LEPTIN AND ACUTE APPETITE CONTROL

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A thesis submitted for the degree of Doctor of Philosophy

to

The University of Glasgow

April 2004

From research conducted at the

Human Nutrition Section, Glasgow Royal Infirmary

Division of Developmental Medicine

University of Glasgow

Glasgow, Scotland

PAGE

NUMBERING

AS ORIGINAL

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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 $kg \cdot m^{-2}$) and ten lean women (mean age ± SD: 37 ± 10 years, mean body mass index ± SD: 22 ± 2 $kg \cdot m^{-2}$) 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 $kg \cdot m^{-2}$, 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 $kg \cdot m^{-2}$) 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 α/β 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 β-adrenoceptor blockade. Any conclusions with respect to the α- adrenoceptor blockade should be drawn with caution since labetalol, an α/β blocker, has greater affinity for β- than α-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 β-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 α-1 adrenoceptors. Labetalol probably was not a sufficiently strong α-adrenoceptor blocker to investigate such effects. A study of a more selective α_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/m^2, while a BMI of > 25 kg/m^2 is designated 'overweight' and a BMI of < 25 kg/m^2 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 *in*activity is believed to contribute to the rising prevalence of obesity, the reasons of appetite and body weight deregulation residing in a physically *in*active 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 *in*activity 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 leptòs *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 hypothlamus 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

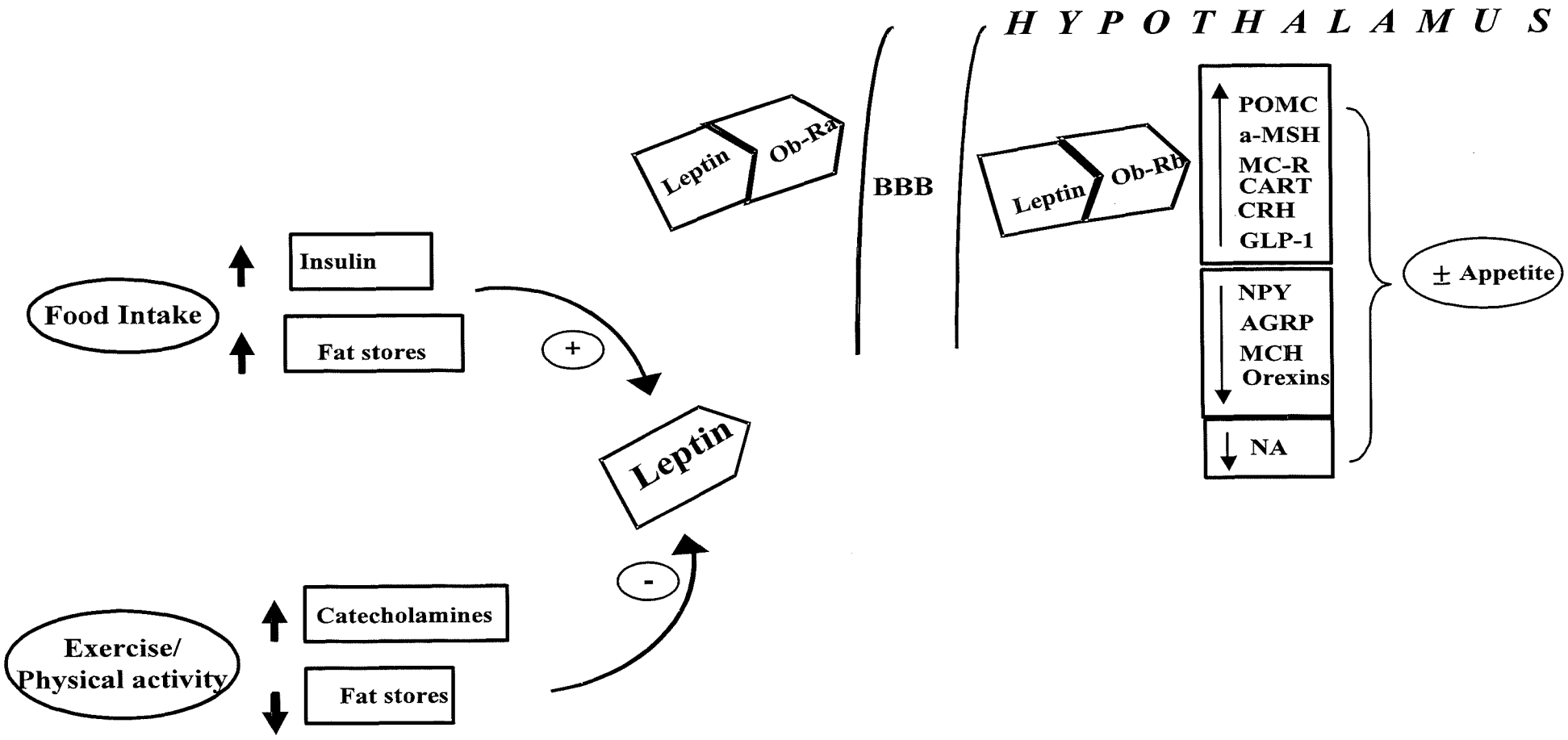
weight, increasing energy expenditure and fat oxidation, thus favoring leaness (Jeanrenaud and Rohner-Jeanrenaud, 2001).

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 paravetricular nucleus is known to stimulate food intake (Leibowitz and Brown, 1980), probably mediated by α_2–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-Ra
BBB
Leptin
Ob-Rb
POMC
a-MSH
MC-R
CART
CRH
GLP-1
NPY
AGRP
MCH
Orexins
NA
± Appetite
Food Intake
Insulin
Fat stores
+
Leptin
-
Exercise/
Physical activity
Catecholamines
Fat stores

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% $\dot{V}O_{2max}$ 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 β_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 β_1- and β_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 weigh 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 etch.) 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 forarm 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 K_3EDTA and into tubes containing coagulation factors. Duplicate aliquots (400 µl) of whole blood from the K_3EDTA tube were rapidly deproteinised in 800 µl of ice-cold 0.3 $mol \cdot l^{-1}$ 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 $P \leq 0.05$.

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 $ml \cdot min^{-1}$) 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 $b \cdot min^{-1}$, 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 K_3EDTA 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_3EDTA tube were rapidly deproteinised in 800 µl of ice-cold 0.3 $mol \cdot l^{-1}$ 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 (BMI ($kg \cdot m^{-2}$) and fat mass (FM (kg) in all trials (BMI r_s = 0.73, P = 0.02, FM r_s = 0.88, P = 0.001 Moderate physical activity; BMI r_s = 0.69, P = 0.02, FM r_s = 0.85, P = 0.002 Snack; BMI r_s = 0.78, P = 0.008, 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) **(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 $mmol \cdot l^{-1}$), 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 Asrtup, 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

Age (years)	50 ± 8
Weight (kg)	96 ± 18
Height (cm)	161 ± 5
BMI ($kg \cdot m^{-2}$)	37 ± 6
Fat mass (kg)	45 ± 10
Fat mass (%)	47 ± 4
Fat free mass (kg)	50 ± 9
Fat free mass (%)	53 ± 4

Values are mean ± SD.

Table 3.2 Self-selected nutrient intake of obese women at dinner after the trial conditions

	Control	**Moderate exercise**	**Snack**
Energy intake (kcal)	724 (509-1369)	683 (509-1011)	657 (541-742)
(kJ)	2751 (2268-3108)	2860 (2134-4234)	2751 (2268-3108)
Protein			
(g)	49 ± 17	45 ±16	39 ± 12
Carbohydrate			
(g)	84 ± 40	75 ± 31	66 ± 24
Fat			
(g)	22 ± 12	23 ± 11	23 ± 13

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

	Baseline (-60 min)	**Pre intervention (0 min)**	**Post intervention (30 min)**	**1 h post-intervention (90 min)**
Control				
Serum leptin ($ng \cdot ml^{-1}$)	45.4 (31.0 - 123.0)	48.1 (30.5 - 122.4)	51.3 (26.2 - 131.6)	55.9 (27.4 - 130.6)
Blood glucose ($mmol \cdot l^{-1}$)	4.9 (4.1 - 5.9)	4.7 (4.1 - 5.9)	4.7 (4.3 - 5.3)	4.6 (4.4 - 5.3)
Plasma FFA ($mmol \cdot l^{-1}$)	0.5 (0.2 - 1.0)	0.6 (0.1 - 1.0)	0.8 (0.2 - 1.4) **	0.8 (0.4 - 1.3) **
Moderate activity				
Serum leptin ($ng \cdot ml^{-1}$)	54.8 (29.1 - 91.4)	56.7 (26.9 - 119.2)	58.7 (33.7 - 121.0)	56.1 (26.0 - 133.0)
Blood glucose ($mmol \cdot l^{-1}$)	5.2 (3.9 - 6.3)	4.7 (4.1 - 6.1)	4.7 (3.9 - 5.5)	4.9 (3.8 - 5.2)
Plasma FFA ($mmol \cdot l^{-1}$)	0.5 (0.1 - 0.8)	0.6 (0.3 - 1.1) §	1.3 (0.5 - 1.9) *, †, §	0.9 (0.4 - 1.2) §
Snack				
Serum leptin ($ng \cdot ml^{-1}$)	44.1 (26.4 - 147.4)	47.6 (22.4 - 136.0)	48.6 (23.2 - 112.0)	54.4 (27.4 - 100.2)
Blood glucose ($mmol \cdot l^{-1}$)	5.2 (4.6 - 6.0)	4.9 (4.3 - 5.4) ||	6.0 (4.7 - 8.0) †, ‡, ||	6.3 (5.0 - 7.3)) †, ‡
Plasma FFA ($mmol \cdot l^{-1}$)	0.5 (0.2 - 0.9)	0.6 (0.1 - 0.9)	0.7 (0.3 - 1.0)	0.2 (0.1 - 0.4) †, ‡, ||

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.

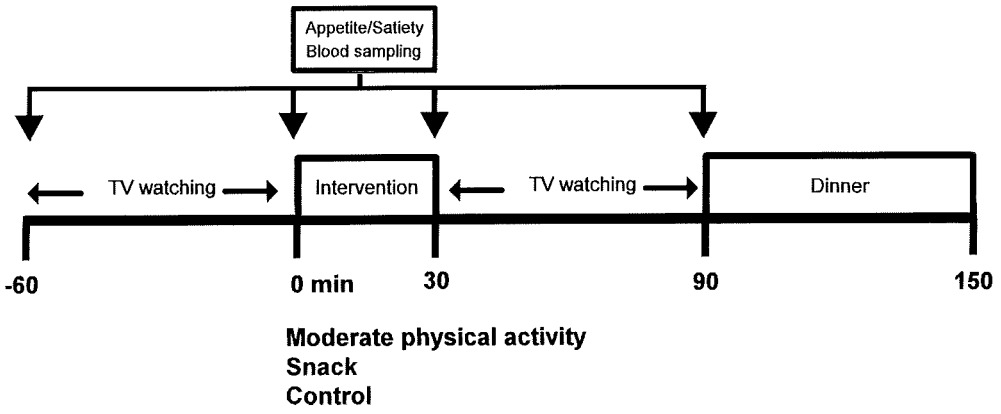


Figure 3.2: Median profiles of self-reported appetite-satiety ratings under the Moderate physical activity (■), Snack (▲) and Control (●) trials; Data were analysed using Kruskal-Wallis test followed by Wilcoxon-signed rank test to determine the differences in ratings between trials. *, †, ‡ indicate 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

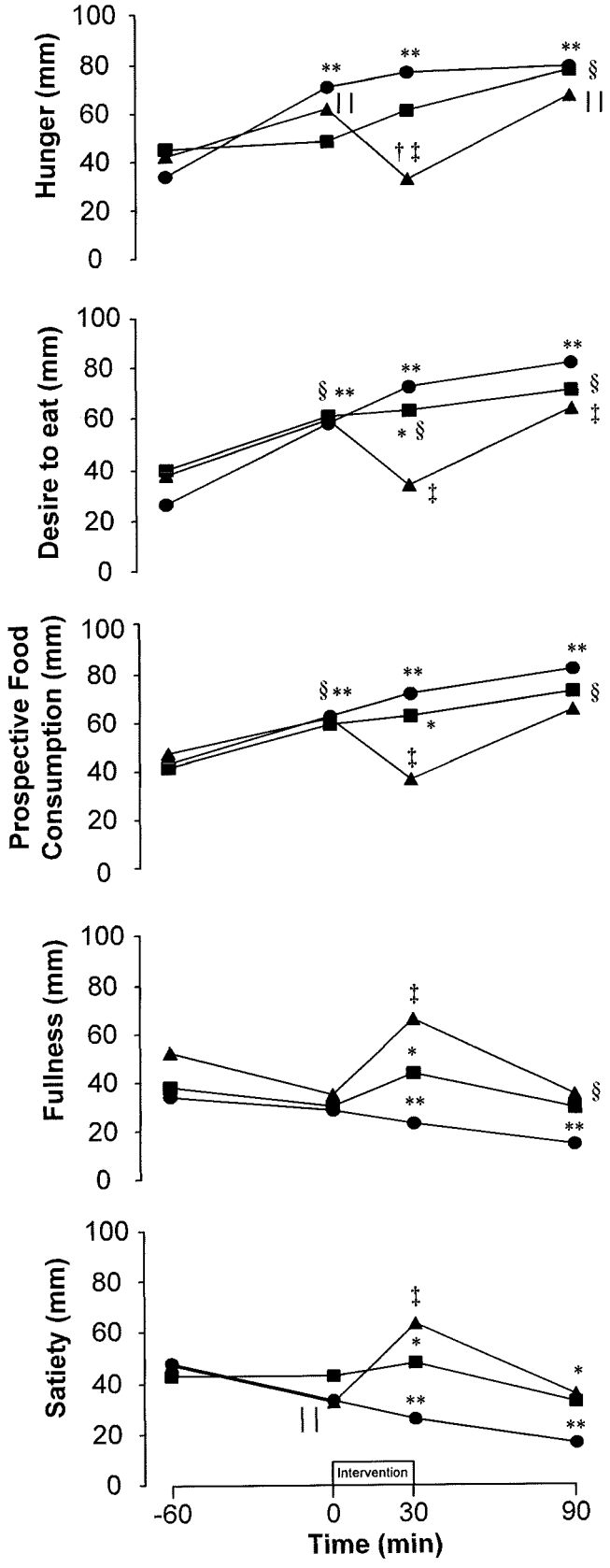
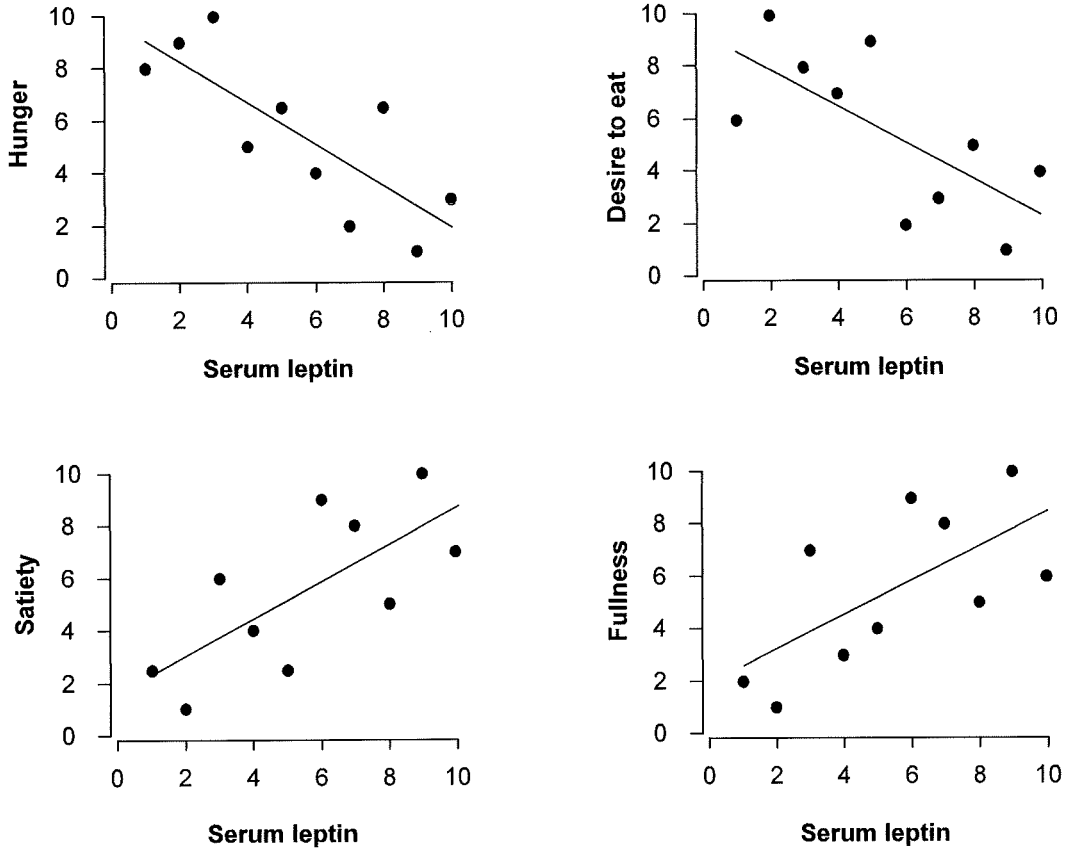

Hunger (mm)
Desire to eat (mm)
Prospective Food Consumption (mm)
Fullness (mm)
Satiety (mm)
100
80
60
40
20
0
Intervention
-60
0
30
90
Time (min)
Moderate physical activity
Snack
Control

Figure 3.3: Spearman rank associations between ranked serum leptin concentrations ($ng \cdot ml^{-1}$) 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 (Vb_{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 $b \cdot min^{-1}$, 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 K_3EDTA 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_3EDTA tube were rapidly deproteinised in 800 µl of ice-cold 0.3 $mol \cdot l^{-1}$ 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).

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% VO_2max, 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

Age (years)	37 ± 10
Weight (kg)	57 ± 6
Height (cm)	163 ± 6
Waist (cm)	73 ± 7
BMI ($kg.m^{-2}$)	22 ± 2
FM (%)	27 ± 7
FM (kg)	15 ± 5
FFM (%)	73 ± 7
FFM (kg)	42 ± 4

Values are mean ± SD

Table 4.2 Self-selected nutrient intake of lean women at dinner after the trial conditions

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

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

	Baseline (-60 min)	**Pre intervention (0 min)**	**Post intervention (30 min)**	**1 hr post-intervention (90 min)**
Control				
Serum leptin $(ng.ml)^{-1}$	7.05 (3-31)	7.1 (3.6-35.2)	7.1 (3.4-39.8)	6.2 (3-32.8)
Blood glucose $(mmol.l)^{-1}$	4.61 (4-5.42)	4.27 (3.85-4.8)	4.23 (3.96-5)	4.28 (3.7-4.8)
Plasma FFA $(mmol.l)^{-1}$	0.44 (0.05-1.41)	0.69 (0.05-1.37)	0.78 (0.1-1.26)	0.77 (0.16-1.3)
Moderate exercise				
Serum leptin $(ng.ml)^{-1}$	8.1 (1.8-16.8)	7.9 (1.4-17.2)	8.1 (1.8-20)	9 (1.6-17.2)
Blood glucose $(mmol.l)^{-1}$	4.55 (3.97-6.38)	4.23 (3.78-4.86)§	4.56 (4.17-4.71)*	4.27 (3.81-4.78)§
Plasma FFA $(mmol.l)^{-1}$	0.34 (0.03-0.93)	0.51 (0.08-1.07)	1.46 (0.15-2.54)$^{*, \dagger, \S}$	0.71 (0.19-0.85)
Snack				
Serum leptin $(ng.ml)^{-1}$	7.1 (2.5-25)	7.5 (2-28.4)	8.1 (2.1-26.8)	9 (1.6-29.4)
Blood glucose $(mmol.l)^{-1}$	4.47 (3.66-5.84)	4.29 (3.78-4.86)	6.22 (4.63-6.68) $^{\ddagger, \dagger, \|}$	6.29 (4.39-7.24) $^{\ddagger, \dagger, \|}$
Plasma FFA $(mmol.l)^{-1}$	0.29 (0.06-1.08)	0.59 (0.11-0.95)	0.61 (0.07-1.13)	0.10 (0.05-0.27) $^{\ddagger, \dagger, \|}$

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

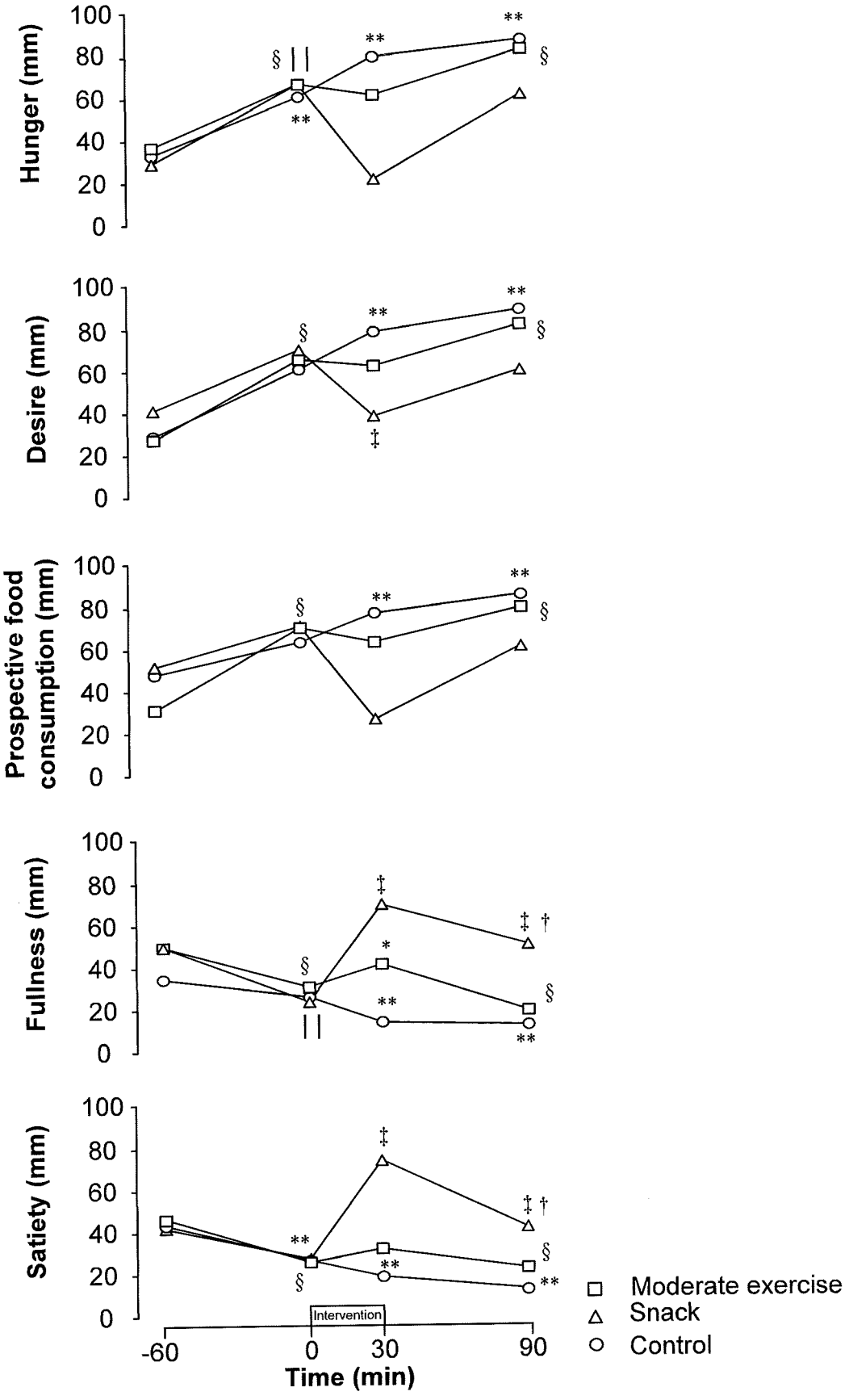
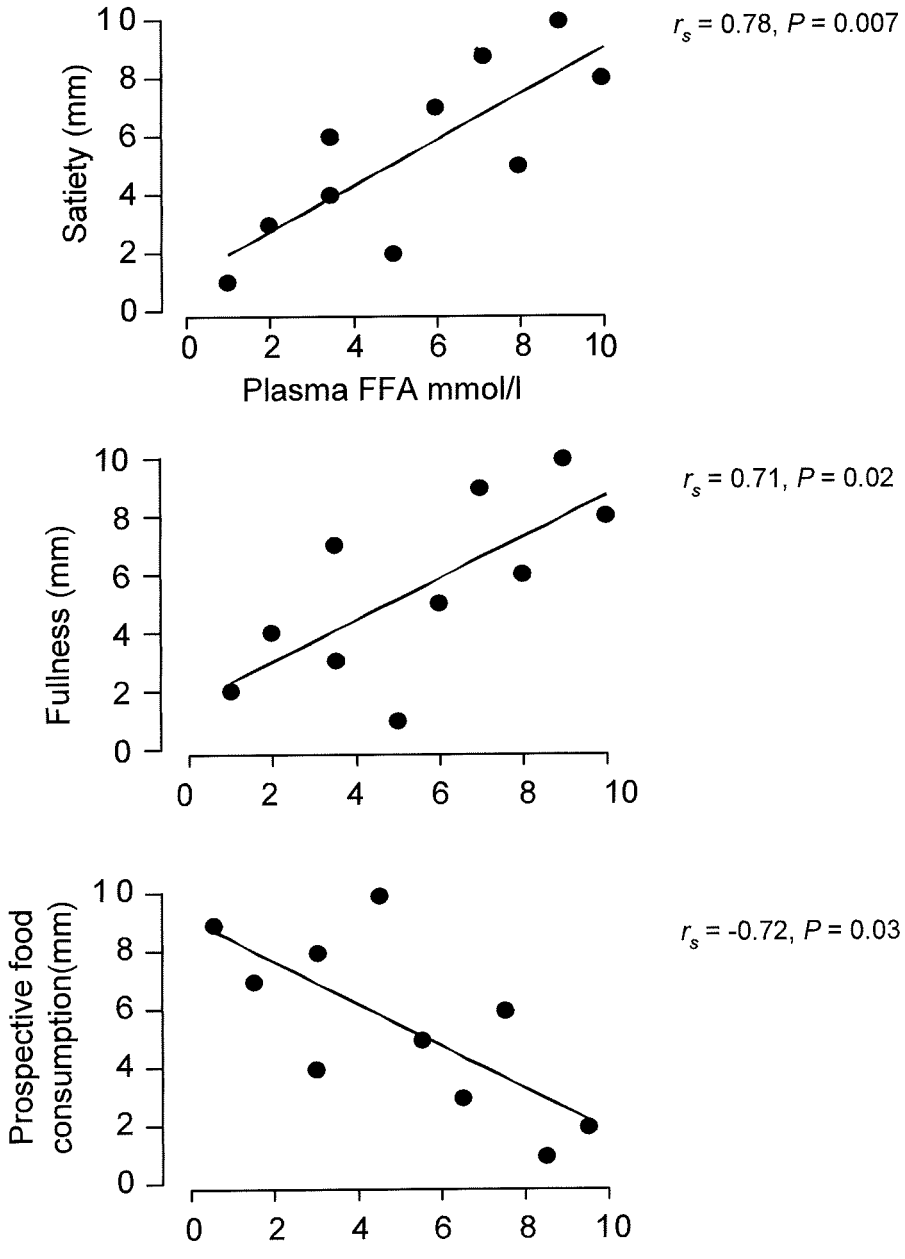

Hunger (mm)
Desire (mm)
Prospective food consumption (mm)
Fullness (mm)
Satiety (mm)
100
80
60
40
20
0
-60
0
30
90
Time (min)
Intervention
Moderate exercise
Snack
Control

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.



Chapter Five

(Study 3)

Effects of circulating adrenaline concentrations and of moderate exercise plus α/β 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 β_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 α_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 β_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_3EDTA 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_3EDTA tube were rapidly deproteinised in 800 µl of ice-cold 0.3 $mol \cdot l^{-1}$ 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 $P \leq 0.05$.

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 ($kg \cdot m^{-2}$), 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 α/β 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 ($kg{\cdot}m^{-2}$), 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 α/β 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 $nmol \cdot ml^{-1}$, 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 $nmol \cdot ml^{-1}$ (approximately 0.8 $nmol \cdot ml^{-1}$ 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 β_1- and β_2- 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 β_1 and β_2 adrenoceptors, and also α_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 intavenous 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 α- 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 α-adrenergic receptor antagonist on appetite/satiety ratings and food intake in obese women?

Table 5.1 Subject characteristics, n = 10	
Age (years)	50.3 ± 6.1
Weight (kg)	90.2 ± 16.5
Height (cm)	158.0 ± 12.8
BMI ($kg \cdot m^{-2}$)	36.1 ± 5.5
Waist circumference (cm)	104.8 ± 12.8
Hip circumference (cm)	115.2 ± 9.7
Fat mass (%) predicted by waist	47.7 ± 5.4
Systolic Blood Pressure (mmHg)	129.6 ± 7.2
Diastolic Blood Pressure (mmHg)	89.2 ± 4.1

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

	Baseline (-60 min)	**Pre intervention (0 min)**	**Post intervention (20 min)**	**1 h post-intervention (80 min)**
Exercise plus α/β blocker				
Serum leptin ($ng \cdot ml^{-1}$)	62.7 ± 22.9	63.4 ± 23.2	68.9 ± 23.8§	65.2 ± 24.8
Blood glucose ($mmol \cdot l^{-1}$)	4.6 ± 0.5	4.8 ± 0.3	4.9 ± 0.2*	4.9 ± 0.2*
Plasma FFA ($mmol \cdot l^{-1}$)	0.69 ± 0.3	0.59 ± 0.18	0.49 ± 0.15$^{* \S}$	0.61 ± 0.16*
Exercise plus placebo				
Serum leptin ($ng \cdot ml^{-1}$)	62.3 ± 22.1	65.7 ± 26.5	73.0 ± 26.7$^{||}$	65.6 ± 23.4
Blood glucose ($mmol \cdot l^{-1}$)	4.9 ± 0.5	4.5 ± 0.2	4.5 ± 0.3	4.5 ± 0.2
Plasma FFA ($mmol \cdot l^{-1}$)	0.67 ± 0.34	0.68 ± 0.22	0.8 ± 0.26*	0.78 ± 0.24*

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

	Baseline (-60 min)	**Pre intervention (0 min)**	**Post intervention (20 min)**	**1 h post-intervention (80 min)**
Adrenaline				
Serum leptin ($ng \cdot ml^{-1}$)	60.9 ± 21.9	60.1 ± 23.4	61.9 ± 24.3	64.2 ± 29.7
Blood glucose ($mmol \cdot l^{-1}$)	4.6 ± 0.7	4.6 ± 0.2	4.7 ± 0.2	4.5 ± 0.1
Plasma FFA ($mmol \cdot l^{-1}$)	0.76 ± 0.3	0.7 ± 0.3	0.95 ± 0.4$^{*\,\S}$	0.75 ± 0.2
Saline				
Serum leptin ($ng \cdot ml^{-1}$)	62.3 ± 23.9	62.3 ± 23.9	61.7 ± 26.6	64.6 ± 24.1
Blood glucose ($mmol \cdot l^{-1}$)	4.9 ± 0.6	4.8 ± 0.3	4.7 ± 0.2	4.6 ± 0.2
Plasma FFA ($mmol \cdot l^{-1}$)	0.63 ± 0.3	0.61 ± 0.3	0.65 ± 0.3	0.66 ± 0.2

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

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

Values are mean ± SD. 2-way ANOVA did not detect differences with $P \leq 0.05$

Table 5.5 Heart rate, perceived breathlessness and leg-tiredness among nine subjects during the 20 min Adrenaline and the Saline infusions, n = 9

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

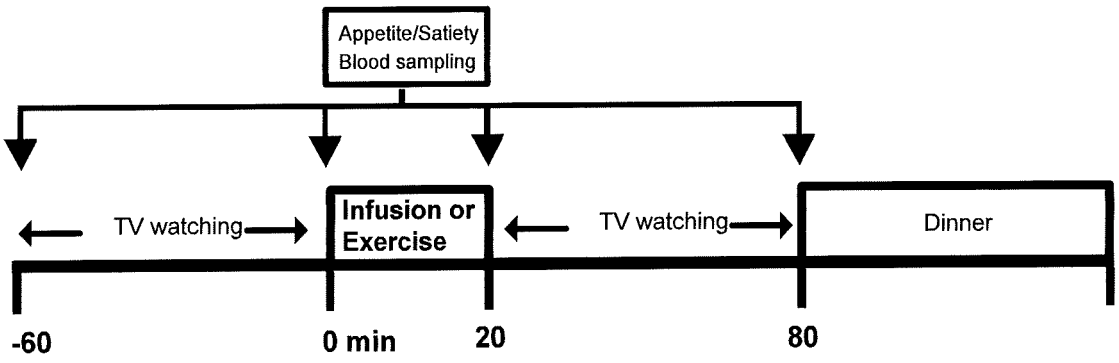
Values are mean ± SD. The superscript symbol * indicates significant differences between trials (* $P < 0.01$: Adrenaline infusion *vs* Saline infusion, Paired t-test). ANOVA treatment × time effect, $P = 0.05$.

Table 5.6 Cardiopulmonary variables for each of the two 20 min exercise trials in EXP 1, n = 10

	Exercise Time (min)	
	8:30 -9:30 min	**18:30 -19:30 min**
Exercise plus α/β blocker		
VO_2 (lt/min)	1.3 ± 0.5	1.4 ± 0.3
VCO_2 (lt/min)	1.1 ± 0.3	1.2 ± 0.2
RER	0.8 ± 0.1	0.8 ± 0.03
%Fat oxidised	65.1 ± 13.8	67.4 ± 12.4
%CHO oxidised	34.9 ± 13.8	32.6 ± 12.4
Exercise plus placebo		
VO_2 (lt/min)	1.4 ± 0.4	1.5 ± 0.3
VCO_2 (lt/min)	1.1 ± 0.4	1.1 ± 0.3
RER	0.8 ± 0.03	0.8 ± 0.1
%Fat oxidised	62.4 ± 12.1	78.6 ± 29.7
%CHO oxidised	37.1 ± 12.1	21.4 ± 29.7

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

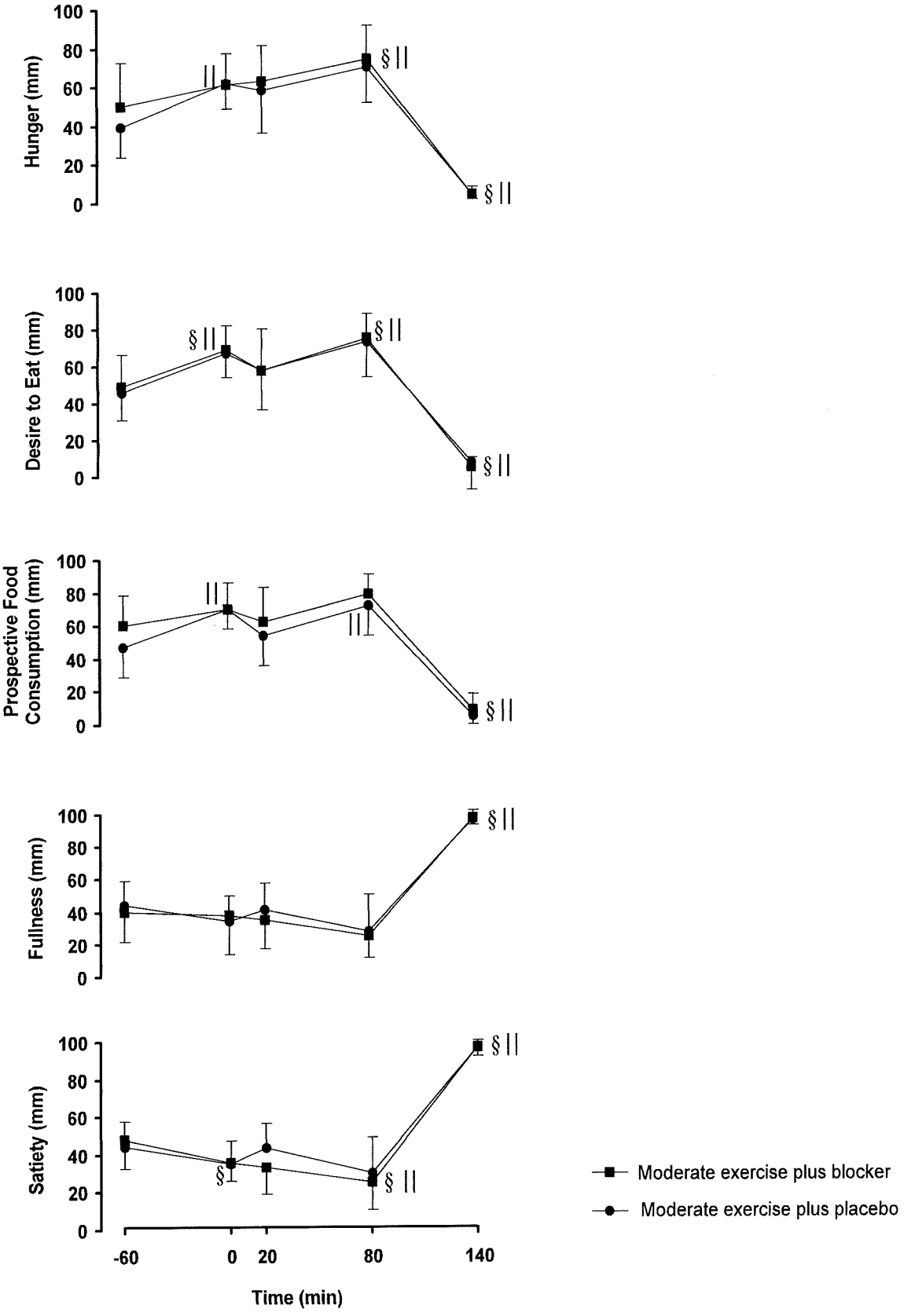

Hunger (mm)
Desire to Eat (mm)
Prospective Food Consumption (mm)
Fullness (mm)
Satiety (mm)
100
80
60
40
20
0
§ ||
||
-60
0
20
80
140
Time (min)
Moderate exercise plus blocker
Moderate exercise plus placebo

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 5.3

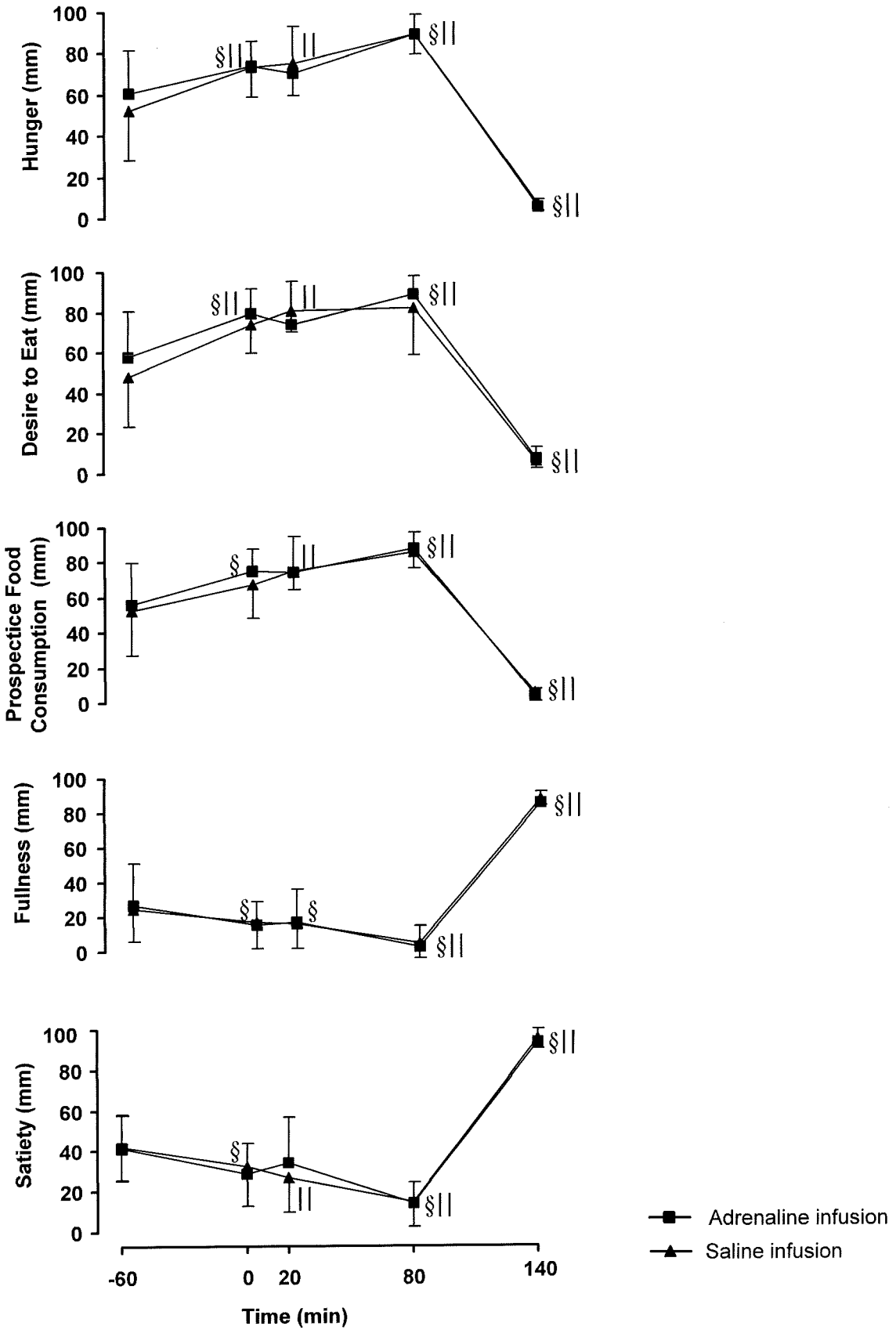

Hunger (mm)
Desire to Eat (mm)
Prospectice Food Consumption (mm)
Fullness (mm)
Satiety (mm)
100
80
60
40
20
0
§||
§
||
-60
0
20
80
140
Time (min)
Adrenaline infusion
Saline infusion

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.

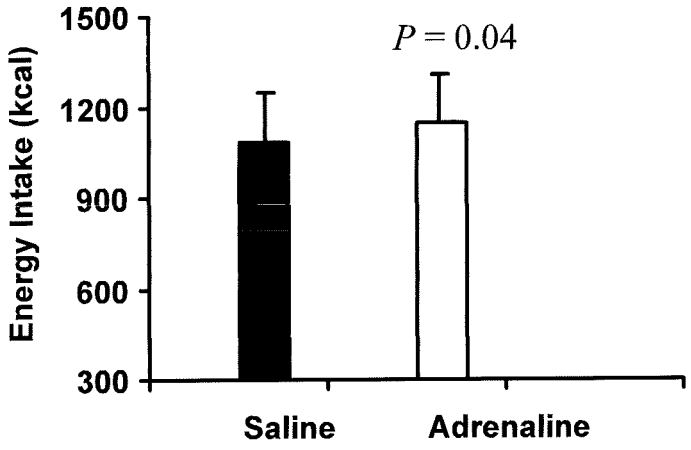
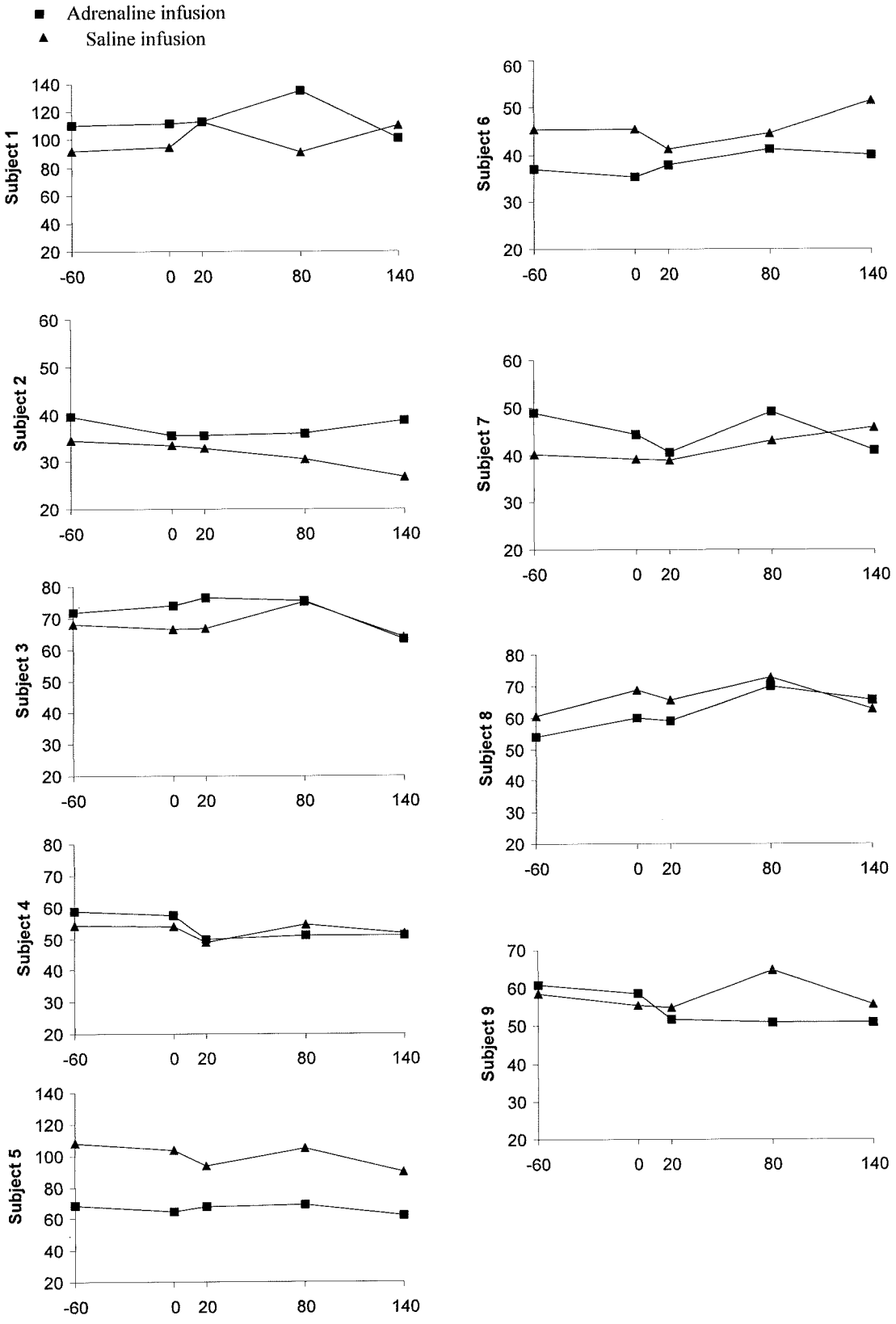


Figure 5.5: Profiles of serum leptin concentrations (ng/ml) during the Adrenaline and Saline infusion trials (from 17:00 pm to 20:20 pm), n = 9



Chapter Six

(Study four)

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 partcipants 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 1074kcal (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: ISI (gly) = 2/[(INS x 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 (Belfliore *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 postaprandially (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 6.1 Subject characteristics, n = 8

Age (years)	28 ± 12
Weight (kg)	73.7 ± 17
Height (cm)	176 ± 0.06
Waist (cm)	79.3 ± 4.1
BMI ($kg \cdot m^{-2}$)	23.6 ± 1.0
Sum of skinfolds (mm)	39.6 ± 9.4
Fat mass (%)	17.0 ± 3.3

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.

	Time scale					
	0 h	**0.5 h**	**1 h**	**2 h**	**4 h**	**6 h**
	Trained					
Plasma Insulin ($\mu U \cdot ml^{-1}$)	4.1 ± 0.4 *	40.3 ± 5.1 *	21.1 ± 4.2	13.4 ± 1.2 *	5.9 ± 1.0 *	4.1 ± 0.6
Plasma Glucose ($mmol \cdot ml^{-1}$)	4.98 ± 0.12	5.86 ± 0.36	4.83 ± 0.27	5.44 ± 0.20	5.13 ± 0.08	5.13 ± 0.09
ISI(gly)	1.17 ± 0.35 *					
	Detrained					
Plasma Insulin ($nmol \cdot ml^{-1}$)	5.7 ± 0.6	56.1 ± 5.3	30.3 ± 4.2	19.1 ± 2.5	7.6 ± 0.9	5.5 ± 0.5
Plasma Glucose ($nmol \cdot ml^{-1}$)	5.0 ± 0.08	5.91 ± 0.25	4.86 ± 0.36	5.22 ± 0.19	5.13 ± 0.07	5.10 ± 0.07
ISI(gly)	0.83 ± 0.18					

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.

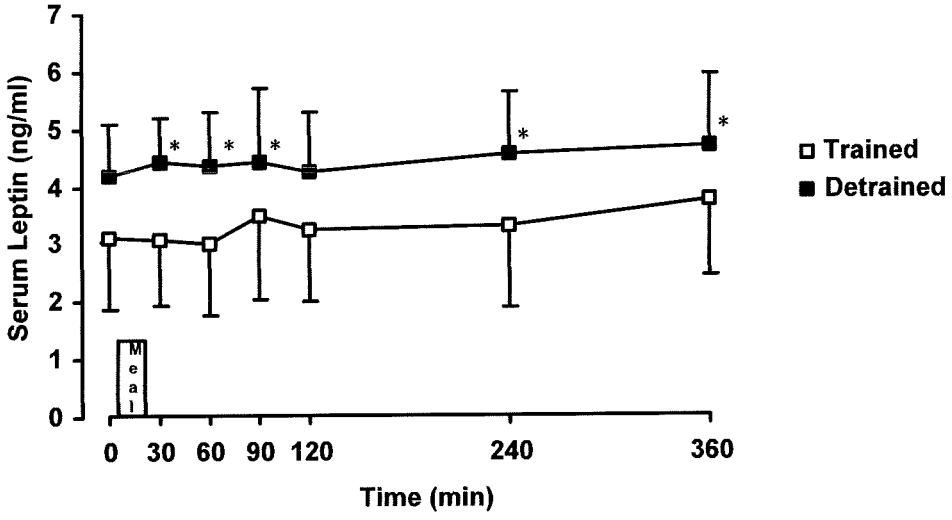


Figure 6.2: Profiles of self-reported appetite-satiety ratings under the Trained (□) and the Detrained (■) conditions. Data were analysed by Students' *t*-test to determine the changes in ratings over time within trials. §|| are significantly different from baseline within the Trained (§ $P < 0.05$) or the Detrained (|| $P < 0.05$) conditions. Values are mean ± SEM, n = 8

Figure 6.2

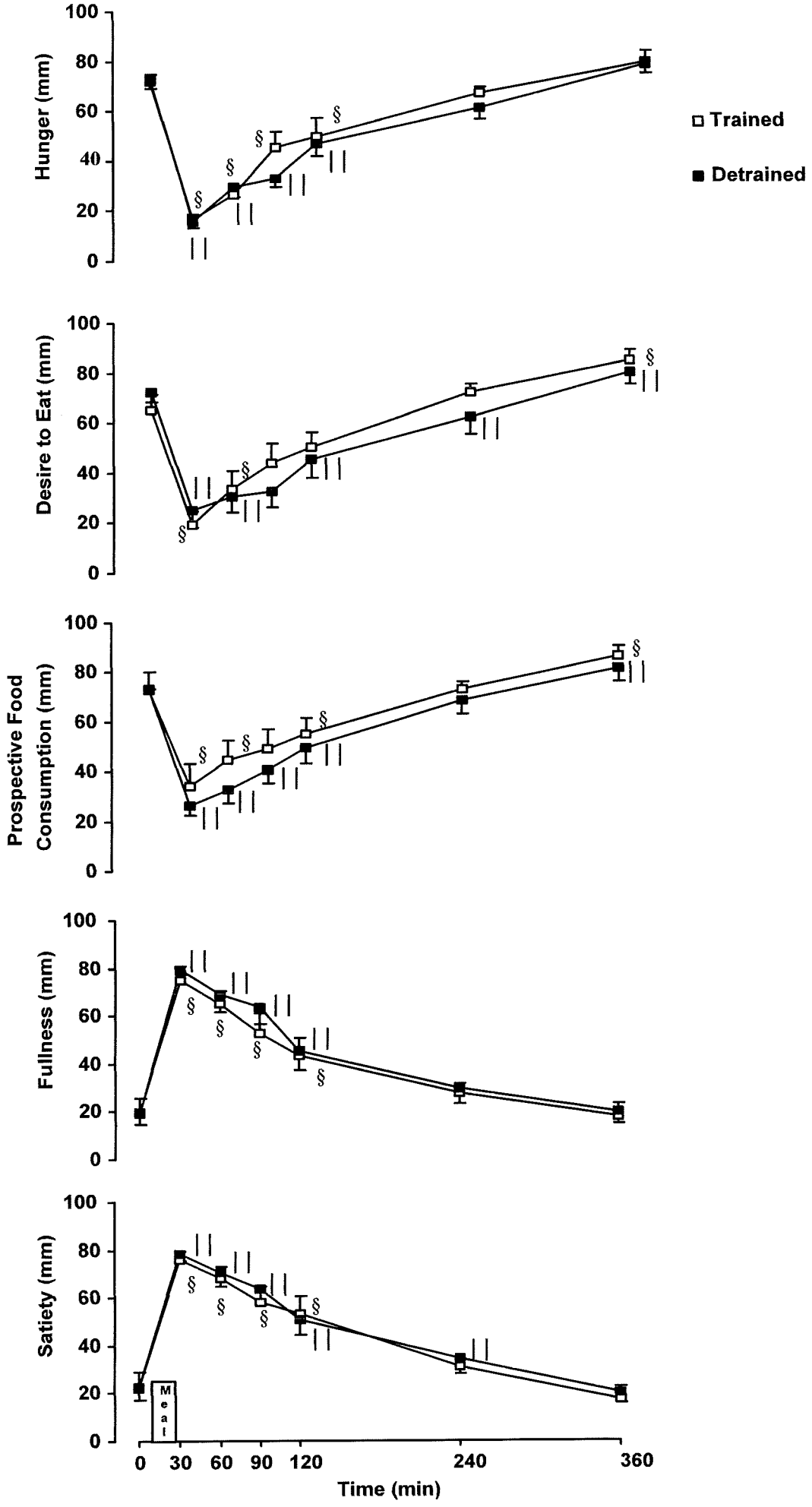

Hunger (mm)
Desire to Eat (mm)
Prospective Food Consumption (mm)
Fullness (mm)
Satiety (mm)
100
80
60
40
20
0
Trained
Detrained
Meal
0
30
60
90
120
240
360
Time (min)

Figure 6.3: Associations between insulin plasma concentrations ($\mu U \cdot ml^{-1}$) 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

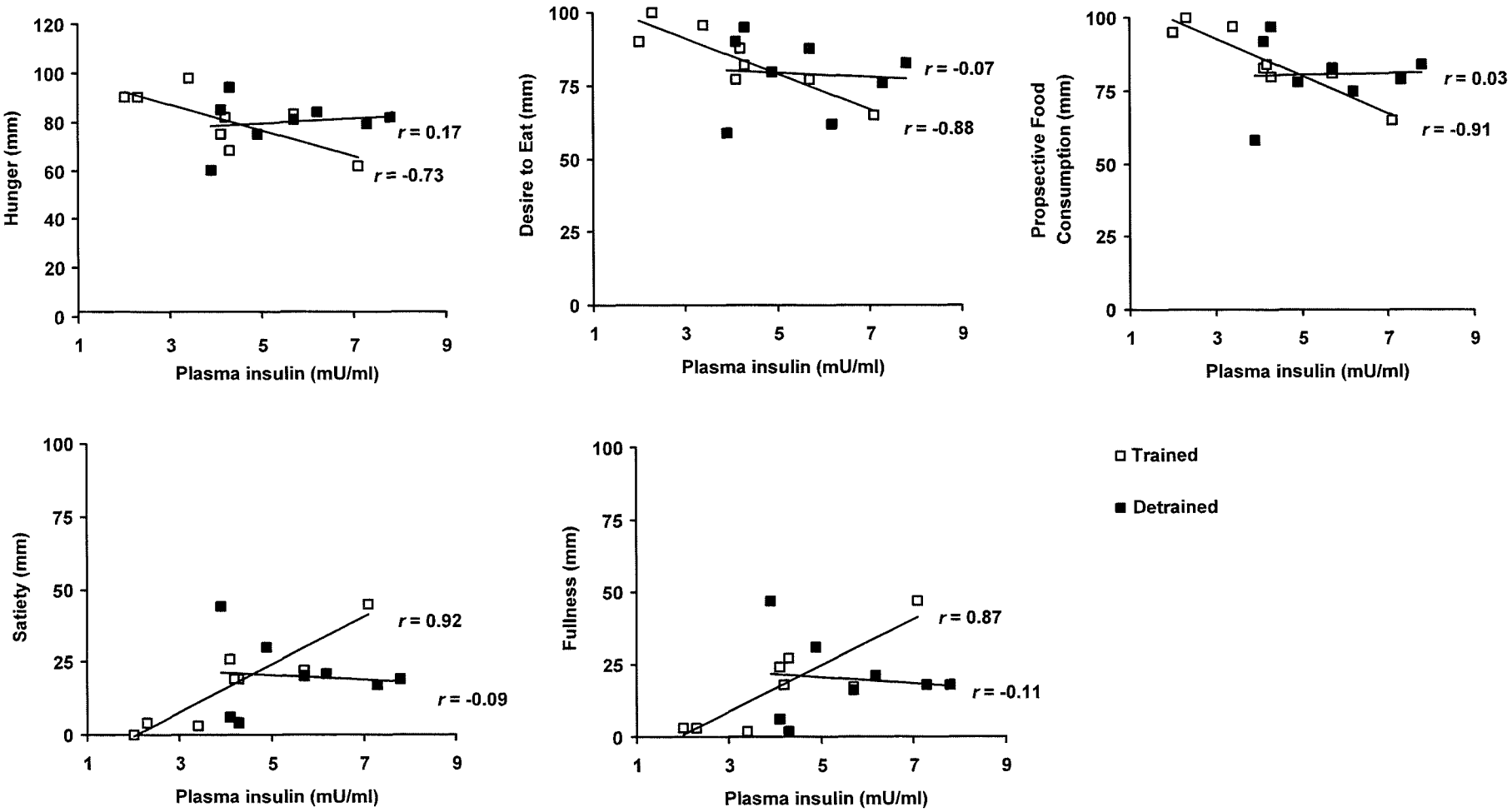
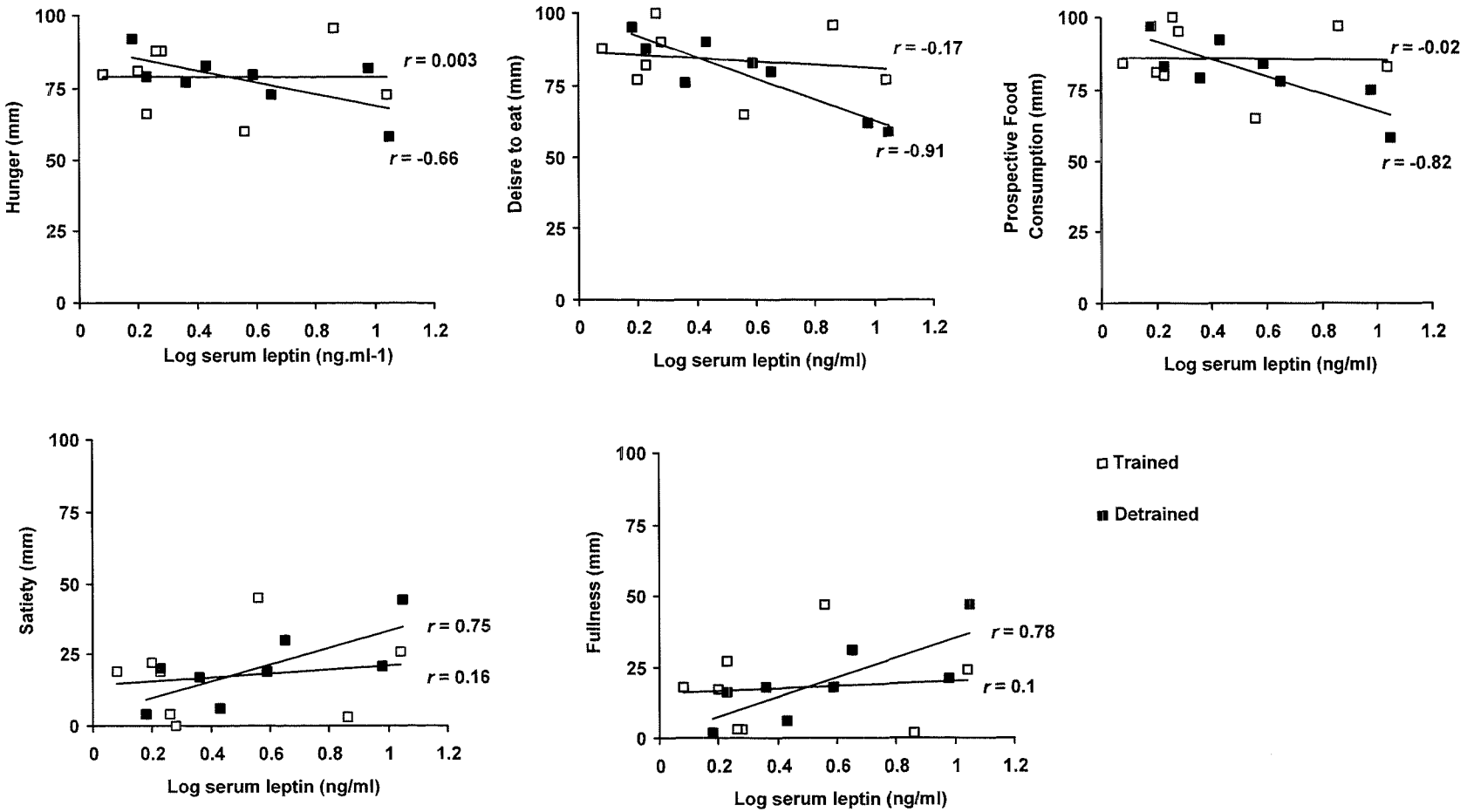

Hunger (mm)
Plasma insulin (mU/ml)
r = 0.17
r = -0.73
Desire to Eat (mm)
r = -0.07
r = -0.88
Propsective Food Consumption (mm)
r = 0.03
r = -0.91
Satiety (mm)
r = 0.92
r = -0.09
Fullness (mm)
r = 0.87
r = -0.11
Trained
Detrained

Figure 6.4: Associations between $\log_{10}$ serum leptin concentrations ($ng \cdot ml^{-1}$) 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.

Figure 6.4


Hunger (mm)
Log serum leptin (ng.ml-1)
r = 0.003
r = -0.66
Deisre to eat (mm)
Log serum leptin (ng/ml)
r = -0.17
r = -0.91
Prospective Food Consumption (mm)
Log serum leptin (ng/ml)
r = -0.02
r = -0.82
Satiety (mm)
Log serum leptin (ng/ml)
r = 0.75
r = 0.16
Fullness (mm)
Log serum leptin (ng/ml)
r = 0.78
r = 0.1
Trained
Detrained

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 *in*activity 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 *in*activity 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 α_1-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 *in*activity 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 α_1 adrenoceptors. Exogenous NA can elicit or reduce eating, depending on the site of infusion (paraventricular nucleus) and the relative balance of postsynaptic α_2-adrenoceptors (stimulate eating) and α_1-adrenoceptors (suppress eating). Recently, α_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 α_1-adrenoceptors. Labetalol probably was not a sufficiently strong α-adrenoceptor blocker to investigate such effects. Probably a more selective α_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 α_1-adrenoceptor antagonist on appetite/satiety ratings and food intake in obese women?'

4. Chapter 6 (study 4): '*What is the effect of short-term detraining on serum leptin, insulin sensitivity and appetite/satiety measures in endurance-trained men?*'

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.

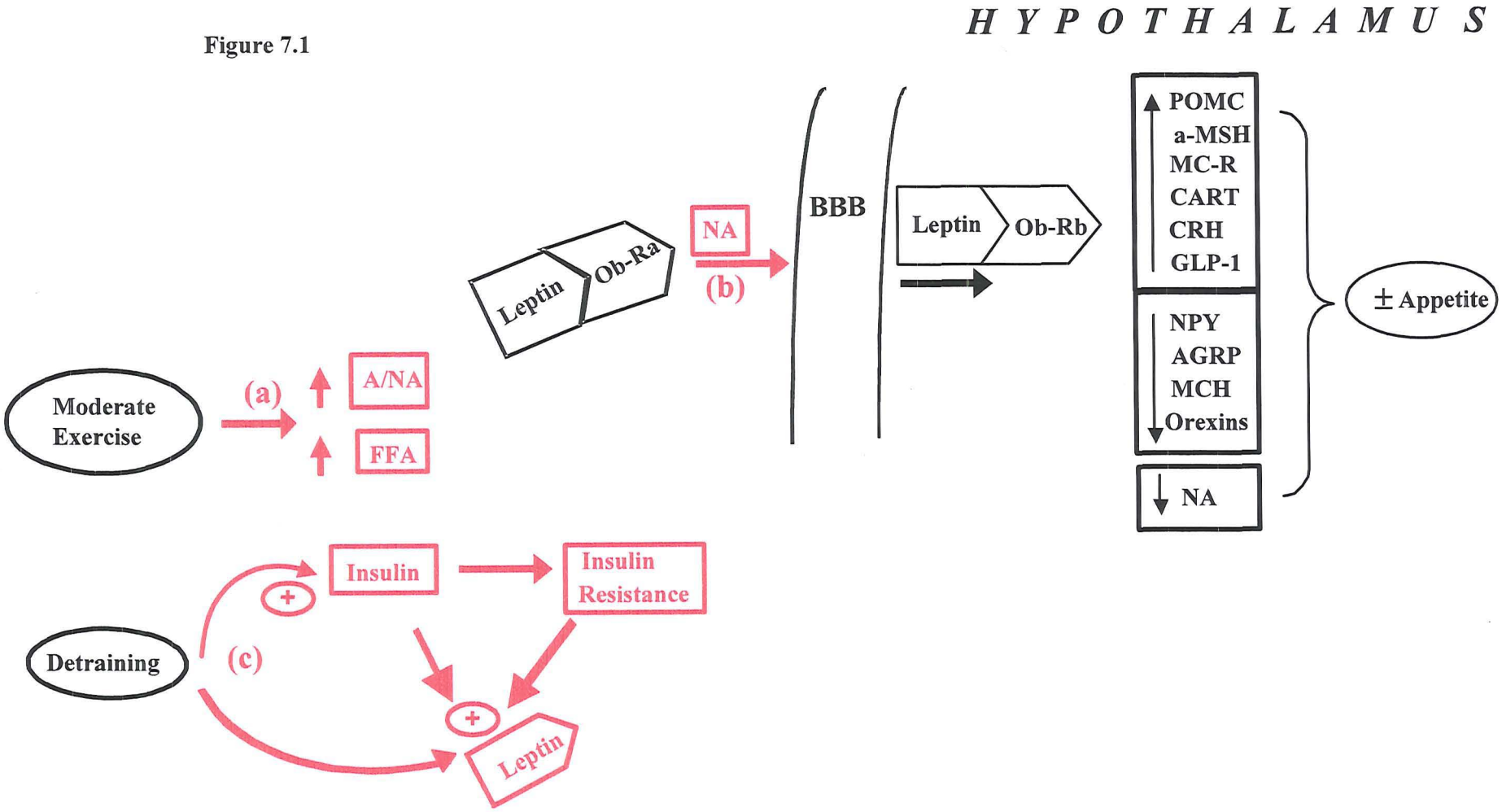
These results in Chapter 6 raised a new research question for investigation in future studies:

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
Moderate Exercise
(a)
A/NA
FFA
Leptin
Ob-Ra
NA
(b)
BBB
Leptin
Ob-Rb
POMC
a-MSH
MC-R
CART
CRH
GLP-1
NPY
AGRP
MCH
Orexins
NA
± Appetite
Detraining
(c)
Insulin
Insulin Resistance
+
+
Leptin

APPENDICES

Acknowledgements

I wish to thank Professor Mike E.J. Lean for his inspired supervision and valuable friendship during my PhD training in the Human Nutrition Department (University of Glasgow). I also thank Dr. Yannis Pitsiladis from the Center for Exercise and Biomedical Sciences (University of Glasgow) for his important co-supervision, companionship and enduring guidance during the experimental studies of my PhD.

I would like to acknowledge Dr. Mike Wallace from the Department of Pathological Biochemistry (University of Glasgow), who offered his advice and leptin expertise to our team. Dr. Dalia Malkova from the Department of Human Nutrition (University of Glasgow) invited me to participate in data collection/analysis in the Detraining Study of this thesis, and therefore she is greatly appreciated. Dr. Jason Gill (Department of Pathological Biochemistry, University of Glasgow) is thanked for the nice collaboration during the Detraining study.

The States Scholarship Foundation (IKY) in Greece and in part Roche Pharmaceuticals, Ltd. have funded my PhD, and I am grateful for their support.

I wish to thank my parents Giorgos and Maria Tsoflios, my brother Yannis and my friends for their support and understanding. I also thank my husband Ippokratis Mitsios for taking care of me during the busy times of my PhD.

Finally, all the volunteers are very much appreciated for their great help and work during the course of the present studies.

References

Ahima, R.S. & Flier, J.S. (2000) Leptin. *Annual Reviews of Physiology*, **62**, 413-437.

Andersen, R.E., Crespo, C.J., Bartlett, S.J., Cheskin, L.J. & Pratt, M. (1998) Relationship of physical activity and television watching with body weight and level of fatness among children: results from the Third National Health and Nutrition. *Journal of the American Medical Association*, **279**, 938-942.

Andersson, I. & Rossner, S. (1996) Meal patterns in obese and normal weight men: the 'Gustaf' study. *European Journal of Clinical Nutrition*, **50**, 639-646.

Arai, T., Kawakami, Y., Matsushima, T., Okuda, Y. & Yamashita, K. (2002) Intracellular fatty acid downregulates ob gene expression in 3T3-L1 adipocytes. *Biochemical Biophysics in Research Communications*, 297, 1291-1296.

Arner, P. (1999) Catecholamine-induced lipolysis in obesity. *International Journal of Obesity and Related Metabolic Disorders*, **23**, 10-13.

Arner, P., Wahrenberg, H., Lonnqvist, F. & Angelin, B. (1993) Adipocyte beta-adrenoceptor sensitivity influences plasma lipid levels. *Arteriosclerosis, thrombosis and vascular biology*, **13**, 967-972.

Astrup, A. (2001) Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. *Public Health Nutrition*, **4**, 499-515.

Astrup, A., Ranneries, C., Simonsen, L., Bulow, J. & Friedman, J. (1997) Leptin: a short-term acting glucostatic hormone? *International Journal of Obesity and Related Metabolic Disorders*, **21**, S13.

Astrup, A., Simonsen, L., Bulow, J., Madsen, J. & Christensen, N.J. (1989) Epinephrine mediates facultative carbohydrate-induced thermogenesis in human skeletal muscle. *American Journal of Physiology*, **257**, E340-345.

van Baak, M.A. (1999) Physical activity and energy balance. *Public Health Nutrition*, **2**, 335-339.

Banks, W.A. (2001) Enhanced leptin transport across the blood-brain barrier by α1 adrenergic agents. *Brain Research*, **899**, 209-217.

Banks, W.A., DiPalma, C.R. & Farrell, C.L. (1999) Impaired transport of leptin across the blood-brain barrier in obesity. *Peptides*, **20**, 1341-1345.

Banks, W.A., Kastin, A.J., Huang, W., Jaspan, J.B. & Maness, L.M. (1996) Leptin enters the brain by a saturable system independent of insulin. *Peptides*, **17**, 305-311.

Barsh, G.S., Farooqi, I.S. & O'Rahilly, S. (2000) Genetics of body-weight regulation. *Nature*, **404**, 644-651.

Belfiore, F., Iannello, S., Camuto, M., Fagone, S. & Cavaleri, A. (2001) Insulin sensitivity of blood glucose versus insulin sensitivity of blood free fatty acids in normal, obese, and obese-diabetic subjects. *Metabolism*, **50**, 573-582.

Bellisle, F. (1999) Food choice, appetite and physical activity. *Public Health Nutrition*, **2**, 357-361.

Bellisle, F. & Dalix, A.M. (2001) Cognitive restraint can be offset by destruction, leading to increased meal intake in women. *American Journal of Clinical Nutrition*, **74**, 197-200.

Blaak, E.E., van Baak, M.A., Kempen, K.P. & Saris, W.H. (1993) Role of alpha- and beta-adrenoceptors in sympathetically mediated thermogenesis. *American Journal of Physiology*, **264**, E11-17.

Blanc, S., Normand, S., Pachiaudi, C., Duvareille, M. & Gharib, C. (2000) Leptin responses to physical inactivity induced by simulated weightlessness. *American Journal of Physiology*, **279**, R891-898.

Blundell, J.E., Goodson, S. & Halford, J.C. (2001) Regulation of appetite: role of leptin in signalling systems for drive and satiety. *International Journal of Obesity and Related Metabolic Disorders*, **25**, S29-34.

Blundell, J.E. & King, N.A. (1996) Overconsumption as a cause of weight gain: behavioural-physiological interactions in the control of food intake (appetite). *Ciba Foundation Symposium*, **201**, 138-154.

Blundell, J.E. & King, N.A. (2000) Exercise, appetite control, and energy balance. *Nutrition*, **16**, 519-522.

Blundell, J.E., Stubbs, R.J., Hughes, D.A., Whybrow, S. & King, N.A. (2003) Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proceedings of the Nutrition Society*, **62**, 651-661.

Boden, G., Chen, X., Mozzoli, M. & Ryan, I. (1996) Effect of fasting on serum leptin in normal human subjects. *Journal of Clinical Endocrinology and Metabolism*, **81**, 3419-3423.

Bonora, E., Kiechl, S., Willeit, J. et al. (2003) Metabolic syndrome: epidemiology and more extensive phenotypic description. Cross-sectional data from the Bruneck Study. *International Journal of Obesity and Related Metabolic Disorders*, **27**, 1283-1289.

Borg, G.A. (1982) Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, **14**, 377-381.

Bramlett, S.B., Zhou, J., Harris, R.B., Hendry, S.L., Witt, T.L. & Zachwieja, J,J. (1999) Does beta(3)-adrenoreceptor blockade attenuate acute exercise-induced reductions in leptin mRNA? *Journal of Applied Physiology*, **87**, 1678-1683.

Bray, G.A. (2000) Afferent signals regulating food intake. *Proceedings of the Nutrition Society*, **59**, 373-384.

Bray GA, Fisler J. & York, D.A. (1990) Neuroendocrine control of the development of obesity: understanding gained from studies of experimental animal models *Frontiers of Neuroendocrinology*, **11**, 128-181.

Brown, D.B. (2000) About Obesity: Incidence, Prevalence and Comorbidity. International Task Force.

Brunetti, L., Michelotto, B., Orlando, G. & Vacca, M. (1999) Leptin inhibits norepinephrine and dopamine release from rat hypothalamic neuronal endings. *European Journal of Pharmacology*, **372**, 237-240.

Buemann, B., Astrup, A., Madsen, J. & Christensen, N.J. (1992) A 24-h energy expenditure study on reduced-obese and nonobese women: effect of beta-blockade. *American Journal of Clinical Nutrition*, **56**, 662-670.

Caixas, A., Bashore, C., Nash, W., Pi-Sunyer, F. & Laferrere, B. (2002) Insulin, unlike food intake, does not suppress ghrelin in human subjects. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1902-1906.

Cammisotto, P.G., Gelinas, Y., Deshaies, Y. & Bukowiecki, L.J. (2003) Regulation of leptin secretion from white adipocytes by free fatty acids. *American Journal of Physiology*, **285**, E521-526.

Campfield, L.A., Smith, F.J., Guisez, J., Devos, R. & Burn, P. (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks *Science* **269**: 546-549.

Caro, J.F., Sinha, M.K., Kolaczynski, J.W., Zhang, P.L. & Considine, R.V. (1996a) Leptin: The tale of an obesity gene. *Diabetes*, **45**, 1455-1462.

Caro, J.F., Kolaczynski, J.W., Nyce, M.R. et al. (1996b) Decreased cerebrospinal fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet*, **348**, 159-161.

Carulli, L., Ferrari, S., Bertolini, M., Tagliafico, E. & Del Rio, G. (1999) Regulation of ob gene expression: evidence for epinephrine-induced suppression in human obesity. *Journal of Clinical Endocrinology and Metabolism*, **84**, 3309-3312.

de Castro, J.M. (2000) Eating behavior: lessons from the real world of humans. *Nutrition*, 16, 800-813.

Christensen, N.J., Trap-Jensen, J., Svendsen, T.L., Rasmussen, S. & Nielsen, P.E. (1978) Effect of labetalol on plasma noradrenaline and adrenaline in hypertensive man. *European Journal of Clinical Pharmacology*, **14**, 227-230.

Clapham, J.C., Smith, S.A., Moore, et al. (1997) Plasma leptin concentrations and OB gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinaemia in lean and obese humans. *International Journal of Obesity and Related Metabolic Disorders*, **21**, 179-183.

Clement, K., Vaisse, C., Lahlou, N. et al. (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**: 398-401.

Clutter, W.E., Bier, D.M., Shah, S.D. & Cryer, P.E. (1980) Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. *Journal of Clinical Investigation*, **66**, 94-101.

Considine, R.V., Cooksey, R.C., Williams, L.B. et al. (2000) Hexosamines regulate leptin production in human subcutaneous adipocytes. *Journal of Clinical Endocrinology and Metabolism*, **85**, 3551-3556.

Considine, R.V., Sinha, M.K., Heiman, M.L. et al. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine*, **334**, 292-295.

Conner, C.S. (1983) Labetalol: an alpha- and beta-blocker. *Drug Intelligence in Clinical Pharmacy*, **17**, 543-544.

Couillard, C., Mauriege, P., Prud'homme, D. et al. (2002) Plasma leptin response to an epinephrine infusion in lean and obese women. *Obesity Research*, **10**, 6-13.

Cowburn, G., Hillsdon, M. & Hankey, C.R. (1997) Obesity management by life-style strategies. *British Medical Bulletin*, **53**, 389-408.

Cryer, P.E. (1993) Adrenaline: a physiological metabolic regulatory hormone in humans? *International Journal of Obesity and Related Metabolic Disorders*, **17**, 43-46.

Dagogo-Jack, S. (2001) Human leptin regulation and promise in pharmacotherapy. *Current Drug Targets*, **2**, 181-195.

Dagogo-Jack, S., Fanelli, C., Paramore, D., Brothers, J. & Landt, M. (1996) Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* **45**: 695-698.

Dallongeville, J.B., Hecquet, P., Lebel, J.L. et al. (1998) Short term response of circulating leptin to feeding and fasting in man: influence of circadian cycle. *International Journal of Obesity and Related Metabolic Disorders*, **22**, 728-733.

Del Rio, G. (2000) Adrenomedullary function and its regulation in obesity. *International Journal of Obesity and Related Metabolic Disorders*, **24**, S89-91.

Dietz, W.H. (2001) The obesity epidemic in young children: reduce television viewing and promote playing. *British Medical Journal*, **322**, 313-314.

Dietz, W.H. Jr & Gortmaker, S.L. (1985) Do we fatten our children at the television set? Obesity and television viewing in children and adolescents. *Pediatrics*, **75**, 807-812.

Dorrian, C.A. (1989) Human proinsulin and insulin: antibody production, assay development and clinical application. University of Glasgow. Ref Type: Thesis/Dissertation.

Doucet, E., Imbeault, P. & St-Pierre, S. (2000) Appetite after weight loss by energy restriction and low-fat diet-exercise follow up. *International Journal of Obesity and Related Metabolic Disorders*, **24**, 906-914.

Duclos, M., Corcuff, J.B., Ruffie, A., Roger, P. & Manier, G. (1999) Rapid leptin decrease in immediate post-exercise recovery. *Clinical Endocrinology*, **50**, 337-342.

Durnin, J.V. & Womersley, J. (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition*, **32**, 77-97.

Durrant, M.L., Royston, J.P. & Wloch, R.T. (1982) Effect of exercise on energy intake and eating patterns in lean and obese humans. *Physiology and Behavior*, **29**, 449-454.

Elias, A.N., Pandian, M.R., Wang, L., Suarez, E., James, N. & Wilson, A.F. (2000) Leptin and IGF-I levels in unconditioned male volunteers after short-term exercise. *Psychoneuroendocrinology*, **25**, 453-461.

Epstein, L.H., Paluch, R.A., Consalvi, A., Riordan, K. & Scholl, T. (2002) Effects of manipulating sedentary behavior on physical activity and food intake. *Journal of Pediatrics*, **140**, 334-339.

Essig, D.A., Alderson, N.L., Ferguson, M.A., Bartoli, W.P. & Durstine, J.L. (2000) Delayed effects of exercise on the plasma leptin concentration. *Metabolism*, **49**, 395-399.

Ewbank, P.P., Darga, L.L. & Lucas, C.P. (1995) Physical activity as a predictor of weight maintenance in previously obese subjects. *Obesity Research*, **3**, 257-263.

Evans, K., Clark, M.L & Frayn, K.N. (2001) Carbohydrate and fat have different effects on plasma leptin concentrations and adipose tissue leptin production. *Clinical Science*, **100**, 493-498.

Farooqi, I.S., Matarese, G., Lord, G.M. et al. (2002) Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *Journal of Clinical Investigation*, **110**, 1093-1103.

Fisher, J.S., Van Pelt, R.E., Zinder, O., Landt, M. & Kohrt, W.M. (2001) Acute exercise effect on postabsorptive serum leptin. *Journal of Applied Physiology*, **91**, 680-686.

Flatt, J.P., Ravussin, E., Acheson, K.J. & Jequier, E. (1985) Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *Journal of Clinical Investigation*, **76**, 1019-1024.

Flier, J.S. (1998) Clinical review 94: What's in a name? In search of leptin's physiologic role. *Journal of Clinical Endocrinology and Metabolism*, **83**, 1407-1413.

Flint, A., Raben, A., Blundell, J.E. & Astrup, A. (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders*, **24**, 38-48.

Fogelholm, M., Kukkonen-Harjula, K., Nenonen, A. & Pasanen, M. (2000) Effects of walking training on weight maintenance after a very-low-energy diet in premenopausal obese women: a randomized controlled trial. *Archives of Internal Medicine*, **160**, 2177-2184.

Forster, H.V., Dempsey, J.A., Thomson, J., Vidruk, E. & DoPico, G.A. (1972) Estimation of arterial PO2, PCO2, pH, and lactate from arterialized venous blood. *Journal of Applied Physiology*, **32**, 134-137.

Friedman, J.M. (2003) A war on obesity, not the obese. *Science*, **299**, 856-858.

Friedman, J.M. & Halaas, J.L. (1998) Leptin and the regulation of body weight in mammals. *Nature*, **395**, 765-770.

Fritsche A, Wahl HG, Metzinger E, Renn W, Kellerer M, Haring H, Stumvoll M (1998) Evidence for inhibition of leptin secretion by catecholamines in man. *Experimental and Clinical Endocrinology and Diabetes*, **106**, 415-418.

Fruehwald-Schultes, B., Oltmanns, K.M., Kern, W., Born, J., Fehm, H.L. & Peters, A. (2002) The effect of experimentally induced insulin resistance on the leptin response to hyperinsulinaemia. *International Journal of Obesity and Related Metabolic Disorders*, **26**, 510-516.

Fung, T.T., Hu, F.B., Yu, J. et al. (2000) Leisure-time physical activity, television watching, and plasma biomarkers of obesity and cardiovascular disease risk. *American Journal of Epidemiology*, **152**, 1171-1178.

George, V.A. & Morganstein, A. (2003) Effect of moderate intensity exercise on acute energy intake in normal and overweight females. *Appetite*, **40**, 43-46.

Gill, J.M., Caslake, M.J., McAllister, C. et al. (2003) Effects of short-term detraining on postprandial metabolism, endothelial function, and inflammation in endurance-trained men: dissociation between changes in triglyceride metabolism and endothelial function. *Journal of Clinical Endocrinology and Metabolism*, **88**, 4328-4335.

Gortmaker, S.L., Must, A., Sobol, A.M., Peterson, K., Colditz, G.A. & Dietz, W.H. (1996) Television viewing as a cause of increasing obesity among children in the United States, 1986-1990. *Archives of Pediatrics and Adolescent Medicine*, **150**, 356-362.

Guldstrand, M., Ahren, B. & Adamson, U. (2003) Improved beta-cell function after standardized weight reduction in severely obese subjects. *American Journal of Physiology-Endocrinology and Metabolism*, **28**, E557-65.

Gustafson, A.B., Farrell, P.A. & Kalkhoff, R.K. (1990) Impaired plasma catecholamine response to submaximal treadmill exercise in obese women. *Metabolism*, **39**, 410-417.

Gutin, B., Ramsey, L., Barbeau, P. et al. (1999) Plasma leptin concentrations in obese children: changes during 4-mo periods with and without physical training. *American Journal of Clinical Nutrition*, **69**, 388-394.

Haffner, S.M., Miettinen, H., Mykkanen, L., Karhapaa, P., Rainwater, D.L. & Laakso, M. (1997) Leptin concentrations and insulin sensitivity in normoglycemic men. *International Journal of Obesity and Related Metabolic Disorders*, **21**, 393-399.

Hakansson, M.L., Brown, H., Ghilardi, N., Skoda, R.C. & Meister, B. (1998) Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. *Journal of Neuroscience*, **18**, 559-572.

Halaas, J., Gajiwala, K., Maffei, M. et al. (1995) Weight reducing effects of the plasma protein encoded by the obese gene. *Science* **269**: 543-546.

Halford, J.C. & Blundell, J.E. (2000) Separate systems for serotonin and leptin in appetite control. *Annals of Medicine*, **32**, 222-232.

Han, T.S., McNeill, G., Seidell, J.C. & Lean, M.E. (1997) Predicting intra-abdominal fatness from anthropometric measures: the influence of stature. *International Journal of Obesity and Related Metabolic Disorders*, **21**, 587-593.

van Harmelen, V., Dicker, A., Ryden, M. et al. (2002) Increased lipolysis and decreased leptin production by human omental as compared with subcutaneous preadipocytes. *Diabetes*, **51**, 2029-2036.

Hartling, O.J., Svendsen, T.L. & Trap-Jensen, J. (1980) Haemodynamic and metabolic effects of combined adrenergic alpha- and beta-receptor blockade with labetalol in the exercising human forearm. *European Journal of Clinical Investigation*, **10**, 431-435.

Heini, A.F., Lara-Castro, C., Kirk, K.A., Considine, R.V., Caro, J.F. & Weinsier, R.L. (1998) Association of leptin and hunger-satiety ratings in obese women. *International Journal of Obesity and Related Metabolic Disorders*, **22**: 1084-1087.

Heitman, B.L. (1990). Evaluation of body fat estimated from body mass index, skinfolds and impedance. A comparative study. *European Journal of Clinical Nutrition*, **44**, 831-837.

Heymsfield, S.B., Greenberg, A.S., Fujioka, K. et al. (1999) Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *Journal of American Medical Association*, **282**, 1568-1575.

Hickey, M.S., Houmard, J.A., Considine, R.V. et al. (1997) Gender-dependent effects of exercise training on serum leptin levels in humans. *American Journal of Physiology*, **272**, E562-566.

Hill, J.O. & Melanson, E.L. (1999) Overview of the determinants of overweight and obesity: current evidence and research issues. *Medicine and Science in Sports and Exercise*, **31**, S515-521.

Hill, A.J. & Rogers, P. (1998) Clinical Obesity. In: Kopelman, P.G. & Stock, M., editors. Food intake and eating behaviour in humans, pages 86-111. London: Blackwell Science Ltd.

Hill, J.O., Wyatt, H.R., Reed, G.W. & Peters, J.C. (2003) Obesity and the environment: where do we go from here? *Science*, **299**, 853-855.

Himaya, A., Fantino, M., Antoine, J.M., Brondel, L. & Louis-Sylvestre, J. (1997) Satiety power of dietary fat: a new appraisal. *American Joural of Clinical Nutrition*, **65**, 1410-1418.

Hoffstedt, J., Arner, P., Hellers, G. & Lonnqvist, F. (1997) Variation in adrenergic regulation of lipolysis between omental and subcutaneous adipocytes from obese and non-obese men. *Journal of Lipid Research*, **38**, 795-804.

Hoffstedt, J., Wahrenberg, H., Thorne, A. & Lonnqvist, F. (1996) The metabolic syndrome is related to beta 3-adrenoceptor sensitivity in visceral adipose tissue. *Diabetologia*, **39**, 838-844.

Holland, B., Welch, A.A., Unwin, I.D., Buss, D.H., Paul, A.A. & Southgate, D.A.T. (1991) McCance and Widdowson's The composition of foods. 5th ed. Cambridge: Goodfellow & Egan Phototypesetting Ltd.

Hubert, P., King, N.A. & Blundell, J.E. (1998) Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity. *Appetite*, **31**, 9-19.

Hukshorn, C.J., Saris, W.H., Westerterp-Plantenga, M.S., Farid, A.R., Smith, F.J., Campfield, L.A. (2000) Weekly subcutaneous pegylated recombinant native human leptin (PEG-OB) administration in obese men. *Journal of Clinical Endocrinology & Metabolism*, **85**, 4003-4009.

Hulver, M.W. & Houmard, J.A. (2003) Plasma leptin and exercise: recent findings. *Sports Medicine*, **33**, 473-482.

Jeanrenaud, B. & Rohner-Jeanrenaud, F. (2001) International Textbook of Obesity. In Per Björntorp, Ltd, editor. Part III Appetite regulation and obesity prevention: Role of Neuropeptide and leptin in food intake and obesity, pages 101-112. West Sussex: John Willey & Sons, UK.

Jebb, S.A. & Moore, M.S. (1999) Contribution of a sedentary lifestyle and inactivity to the etiology of overweight and obesity: current evidence and research issues. *Medicine and Science in Sports and Exercise*, **31**, S534-541.

Jequier, E. (1994) Carbohydrates as a source of energy. *American Journal of Clinical Nutrition*, **59**, 682S-685S.

Jequier, E. (2002) Leptin signaling, adiposity, and energy balance. *Annals New York Academy of Sciences*, **967**, 379-388.

Joannic, J.L., Oppert, J.M., Lahlou, N. et al. (1998) Plasma leptin and hunger ratings in healthy humans. *Appetite*, **30**, 129-138.

Karhunen, L., Haffner, S., Turpeinen, A., Miettinen, H. & Uusitupa, M. (1997) Serum leptin and short-term regulation of eating in obese women. *Clinical Science*, **92**, 573-578.

Karonen, S.L., Koistinen, H.A., Nikkinen, P. & Koivisto, V.A. (1998) Is brain uptake of leptin in vivo saturable and reduced by fasting. *European Journal of Nuclear Medicine*, **25**, 607-612.

Keim, N.L., Stern, J.S. & Havel, P.J. (1998) Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *American Journal of Clinical Nutrition*, **68**, 794-801.

Kraemer, R.R., Johnson, L.G., Haltom, R. et al. (1999) Serum leptin concentrations in response to acute exercise in postmenopausal women with and without hormone replacement therapy. *Proceedings of the Society for Experimental Biology and Medicine*, **221**, 171-177.

King, N.A. & Blundell, J.E. (1995) High-fat foods overcome the energy expenditure induced by high-intensity cycling or running. *European Journal of Clinical Nutrition*, **49**, 114-123.

King, N.A., Burley, V.J. & Blundell, J.E. (1994) Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition*, **48**, 715-724.

King, N.A., Snell, L., Smith, R.D. & Blundell, J.E. (1996) Effects of short-term exercise on appetite responses in unrestrained females. *European Journal of Clinical Nutrition*, **50**, 663-667.

Kissileff, H.R., Pi-Sunyer, F.X., Segal, K., Meltzer, S. & Foelsch, P.A. (1990) Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition*, **52**, 240-245.

Kohrt, W.M., Landt, M. & Birge, S.J. Jr (1996) Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. *Journal of Clinical Endocrinology Metabolism*, **81**, 3980-3985.

Koistinen, H.A., Karonen, S.L., Iivanainen, M. & Koivisto, V.A. (1998) Circulating leptin has saturable transport into intrathecal space in humans. *European Journal of Clinical Investigation*, **28**, 894-897.

Koistinen, H.A., Tuominen, J.A., Ebeling, P., Heiman, M.L., Stephens, T.W. & Koivisto, V.A. (1998) The effect of exercise on leptin concentration in healthy men and in type 1 diabetic patients. *Medicine and Science in Sports and Exercise*, **30**, 805-810.

Kolaczynski, J.W., Ohannesian, J.P., Considine, R.V., Marco, C.C. & Caro, J.F. (1996) Response of leptin to short-term and prolonged overfeeding in humans. *Journal of Clinical Endocrinolology and Metabolism*, **81**, 4162-4165.

Korbonits, M., Trainer, P.J., Little, J.A. et al. (1997) Leptin levels do not change acutely with food administration in normal or obese subjects, but are negatively correlated with pituitary-adrenal activity. *Clinical Endocrinology*, **46**, 751-7.

Kolaczynski, J.W., Ohannesian, J.P., Considine, R.V., Marco, C.C. & Caro, J.F. (1996) Response of leptin to short-term and prolonged overfeeding in humans. *Journal of Clinical Endocrinolology and Metabolism*, **81**, 4162-4165.

Kushner, R.F. & Schoeller, D.A. (1986) Estimation of total body water by bioelectrical impedance analysis. *American Journal of Clinical Nutrition*, **44**, 417-424.

La-Forgia, J., Withers, R.T., Williams, A.D. et al. (1999) Effect of 3 weeks of detraining on the resting metabolic rate and body composition of trained males. *European Journal of Clinical Nutrition*, **53**, 126-133.

Landt, M., Lawson, G.M. & Helgeson, J.M. (1997) Prolonged exercise decreases serum leptin concentrations. *Metabolism*, **46**, 1109-1112.

Larsson, H., Elmstahl, S. & Ahren, B. (1996) Plasma leptin levels correlate to islet function independently of body fat in postmenopausal women. *Diabetes*, **45**, 1580-1584.

Leal-Cerro, A., Garcia-Luna, P.P., Astorga, R. et al. (1998) Serum leptin levels in marathon athletes before and after the marathon run. *Journal of Clinical Endocrinology and Metabolism*, **83**, 2376-2379.

Lean, M.E.J. (2000) Pathophysiology of obesity. *Proceedings of the Nutrition Society*, **59**, 331-336.

Lean, M.E.J. (2001) How does sibutramine work? *International Journal of Obesity and Related Metabolic Disorders*, **25**, S8-11.

Lean, M.E., Han, T.S. & Deurenberg, P. (1996) Predicting body composition by densitometry from simple anthropometric measurements. *American Journal of Clinical Nutrition*, **63**, 4-14.

Lee, D.W., Leinung, M.C., Rozhavskaya-Arena, M. & Grasso, P. (2002) Leptin and the treatment of obesity: its current status. *European Journal of Pharmacology*, **440**, 129-139.

Leibowitz, S.F. & Brown, L.L. (1980) Histochemical and pharmacological analysis of noradrenergic projections to the paraventricular hypothalamus in relation to feeding stimulation. *Brain Research*, **201**, 289-314.

Levin, B.E., Dunn-Meynell, A.A. & Banks, W.A. (2004) Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling prior to obesity onset. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, **286**, R143-150.

Lluch, A., Hubert, P., King, N.A. & Blundell, J.E. (2000) Selective effects of acute exercise and breakfast interventions on mood and motivation to eat. *Physiology and Behavior*, **68**, 515-520.

Long, S.J., Hart, K. & Morgan, L.M. (2002) The ability of habitual exercise to influence appetite and food intake in response to high- and low-energy preloads in man. *British Journal of Nutrition*, **87**, 517-523.

Lonnqvist, F., Thome, A., Nilsell, K., Hoffstedt, J. & Arner, P. (1995) A pathogenic role of visceral fat beta 3-adrenoceptors in obesity. *Journal of Clinical Investigation*, **95**, 1109-1116.

Ma, Z., Gingerich, R.L., Santiago, J.V., Klein, S., Smith, C.H. & Land, M. (1996) Radioimmunoassay of leptin in human plasma. *Clinical Chemistry* **42**: 942-946.

Malkova, D., Evans, R.D., Frayn, K.N., Humphreys, S.M., Jones, P.R. & Hardman A.E. (2000) Prior exercise and postprandial substrate extraction across the human leg. *American Journal of Physiology-Endocrinology and Metabolism*, **279**, E1020-1028.

Malmstrom, R., Taskinen, M.R., Karonen, S.L. & Yki-Jarvinen, H. (1996) Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. *Diabetologia*, **39**, 993-996.

Mantzoros, C.S. & Flier, J.S. (2000) Editorial: leptin as a therapeutic agent-trials and tribulations. *Journal of Clinical Endocrinology and Metabolism*, **85**, 4000-4002.

Marmonier, C., Chapelot, D. & Louis-Sylvestre, J. (2000) Effects of macronutrient content and energy density of snacks consumed in a satiety state on the onset of the next meal. *Appetite*, **34**, 161-168.

Martinez-Gonzalez, M.A., Martinez, J.A., Hu, F.B., Gibney, M.J. & Kearney, J. (1999) Physical inactivity, sedentary lifestyle and obesity in the European Union. *International Journal of Obesity and Related Metabolic Disorders*, **23**, 1192-1201.

Maughan, R.J., Robertson, J.D. & Bruce, A.C. (1989) Dietary energy and carbohydrate intake of runners in relation to training load. *Proceedings of the Nutrition Society*, **48**, 170A.

Maughan, R.J. (1982) A simple, rapid method for determination of glucose, lactate, pyruvate, alanine, 3-hydroxybutyrate and acetoacetate in a single 2 µl blood sample. *Clinica Chemica Acta*, **122**, 231-240.

Mauriege, P., Despres, J.P., Prud'homme, D. et al. (1991) Regional variation in adipose tissue lipolysis in lean and obese men. *Journal of Lipid Research*, **32**, 1625-1633.

Mayer, J. (1953) Decreased activity and energy balance in the hereditary obesity-diabetes syndrome of mice. *Science*, **117**, 504-505.

Mayer, J. (1955) Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. *Annals New York Academy of Sciences*, **63**, 15-43.

Mayer, J., Marshall, N.B., Vitale, J.J., Christensen, J.H., Mashayekhi, M.B. & Stare F.J. (1954) Exercise, food intake and body weight in normal rats and genetically obese adult mice. *American Journal of Physiology*, **177**, 544-548.

Mayer, J. & Thomas, D.W. (1967) Regulation of food intake and obesity. *Science*, **156**, 328-337.

Mayer, J., Roy, P. & Mitra, K.P. (1956) Relation between calorie intake, body weight and physical work: studies in an industrial male population in West Bengal. *American Journal of Clinical Nutrition*, **4**, 169-175.

McClain, D.A. & Crook, E.D. (1996) Hexosamines and insulin resistance. *Diabetes*, **45**, 1003-1009.

McConway, M.G., Johnson, D., Kelly, A., Griffin, D., Smith, J. & Wallace, A.M. (2000) Differences in circulating concentrations of total, free and bound leptin relate to gender and body composition in adult humans. *Annals of Clinical Biochemistry*, **37**, 717-723.

McCrory, M.A., Gomez, T.D., Bernauer, E.M. & Mole, P.A. (1995) Evaluation of a new air displacement plethysmograph for measuring human body composition. *Medicine and Science in Sports and Exercise*, **27**, 1686-1691.

McLoughlin, P., Popham, P., Linton, R.A., Bruce, R.C. & Band, D.M. (1992) Use of arterialized venous blood sampling during incremental exercise tests. *Journal of Applied Physiology*, **73**, 937-940.

Mikines, K.J., Sonne, B., Tronier, B. & Galbo, H. (1989) Effects of training and detraining on dose-response relationship between glucose and insulin secretion. *American Journal of Physiology*, **256**, E588-596.

Moller, N., O'Brien, P. & Nair, K.S. (1998) Disruption of the relationship between fat content and leptin levels with aging in humans. *Journal of Clinical Endocrinology and Metabolism*, **83**, 931-934.

Montague, C.T., Farooqi, I.S., Whitehead, J.P. et al. (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**: 903-908.

Mujika, I. & Padilla, S. (2000) Detraining: loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus. *Sports Medicine*, **30**, 79-87.

Murgatroyd, P.R., Goldberg, G.R., Leahy, F.E., Gilsenan, M.B. & Prentice AM. (1999) Effects of inactivity and diet composition on human energy balance. *International Journal of Obesity and Related Metabolic Disorders*, **23**, 1269-1275.

Nagaya, N., Uematsu, M., Kojima, M. et al. (2001) Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation*, **104**, 2034-2038.

National Audit Office (2001). Tackling Obesity in England. Report by the Comptroller and Auditor General HC 220 Session 2000-2001: 15 February 2001.

Nindl, B.C., Kraemer, W.J., Arciero, P.J. et al. (2002) Leptin concentrations experience a delayed reduction after resistance exercise in males. *Medicine and Science in Sports and Exercise*, **34**, 608–613.

Okazaki, T., Himeno, E., Nanri, H., Ogata, H. & Ikeda, M. (1999) Effects of mild aerobic exercise and a mild hypocaloric diet on plasma leptin in sedentary women. *Clinical and Experimental Pharmacology and Physiology*, **26**, 415-420.

Oliver, J.L. & Miller, G.D. (2001) Differential effects of maximal- and moderate-intensity runs on plasma leptin in healthy trained subjects. *Nutrition*, **17**, 365–369.

O'Rahilly, S., Farooqi, I.S., Yeo, G.S. & Challis, B.G. (2003) Minireview: human obesity-lessons from monogenic disorders. *Endocrinology*, **144**, 3757-3764.

Orban, Z., Remaley, A.T., Sampson, M., Trajanoski, Z. & Chrousos, G.P. (1999) The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. *Journal of Clinical Endocrinology and Metabolism*, **84**, 2126-2133.

Pasman, W.J., Westerterp-Plantenga, M.S. & Saris, W.H. (1998) The effect of exercise training on leptin levels in obese males. *American Journal of Physiology*, **274**, E280-286.

Pelleymounter, M.A., Cullen, M.J. & Baker, M.B. (1995) Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* **269**: 540-543.

Perusse, L., Collier, G. & Gagnon, J. (1997) Acute and chronic effects of exercise on leptin levels in humans. *Journal of Applied Physiology*, **83**, 5-10.

Pico, C., Oliver, P., Sanchez, J. & Palou, A. (2003) Gastric leptin: a putative role in the short-term regulation of food intake. *British Journal of Nutrition*, **90**, 735-741.

Pijl, H., de Meijer, P.H., Langius, J. et al. (2001) Food choice in hyperthyroidism: potential influence of the autonomic nervous system and brain serotonin precursor availability. *Journal of Clinical Endocrinology and Metabolism*, **86**, 5848-5853.

Pollock, M.L. (1988) Prescribing exercise for fitness and adherence. In: Dishman RK, editors. Exercise Adherence, pages 259-277. Champain, Ill: Human Kinetics Publishers.

Porrini, M., Santangelo, A., Crovetti, R., Riso, P., Testolin, G. & Blundell, J.E. (1997) Weight, protein, fat, and timing of preloads affect food intake. *Physiology and Behavior*, **62**, 563-570.

Prentice, A.M. (1998) Manipulation of dietary fat and energy density and subsequent effects on substrate flux and food intake. *American Journal of Clinical Nutrition*, **67**, 535S-541S.

Prentice, A.M. & Jebb, S,A. (1995) Obesity in Britain: gluttony or sloth? *British Medical Journal*, **311**, 437-439.

Putnam, J., Allshouse, J. & Kantor L.S. (2002) U.S. per capita food supply trends: More calories, refined carbohydrates and fats. *Food Review*, **25**, 2-15.

Raben, A. & Astrup, A. (2000) Leptin is influenced both by predisposition to obesity and diet composition. *International Journal of Obesity and Related Metabolic Disorders*, **24**, 450-459.

Raben, A., Kiens, B. & Richter, E.A. (1994) Differences in glycaemia, hormonal response and energy expenditure after a meal rich in mono- and disaccharides compared to a meal rich in polysaccharides in physically fit and sedentary subjects. *Clinical Physiology*, **14**, 267-280.

Raben A, Macdonald I, Astrup A. (1997) Replacement of dietary fat by sucrose or starch: effects on 14 d ad libitum energy intake, energy expenditure and body weight in formerly obese and never-obese subjects. *International Journal of Obesity and Related Metabolic Disorders*, **21**, 846-859.

Racette, S.B., Coppack, S., Landt, M. & Klein, S. (1997) Leptin production during moderate-intensity aerobic exercise. *Journal of Clinical Endocrinology and Metabolism*, **82**, 2275-2277.

Rayner, D.V. (2001) The sympathetic nervous system in white adipose tissue regulation. *Proceedings of the Nutrition Society*, **60**, 357-364.

Rentsch, J. & Chiesi, M. (1996) Regulation of ob gene mRNA levels in cultured adipocytes. *FEBS Letters*, **379**, 55-59.

Reseland, J.E., Anderssen, S.A, Solvoll, K. et al. (2001) Effect of long-term changes in diet and exercise on plasma leptin concentrations. *American Journal of Clinical Nutrition*, **73**, 240-245.

Reynisdottir, S., Ellerfeldt, K., Wahrenberg, H., Lithell, H. & Arner, P. (1994) Multiple lipolysis defects in the insulin resistance (metabolic) syndrome. *Journal of Clinical Investigation*, **93**, 2590-2599.

Ricci, M.R. & Fried, S.K. (1999) Isoproterenol decreases leptin expression in adipose tissue of obese humans. *Obesity Research*, 7, 233-240.

Richards, D.A. & Prichard, B.N (1979) Clinical pharmacology of labetalol. *British Journal of Clinical Pharmacology*, **8**, 89S-93S.

Richards, D.A., Woodings, E.P., Stephens, M.D.M. & Maconochie, J.G. (1974) The effects of oral AH 5158 a combined α- and β- adrenoceptor antagonist in healthy volunteers. *British Journal of Clinical Pharmacology*, **1**, 505-510.

Richards, D.A., Woodings, E.P. & Maconochie, J.G. (1977) Comparison of the effects of labetalol and propranolol in healthy men at rest and during exercise. *British Journal of Clinical Pharmacology*, **4**, 15-21.

Rissanen, A.M., Heliovaara, M., Knekt, P., Reunanen, A. & Aromaa, A. (1991) Determinants of weight gain and overweight in adult Finns. *European Journal of Clinical Nutrition*, **45**, 419-430.

Ritter, R.C. & Epstein, A.N. (1975) Control of meal size by central noradrenergic action. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 3740-3743.

Rolls, B.J. (2000) The role of energy density in the overconsumption of fat. *Journal of Nutrition*, **130**, 268S-271S.

Rolls B.J., Kim, S., McNelis A.L., Fischman, M.W., Foltin, R.W. & Moran, T.H. (1991) Time course of effects of preloads high in fat or carbohydrate on food intake and hunger ratings in humans. *American Journal of Physiology*, **260**, R756-763.

Romon, M., Lebel, P., Velly, C., Marecaux, N., Fruchart, J.C. & Dallongeville, J. (1999) Leptin response to carbohydrate or fat meal and association with subsequent satiety and energy intake. *American Journal of Physiology*, **277**, E855-861.

Ross, R. & Janssen, I. (2001) Physical activity, total and regional obesity: dose-response considerations. *Medicine and Science in Sports and Exercise*, **33**, S521-529.

Saad, M.F., Khan, A., Sharma, A. et al. (1998) Physiological insulinemia acutely modulates plasma leptin. *Diabetes*, **47**, 544-549.

Saladin, R., De Vos, P., Guerre-Millo, M., et al. (1995) Transient increase in obese gene expression after food intake or insulin administration. *Nature*, **377**, 527-529.

Salvadori, A., Fanari, P., Giacomotti, E. et al. (2003) Kinetics of catecholamines and potassium, and heart rate during exercise testing in obese subjects. Heart rate regulation in obese during exercise. *European Journal of Nutrition*, **42**, 181-187.

Sandoval, D.A. & Davis, S.N. (2003) Leptin. Metabolic control and regulation. *Journal of Diabetes and its Complications*, **17**, 108-113.

Scarpace, P.J., Matheny, M., Zhang, Y. et al. (2002) Central leptin gene delivery evokes persistent leptin signal transduction in young and aged-obese rats but physiological responses become attenuated over time in aged-obese rats. *Neuropharmacology*, **42**, 548-561.

Schwartz, M.W., Marks, J.L., Sipols, A.J. et al. (1991) Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. *Endocrinology*, **128**, 2645-2647.

Schwartz, M.W., Peskind, E., Raskind, M., Boyko, E.J. & Porte, D. Jr. (1996) Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Medicine*, **2**, 589-593.

Schwartz, R.S., Shuman, W.P., Bradbury, V.L. et al. (1990) Body fat distribution in healthy young and older men. *Journal of Gerontology*, **45**, 181-185.

Scriba, D., Aprath-Husmann, I., Blum, W.F. & Hauner, H. (2000) Catecholamines suppress leptin release from in vitro differentiated subcutaneous human adipocytes in primary culture via beta1- and beta2-adrenergic receptors. *European Journal of Endocrinology*, **143**, 439-445.

Segal, K.R., Landt, M. & Klein, S. (1996) Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. *Diabetes*, **45**, 988-991.

Shiiya, T., Nakazato, M., Mizuta, M. et al. (2002) Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *Journal of Clinical Endocrinology and Metabolism*, 7, 240-244.

Shin, M.S., Kim, H., Chang, H.K. et al. (2003) Treadmill exercise suppresses diabetes-induced increment of neuropeptide Y expression in the hypothalamus of rats. *Neuroscience Letters*, **346**, 157-160.

Shoemaker, J.K., Green, H.J., Ball-Burnett, M. & Grant, S. (1998) Relationships between fluid and electrolyte hormones and plasma volume during exercise with training and detraining. *Medicine and Science in Sports and Exercise*, **30**, 497-505.

Steinberg, G.R., Smith, A.C., Wormald, S., Malenfant, P., Collier, C. & Dyck, D.J. (2004) Endurance training partially reverses dietary-induced leptin resistance in rodent skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, **286**, E57-63.

Strobel, A., Issad, T., Camoin, L., Ozata, M. & Strosberg, A.D. (1998) A leptin missense mutation associated with hypogonadism and morbid obesity *Nature Genetics* **18**: 213-215.

Stubbs, R.J., Hughes, D.A., Johnstone, A.M. et al. (2000) The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *British Journal of Nutrition*, **84**, 405-415.

Stubbs, R.J., Sepp, A., Hughes, D.A. et al. (2002a) The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. *European Journal of Clinical Nutrition*, **56**, 129-40.

Stubbs, R.J., Sepp, A., Hughes, D.A. et al. (2002b) The effect of graded levels of exercise on energy intake and balance in free-living women. *International Journal of Obesity and Related Metabolic Disorders*, **26**, 866-869.

Swinburn, B. & Egger, G. (2002) Preventive strategies against weight gain and obesity. *Obesity Reviews*, **3**, 289-301.

Thong, F.S., McLean, C. & Graham, T.E. (2000) Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. *Journal of Applied Physiology*, **88**, 2037-2044.

Thompson, D.A., Wolfe, L.A. & Eikelboom, R. (1988) Acute effects of exercise intensity on appetite in young men. *Medicine and Science in Sports and Exercise*, **20**, 222-227.

Trayhurn, P., Duncan, J.S., Hoggard, N. & Rayner, D.V. (1998) Regulation of leptin production: a dominant role for the sympathetic nervous system? *Proceedings of the Nutrition Society*, **57**, 413-419.

Trayhurn, P., Duncan, J.S. & Rayner, D.V. (1995) Acute cold-induced suppression of ob (obese) gene expression in white adipose tissue of mice: mediation by the sympathetic system. *Biochemical Journal*, **311**, 729-733.

Tremblay, A., Despres, J.P. & Bouchard, C. (1984) Adipose tissue characteristics of ex-obese long-distance runners. *International Journal of Obesity*, **8**, 641-648.

Tremblay, A., Doucet, E. & Imbeault, P. (1999) Physical activity and weight maintenance. *International Journal of Obesity and Related Metabolic Disorders*, **23**, S50-54.

Tremblay, A., Despres, J.P. & Bouchard, C. (1985) The effects of exercise-training on energy balance and adipose tissue morphology and metabolism. *Sports Medicine*, **2**, 223-233.

Tuominen, J.A., Ebeling, P., Stenman, U.H., Heiman, M.L., Stephens, T.W. & Koivisto, V.A. (1997) Leptin synthesis is resistant to acute effects of insulin in insulin-dependent diabetes mellitus patients. *Journal of Clinical Endocrinology and Metabolism*, **82**, 381-382.

U.S. Department of Agriculture, Agricultural Research Service. Pyramid servings Data: Results From USDA's 1994-1996.Continuing Survey of Food Intakes by Individuals, ARS Food Surveys Research Group, February 1999. http://www.barc.usda.gov/bhnrc/foodsurvey/pdf/3yr-py.pdf.

Vukovich, M.D., Arciero, P.J., Kohrt, W.M., Racette, S.B., Hansen, P.A. & Holloszy, J.O. (1996) Changes in insulin action and GLUT-4 with 6 days of inactivity in endurance runners. *Journal of Applied Physiology*, **80**, 240-244.

Wallace, A.M., McMahon, A.D., Packard, C.J. et al. (2001) Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation*, **104**, 3052-3056.

Wallin, J.D. & O'Neill, W.M. Jr. (1983) Labetalol. Current research and therapeutic status. *Archives of Internal Medicine*, **143**, 485-490.

Walsh, K.M., Adams, C., Sinclair, A., Leen, E. & Lean, M.E. (1998) Influences on adrenaline-induced thermogenesis in obese women and relationship to cardiovascular responses. *Clinical Science*, **94**, 121-127.

Wang, J., Yuen, V.G. & McNeill, J.H. (2001) Effect of vanadium on insulin sensitivity and appetite. *Metabolism*, **50**, 667-673.

Webber, J. & Macdonald, I.A. (1993) A comparison of the cardiovascular and metabolic effects of incremental versus continuous dose adrenaline infusions in men and women. *International Journal of Obesity and Related Metabolic Disorders*, **17**, 37-43.

Webber, J., Taylor, J., Greathead, H., Dawson, J., Buttery, P.J. & Macdonald, I.A. (1994) A comparison of the thermogenic, metabolic and haemodynamic responses to infused adrenaline in lean and obese subjects. *International Journal of Obesity and Related Metabolic Disorders*, **18**, 17-24.

Weigle, D.S., Duell, P.B., Connor, W.E., Steiner, R.A., Soules, M.R. & Kuijper, J.L. (1996) Effect of fasting, refeeding, and dietary fat restriction in plasma leptin levels. *Journal of Clinical Endocrinology and Metabolism*, **82**, 561-565.

Wellhoener, P., Fruehwald-Schultes, B., Kern, W. et al. (2000) Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. *Journal of Clinical Endocrinology and Metabolism*, **85**, 1267-1271.

Wellman, P.J. (2000) Norepinephrine and the control of food intake. *Nutrition*, **16**, 837-842.

Wellman, P.J., Davies, B.T., Morien, A. & McMahon, L. (1993) Modulation of feeding by hypothalamic paraventricular nucleus alpha 1- and alpha 2-adrenergic receptors. *Life Sciences*, **53**, 669-679.

Weltman, A., Pritzlaff, C.J., Wideman, L. et al. (2000) Intensity of acute exercise does not affect serum leptin concentrations in young men. *Medicine and Science in Sports and Exercise*, **32**, 1556-1561.

Westerterp, K.R. (1998) Alterations in energy balance with exercise. *American Journal of Clinical Nutrition*, **68**, 970S-974S.

Westerterp, K.R., Meijer, G.A., Janssen, E.M., Saris, W.H. & Ten Hoor, F. (1992) Long-term effect of physical activity on energy balance and body composition. *British Journal of Nutrition*, **68**, 21-30.

Westerterp-Plantenga, M.S., Saris, W.H., Hukshorn, C.J. & Campfield, L.A. (2001) Effects of weekly administration of pegylated recombinant human OB protein on appetite profile and energy metabolism in obese men. *American Journal of Clinical Nutrition*, **74**, 426-434.

Wing, R.R. & Hill, J.O. (2001) Successful weight loss maintenance. *Annual Review of Nutrition*, **21**, 323-341.

Woo, R. & Pi-Sunyer, F.X. (1985) Effect of increased physical activity on voluntary intake in lean women. *Metabolism*, **34**, 836-841.

Woo, R., Garrow, J.S. & Pi-Sunyer, F.X. (1982a) Effect of exercise on spontaneous calorie intake in obesity. *American Journal of Clinical Nutrition*, **36**, 470-477.

Woo, R., Garrow, J.S. & Pi-Sunyer, F.X. (1982b) Voluntary food intake during prolonged exercise in obese women. *American Journal of Clinical Nutrition*, **36**, 478-484.

World Health Organisation (1995) Expert Committee on Physical Status: The use and interpretation of anthropometry: report of a WHO expert committee. Geneva: WHO.

World Health Organisation (1997) International Statistical Classification of Disease and Related Health Problems, 10th ed. Geneva: WHO.

World Health Organization (1998). Obesity: Preventing and Managing the Global Epidemic. Report of WHO Consultation on Obesity. Geneva: WHO.

Williamson, D.F. (1996) Dietary intake and physical activity as "predictors" of weight gain in observational, prospective studies of adults. *Nutrition Reviews*, **54**, S101-109.

Zaccaria, M., Ermolao, A., Roi, G.S., Englaro, P., Tegon, G., Varnier, M. (2002) Leptin reduction after endurance races differing in duration and energy expenditure. *European Journal of Applied Physiology*, **87**, 108-111.

Zafeiridis, A., Smilios, I., Considine, R.V. & Tokmakidis, S.P. (2003) Serum leptin responses after acute resistance exercise protocols. *Journal of Applied Physiology*, **94**, 591-597.

Zhang, Y.Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. & Friedman, J.M. (1994) Positional cloning of the obese gene and its human homolog. *Nature* **372**: 425-432.

Zimmet, P.Z., Collins, V.R., de Courten, M.P. et al. (1998). Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean nation of Mauritius. Mauritius NCD Study Group. *International Journal of Obesity and Related Metabolic Disorders*, **22**, 171-177.

Zlokovic, B.V., Jovanovic, S., Miao, W., Samara, S., Verma, S. & Farrell, C.L. (2000) Differential regulation of leptin transport by the choroid plexus and blood-brain barrier and high affinity transport systems for entry into hypothalamus and across the blood-cerebrospinal fluid barrier. *Endocrinology*, **141**, 1434-1441.

Publications achieved from this PhD thesis

Peer reviewed original refereed papers published

Tsofliou F, Pitsiladis YP, Malkova D, Wallace AM, Lean ME (2003). Moderate physical activity permits acute coupling between serum leptin and appetite-satiety measures in obese women. *International Journal of Obesity* 27: 1332-9.

Peer reviewed original refereed papers to be submitted

Tsofliou 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.

Tsofliou F, Pitsiladis YP, Fuld J, Wallace AM & Lean MEJ. The effects of moderate exercise plus α/β adrenoceptor blockade on serum leptin, appetite/satiety measures and subsequent food intake in obese women.

Tsofliou F, Gill JMR, McAllister C, Wallace AM, Lean MEJ & Malkova D. Effects of 7 days detraining on serum leptin and hunger/satiety measures in endurance-trained men.

Tsofliou 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

Tsofliou F, Pitsiladis YP, Malkova D, Wallace AM & Lean MEJ (2001) Low-intensity exercise permits coupling between serum leptin and hunger/satiety measures in middle aged obese women. *Proceedings of the Nutrition Society* 60: 204 A.

Tsofliou F, Malkova D, McAllister C, Pitsiladis YP, Wallace AM, Lean MEJ & Gill JMR (2002). Effects of 7 days detraining on serum leptin and hunger/satiety measures in endurance-trained men. *Proceedings of the Nutrition Society* 61: 166 A.

Tsofliou F, Pitsiladis YP, Malkova D, Wilson R, Wallace AM & Lean MEJ (2002) Light-Exercise links serum leptin with acute appetite regulation in obese but not in lean women. *International Journal of Obesity* 26 (S1): S182 A.

Tsofliou F, Pitsiladis YP, Malkova D & Lean MEJ (2003). Appetite and satiety after physical activity and snack intake. *International Journal of Obesity* 27 (S1): S136 A.

Tsofliou F, Pitsiladis YP, Hadjicharalmbous M, Fuld J, Wallace AM & Lean MEJ (2004). The acute effects of increased adrenaline concentrations on serum leptin, appetite/satiety measures and subsequent food intake in obese women. *International Journal of Obesity* 28 (S1): S47 A.

Tsofliou F, Pitsiladis YP, Fuld J, Wallace AM & Lean MEJ (2004). The effects of moderate exercise plus α/β adrenoceptor blockade on serum leptin, appetite/satiety measures and subsequent food intake in obese women. *International Journal of Obesity* 28 (S1): S211 A.

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