effect of adrenaline infusion on appetite/satiety ratings, subsequent food intake and serum leptin in obese women?'

Elevated plasma adrenaline concentrations increased caloric intake in obese women acutely. The β-adrenergic stimulation does not have a specific role in linking physical activity to appetite regulation via enhanced leptin transport. On the other hand it may be related with central orexigenic mechanisms.
The results in Chapter 5 (EXP 1 and 2) raised some new research questions for future studies:

1. *What is the effect of noradrenaline infusion on appetite/satiety ratings, subsequent food intake and serum leptin in obese women?*

2. *What is the effect of moderate exercise performed with a selective α1-adrenoceptor antagonist on appetite/satiety ratings and food intake in obese women?*


A short-term detraining increased serum leptin concentrations, despite the decline in insulin sensitivity, but had no effect on fasting and postprandial appetite and satiety ratings. Insulin resistance does not appear to block the detraining-induced leptin increase. Endurance exercise is needed not only to prevent high levels of insulinemia but also leptinemia in trained individuals.

These results in Chapter 6 raised a new research question for investigation in future studies:

1. *What is the influence of long-term detraining on serum leptin concentrations, insulin sensitivity and energy balance in lean and obese individuals?*
Figure 7.1: Schematic view of the main conclusions from this thesis.

Moderate exercise induced coupling between circulating leptin and suppressed appetite in obese women. It is speculated that the catecholamine-stimulated lipolysis following exercise (increased A, NA, FFA) has facilitated the function of leptin by enhancing the leptin transport through the BBB (a). Noradrenaline (NA) is more likely to be the exercise factor responsible for the coupling between leptin and suppressed appetite since adrenaline infusion stimulated an increase in subsequent energy intake (b). Detraining factor(s) induced hyperleptinemia, increased circulating insulin concentrations and insulin resistance, which indicate the interrelationship between leptin and insulin action in the control of energy balance (c). The red indicates links strengthened by work in the present thesis.
Figure 7.1

HYPOTHALAMUS

± Appetite

POMC
α-MSH
MC-R
CART
CRH
GLP-1

NPY
AGRP
MCH
Orexins

NA

Leptin
Ob-Rb

Leptin

BBB

Insulin

Insulin Resistance

Insulin Resistance

A/NA
FFA

Moderate Exercise

Detraining

HYPOTHALAMUS

POMC
α-MSH
MC-R
CART
CRH
GLP-1

NPY
AGRP
MCH
Orexins

NA

Leptin
Ob-Rb

Leptin

BBB

Insulin

Insulin Resistance

Insulin Resistance

A/NA
FFA

Moderate Exercise

Detraining

HYPOTHALAMUS

POMC
α-MSH
MC-R
CART
CRH
GLP-1

NPY
AGRP
MCH
Orexins

NA

Leptin
Ob-Rb

Leptin

BBB

Insulin

Insulin Resistance

Insulin Resistance

A/NA
FFA

Moderate Exercise

Detraining
APPENDICES
Acknowledgements

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References


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Publications achieved from this PhD thesis

Peer reviewed original refereed papers published


Peer reviewed original refereed papers to be submitted

Tsouliou F, Pitsiladis YP, Hadjicharalmbous M, Fuld J, Wallace AM & Lean MEJ. The acute effects of increased adrenaline concentrations on serum leptin, appetite/satiety measures and subsequent food intake in obese women.


Tsouliou F, Pitsiladis YP, Wallace AM & Lean MEJ. Light-Exercise links serum leptin with acute appetite regulation in obese but not in lean women.

Abstracts from presentations at scientific meetings


APPETITE QUESTIONNAIRE

Subject Number:................. Study code..............
Name: __________________ Date: ___/___/___ Visit: _________________________

Please answer the following questions by placing a vertical mark through the line for each question. Regard the end of each line as indicating the most extreme sensation you have ever felt and mark how you feel NOW.

Example
This is how to mark this line.
e.g. How happy are you (now)?

Not at all happy

As happy as I have ever been

Time: __________

1. How hungry do you feel (now)?
I am not hungry at all

I have never been more hungry

2. How satisfied do you feel (now)?
I am completely empty

I cannot eat another bite

3. How full do you feel (now)?
Not at all full

Totally full

4. How much do you think you can eat (now)?
Nothing at all

A lot

5. How strong is your desire to eat (now)?
Not at all strong

Very strong