2005

The Effects Of A Single Exercise Bout On Plasma Leptin Concentration In Obese Males

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THE EFFECTS OF A SINGLE EXERCISE BOUT ON PLASMA LEPTIN CONCENTRATION IN OBESE MALES

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A thesis proposal submitted in partial fulfillment of the requirements
for the degree of Master of Arts
in the Department of Child Family and Community Sciences
in the College of Education
at the University of Central Florida
Orlando, Florida

Spring 2005

ABSTRACT

Recent findings suggest that leptin may be regulated in response to abrupt changes in energy homeostasis. Therefore, it is conceivable that transient changes in energy balance induced by exercise may also regulate leptin synthesis and secretion. As such, we hypothesized that acute increases energy expenditure (i.e. exercise), may regulate leptin concentrations in obese individuals. Fifteen healthy obese males underwent either a single exercise session of moderate intensity (58.4 % ± 4.0 of VO_2max) for 60 min (n=8), or served as controls (n=7). The exercise session elicited an energy expenditure of 567±80 Kcal. No significant changes in plasma leptin (pre 23.5± 30.2; post 24.3± 34.3; 24h-post 34.9± 66.6; 48h-post 33.8±64.0 ng/ml), or insulin levels (pre 16.1± 9.2 vs. post 8.1± 9.1; 24h-post 14.3± 9.9; 48h-post 13.8± 10.2 μU/ml) were detected immediately after the intervention. Baseline plasma leptin levels were positively correlated with BMI ($r=0.65$; $p<0.01$), body weight ($r=0.64$; $p<0.01$), % body fat ($r=0.90$; $p<0.01$) and were negatively correlated with VO_2max ($r=-0.82$; $p<0.01$). The results of the present study suggest that acute exercise of moderate intensity and duration may not affect leptin concentration.

This effort is dedicated in memory to my beloved father and childhood hero, who taught me to pursue my dreams with no fear of failing

ACKNOWLEDGEMENTS

I would like to express my gratitude to several people who supported me throughout these years and contributed to the completion of this project. Fist and foremost, I would like to thank my advisor and friend Dr. Theodore J. Angelopoulos for believing in me and for giving me the opportunity to explore my limits and, in many instances, go beyond them. It is due to his excellent academic guidance and encouragement that I have now expanded my academic pursuits. I would also like to thank my friend George Ekonomou for being a great mentor and especially for encouraging me to fulfill my dreams in the United States. This project would not have been possible without Jonathan Caplan who tolerantly stood beside me in difficult times and Joshua Lowdes, and Richard Carpenter for their great help with the completion of this project. Finally, I would like to express my gratitude and respect to my mother Diana and father Apostolos for their constant support, encouragement and excellent parenthood.

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INTRODUCTION

The isolation of the *ob* gene in mice opened new horizons in the pathophysiology of obesity (Zhang et al., 1994). Leptin, the only known *ob* gene product, is a hormone that is secreted primarily by white adipose tissue (Kolaczynski et al., 1996) and postulated to act as part of a feedback signal to the central nervous system, in particular the satiety center in the hypothalamus. This feedback signal functions primarily to ensure that energy intake and output are balanced over time (Schwartz, Seeley, Campfield, Burn, & Baskin, 1996).

Serum leptin concentration is primarily, but not exclusively, determined by the amount of adipose tissue in the body. As such, obese individuals (BMI >30 kg/m^2) have on average four-fold more serum leptin concentration than their lean counterparts (Considine et al., 1996). This difference in serum leptin levels may be explained by the greater concentration of *ob* mRNA found in adipocytes taken from obese individuals (Considine et al., 1996; Lonnqvist, Arner, Nordfors, & Schalling, 1995). In addition to adipose tissue volume, gender has been shown to influence serum leptin. Females have higher concentrations than males and these differences exist after adjusting for differences in adiposity (Couillard et al., 1997; Hickey et al., 1996).

Regular physical activity has been shown to be effective in the prevention and treatment of obesity and its comorbidities (DiPietro, 1999; Rippe & Hess, 1998). Therefore, it is reasonable to speculate that chronic physical activity may directly or indirectly regulate leptin metabolism. Chronic exercise training (9-36 weeks) (Kohrt, Landt, & Birge, Jr., 1996; Kraemer et al., 1999), however, failed to demonstrate an independent effect on serum leptin levels in obese women.

Decreases in serum leptin were observed only as a result of an exercise-induced reduction in body fat mass (Christensen, Svendsen, Hassager, & Christiansen, 1998; Thong, Hudson, Ross, Janssen, & Graham, 2000). Similarly, diet-induced fat loss decreased serum leptin concentrations proportionally to the reduction in adiposity in obese men and women (Geldszus et al., 1996; Havel et al., 1996; Wadden et al., 1998). It should be noted however, that chronic exercise training often results in significant decreases in adipose tissue, especially in obese individuals (Wilmore et al., 1999). As such, it is difficult to evaluate the independent effect of exercise on leptin levels.

The effect of acute exercise on serum leptin has been examined by several investigators. Acute exercise is unlikely to alter body fat mass and therefore may operate independently of adiposity in altering leptin metabolism. It has been proposed that acute exercise may play a role in leptin metabolism by transiently disrupting the equilibrium between energy intake and expenditure. For example, significant decreases in serum leptin concentrations (32%) have been reported in lean trained subjects immediately after a 36-hour ultra-distance race (Landt et al., 1997). These data suggest that a substantial energy deficit does result in changes in serum leptin levels, but does not shed light on exercise of a more typical duration, and therefore energy expenditure. The majority of early studies in lean sedentary males and females reported little or no effect on serum leptin levels when measured immediately following an exercise bout of shorter duration (10 to 60 minutes) and moderate intensity (50-75% of maximal oxygen consumption) (Perusse et al., 1997; Racette, Coppack, Landt, & Klein, 1997; Weltman et al., 2000). The absence of significant changes in circulating leptin immediately after a single exercise bout does not

preclude the possibility of a delayed effect of exercise on serum leptin levels. A significant decrease (34%) in plasma leptin levels in lean trained males was reported, 44 hours following a 2-hour treadmill session at 75% of VO_2 max (Tuominen et al., 1997). Similar delayed (48h post) responses in leptin levels were observed in a recent study in which lean trained males exercised at 70% of their VO_2 max, and expended between 800 to 1,500 Kcal (Essig, Alderson, Ferguson, Bartoli, & Durstine, 2000). Finally, a decrease in leptin concentration 24-48 hours after a 2-hour exercise session (50% Vo_2 max) has been reported in lean sedentary males (van Aggel-Leijssen, van Baak, Tenenbaum, Campfield, & Saris, 1999). These delayed effects on serum leptin levels may be due to factors other than adiposity, since a single bout of exercise of moderate duration and intensity has not been shown to alter body fat. Fasting and refeeding studies have also indicated a transient effect on serum leptin concentration. For example, short term fasting (12-36h) significantly decreased serum leptin concentration (Boden, Chen, Mozzoli, & Ryan, 1996). Nevertheless, these changes in serum leptin levels were restored to pre-fasting levels (within 24h) by successive refeeding (Kolaczynski et al., 1996; Weigle et al., 1997). Based on the above observations it is reasonable to speculate that acute fluctuations in energy balance (i.e. caused by exercise, and/or fasting) may play a role in the short-term regulation of leptin.

In summary, the role of leptin in human obesity is not completely understood. Among other factors, exercise may have a potential involvement in leptin metabolism. The effects of exercise in leptin metabolism may be of particular clinical significance for obese individuals. The role of chronic exercise training on circulating leptin levels is unclear, since changes in leptin levels

are often the result of an exercise-induced decrease in fat mass. The effects of acute exercise, if any, are independent of changes in adipose tissue and may clarify how regular exercise may affect leptin metabolism.

In summary, in order to clarify the role of chronic exercise on systemic leptin levels in obese individuals, the effects of acute exercise need to be adequately characterized. Interestingly, to date, no studies are available that address the effects of acute exercise on leptin metabolism in obese individuals. Therefore, this is the first attempt to characterize the effects of acute exercise on systemic leptin levels in obese individuals. Such an attempt may shed new light on the role of exercise in leptin metabolism and the physiological mechanisms that link leptin metabolism to obesity.

REVIEW OF THE LITERATURE

Introduction

Obesity has emerged as a serious health problem in Western societies, affecting up to 35% of the adult population in the United States. Physiologically, obesity is a disorder characterized by excess body fat accumulation, developing when energy intake exceeds energy expenditure (Ostlund et al., 1996). It is believed that obesity is a multi-factorial disorder influenced by metabolic abnormalities, dietary patterns, lack of adequate amount of physical activity and genetic defects (Weinsier et al., 1995).

It has been suggested that regulation of body weight may involve the direct or indirect actions of several hormones (i.e. insulin, glucocorticoids). These hormones, after receiving information from the peripheral tissues, modulate the neuronal pathways in the hypothalamus that have potent effects on both energy intake and expenditure. This feedback system between the brain and the energy status of the body functions primarily to ensure that energy intake and expenditure are matched over time, maintaining a stable body weight.

In 1953, Kennedy proposed the 'lipostatic theory' in which total body mass is maintained by regulating body fat content (Kennedy, 1953). He suggested that an unknown circulating factor provides the hypothalamus with information on the amount of body fat stores. If changes in adiposity occur over time, the hypothalamus would make appropriate adjustments in food intake in order to restore body fat content.

Leptin: structure, synthesis and degradation

In the past two decades, several advances in molecular biology and biotechnology have allowed the study and identification of a number of genes linked to obesity (Bessesen & Faggioni, 1998). Among them is the identified obese gene (*ob* gene) and its protein product leptin (OB protein) (Zhang et al., 1994). The discovery of leptin provided a molecular basis for the aforementioned lipostatic theory of energy balance regulation.

The *ob* gene was identified by positional cloning and consists of 3 exons and 2 introns, which encode a 4.5 kb mRNA expressed primarily in white adipocytes. Emerging evidence, however, suggests that leptin transcription and translation also occur in the placenta and the gastrointestinal tract (Masuzaki et al., 1997). The *ob* gene product, leptin, is a 167 amino acid peptide with an amino-terminal secretory signal sequence of 21 amino acids, which is subsequently removed prior to release of leptin into the circulation. Thus, circulating leptin is a peptide of 146 amino acids with a molecular mass of 16 kDa. Leptin is secreted into the circulation, where it can be measured using an immunoprecipitation assay or a radioimmunoassay (Ma et al., 1996; Maffei et al., 1995). Furthermore, serum leptin circulates in part bound to transport proteins in the serum of both rodents and humans (Houseknecht et al., 1996), but obese individuals have most of leptin circulating in its free bioactive form (Sinha et al., 1996). Human leptin is 84% homologous to the mouse protein and has a half-life of 24.9±4.4 min with no differences between obese and lean individuals (Klein, Coppack, Mohamed-Ali, & Landt, 1996). The short half-life of leptin in the circulation is mainly

determined by renal clearance, which is mediated by renal flow and glomerular filtration.

Biological functions of Leptin

It has been postulated that leptin acts as part of a feedback signal to the central nervous system (CNS), in particular the satiety center in the hypothalamus (Kolaczynski et al., 1996). This speculation is further supported by the existence of a saturable transport system for leptin across the blood-brain barrier, mainly at the choroid plexus and the hypothalamus (Banks, Kastin, Huang, Jaspan, & Maness, 1996). After entering the CNS, leptin binds to specific receptors found in the arcuate nucleus and in the ventromedial and dorsomedial hypothalamus (Schwartz, Seeley, Campfield, Burn, & Baskin, 1996). The leptin receptor (*ob*-R) belongs to the family of cytokine receptors (i.e. hGH, IGF-1) and is likely to signal via the JAK-STAT signal transduction pathway in the hypothalamus. The leptin receptor gene encodes at least five alternatively spliced forms, with two of these forms (one short and one long) found in the hypothalamus. The short *ob*-R may be involved in leptin transport and the long *ob*-R may mediate leptin action (Halaas & Friedman, 1997; Tartaglia et al., 1995).

In rodents, leptin has substantial effects on food intake and energy expenditure. Injection of leptin in *ob/ob* mice (absence of functional leptin) resulted in reduced body weight, fat mass, and percent body fat mostly mediated through a reduction in food intake (Pelleymounter et al., 1995). These effects are exhibited via the orexigenic neuropeptide Y (NPY) (Schwartz, Seeley, Campfield, Burn, & Baskin, 1996). Leptin has been shown to decrease both the release of NPY and the expression of NPY mRNA *in vivo* (Stephens et al., 1995). Nevertheless, NPY is not the

only target of leptin in the hypothalamus. Leptin has been shown to up-regulate the secretion of corticotropin-releasing hormone (CRH), pro-opiomelanocortin (POMC), cocaine-and amphetamine-regulated transcript (CART) and other peptides which have been shown to increase or maintain energy expenditure and thermogenesis in rats (Costa et al., 1997; Thornton, Cheung, Clifton, & Steiner, 1997).

Interestingly, leptin may also act peripherally, with receptors found in numerous non-hypothalamic tissues. Administration of recombinant leptin restored the fertility of the otherwise infertile *ob/ob* mice (Chehab, Lim, & Lu, 1996). Moreover, leptin appears to inhibit insulin secretion from the β-cells of the pancreas (Fehmann et al., 1997), stimulate glucose utilization in the skeletal muscle (Kamohara, Burcelin, Halaas, Friedman, & Charron, 1997), stimulate lipolysis in adipocytes (Fruhbeck, Aguado, & Martinez, 1997) and glucose transport across the small intestine (Lostao, Urdaneta, Martinez-Anso, Barber, & Martinez, 1998). Finally, leptin may be a stimulatory factor in haematopoiesis (Gainsford et al., 1996).

Methods of Leptin Detection and Measurement

Two methods have been developed to measure total human leptin in serum, plasma or tissue culture media; a) Human Leptin radioimmunoassay (RIA) and b) Human Leptin Enzyme Linked Immunosorbent Assay (ELISA).

In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited. For example, only 50% of the total tracer concentration may be bound by

antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A calibration or standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.

Human Leptin ELISA is a two-site immunoassay using a high affinity polyclonal capture antibody and a monoclonal detecting antibody. Signal is derived by monitoring (405 nm) alkaline phosphatase activity in the presence of the substrate p-nitrophenyl phosphate. Human leptin ELISA assay is based, sequentially, on: 1) capture of human leptin molecules from samples to the wells of a microtiter plate coated by pre-titered amount of polyclonal rabbit anti-human leptin antibodies, 2) wash away of unbound materials from samples, 3) binding of a biotinylated monoclonal antibody to the captured human leptin, 4) conjugation of alkaline phosphatase to biotinylated antibodies, 5) wash away of free antibody-enzyme conjugates, and 6) quantification of immobilized antibody-enzyme conjugates by monitoring alkaline phosphatase activities in the presence of the substrate p-nitrophenyl phosphate. The enzyme

activity is measured spectrophotometrically by the increased absorbency at 405 nm due to production of the yellow colored product p-nitrophenol. Since the increase in absorbency is directly proportional to the amount of captured human leptin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human leptin. This assay produces results identical to the gold standard Human Leptin RIA and offers an alternative for laboratories interested in reducing use of radioisotopes yet maintaining data consistency to previously published RIA results.

Leptin regulation in humans

The physiologic role of leptin in humans has not yet been fully elucidated. Serum leptin concentration is related to the amount of adipose tissue in the body (Considine et al., 1996; Maffei et al., 1995). Moreover, there is evidence that the amount of *ob* mRNA expressed by the adipose tissue is determined by the number and size of adipose cells. Thus, obese individuals have proportionally greater concentration of ob mRNA in their adipocytes, which accordingly increases the rate of leptin production. As such, obese individuals (BMI >30kg/m^2) have on average four-fold serum leptin concentration than their lean counterparts (Considine et al., 1996). Since leptin is proportionally related to body fat content it is reasonable to expect that changes in leptin concentration will parallel changes in body fat mass. Diet-induced weight loss decreases leptin concentration proportionally to the reductions in body fat mass (Geldszus et al., 1996;Wadden et al., 1998). In obese subjects, however, changes in leptin concentration with

weight loss are more dramatic, given that a fixed reduction in adiposity induces a 3 to 10-fold decrease in leptin concentration (Havel et al., 1996; Wadden et al., 1998).

Nevertheless, a large portion of the variability observed in leptin concentrations between different individuals cannot be explained by differences in body fat alone. Gender influences serum leptin independent of adiposity. As such females have higher concentrations of circulating leptin than males (Castracane, Kraemer, Franken, Kraemer, & Gimpel, 1998; Couillard et al., 1997). Moreover, plasma leptin in women increases during the luteal phase of the menstrual cycle, adding more evidence to the hypothesis that sex hormones (i.e. estradiol, progesterone) may regulate leptin secretion (Cella, Giordano, & Cordera, 2000). Similarly, it has been demonstrated that testosterone in men may have a suppressive effect on leptin production by the adipocytes (Behre, Simoni, & Nieschlag, 1997; Haffner, Miettinen, Karhapaa, Mykkanen, & Laakso, 1997). In a recent report, however, gender difference in leptin concentrations were not apparent when the relationship between log transformed plasma leptin levels and Dual Energy X-ray Absorbance (DEXA)-derived percent body fat were examined, indicating that the method of assessing body composition may greatly affect the relationship between leptin and adiposity (Jensen, Hensrud, O'Brien, & Nielsen, 1999).

Conflicting reports exist regarding the association between circulating leptin levels and aging. In two recent studies, BMI and fat mass-adjusted leptin levels were found to gradually decline with aging, with more notable reduction in women compared to men (Isidori et al., 2000; Neuhauser-Berthold et al., 2000). In contrast, others have reported no effect of age on the relationship between circulating leptin and body fat mass (Roberts et al., 1997; Sumner,

Falkner, Kushner, & Considine, 1998).

Leptin in humans may also be altered by circadian rhythms. Plasma leptin concentrations reach maximal values shortly after midnight (Sinha et al., 1996). These fluctuations in plasma leptin concentrations were not altered between normal-size meals, suggesting that meal ingestion does not interfere with leptin metabolism, and therefore it is unlikely that leptin can act as an acute satiety factor (Considine et al., 1996). Short-term fasting, however, decreases leptin levels without any appreciable changes in body fat content (Boden, Chen, Mozzoli, & Ryan, 1996). Leptin levels begin a steady decline after 12h of fasting, reaching a nadir at 36h and returning to pre-fasting levels 6-12h after refeeding (Kolaczynski et al., 1996; Pratley, Nicolson, Bogardus, & Ravussin, 1997; Weigle et al., 1997). Leptin level alterations with fasting and refeeding may be dependent on insulin, since these changes occur parallel to changes in plasma insulin (Patel, Koenig, Kaplan, & Hooi, 1998). The majority of the data, however, suggest that, at least short-term, insulin does not have an effect on leptin secretion in humans. A 3-hour euglycemic hyperinsulinemic clamp did not increase leptin mRNA levels in human adipose tissue (Vidal et al., 1996). Similarly, neither a hyperglycemic (approximately 10 mmol/L) nor a hyperinsulinemic- euglycemic (400-3000 pmol/L) clamp affected plasma leptin levels (Ryan & Elahi, 1996). Finally, leptin gene expression and secretion in humans may be regulated by glucocorticoids (Casanueva & Dieguez, 1999) and catecholamines (Del Rio, 2000).

The effects of acute exercise training on leptin concentrations in humans

The fact that energy intake can influence leptin levels may suggest that energy expenditure may also have a potentially independent role in leptin metabolism. Many studies have addressed the effects of prolonged exercise (>120min) on leptin levels. For example, significantly decreased serum leptin concentrations (32%) was reported immediately post-exercise, when lean trained males had participated in a 36-hour ultra-distance race (Landt et al., 1997). In addition, energy expenditure of 2800 Kcal induced by a Marathon run reduced serum leptin levels in male athletes (Leal-Cerro et al., 1998). No significant changes in serum leptin levels were reported in highly trained male athletes immediately after a 20-mile run, suggesting that relatively chronic changes in energy expenditure are required to alter leptin metabolism (Hickey et al., 1996),. Similarly, acute exercise of shorter duration (60 minutes) did not change plasma leptin concentrations in sedentary male subjects after a moderate-intensity (50% of maximum heart rate) cycle ergometer training (Racette, Coppack, Landt, & Klein, 1997) or treadmill exercise (Torjman, Zafeiridis, Paolone, Wilkerson, & Considine, 1999). Moreover, 30 minutes of acute exercise at varying intensities and caloric expenditures, did not affect leptin concentrations in active men during or immediately after the recovery (Weltman et al., 2000). Finally, a maximal exercise test (12-15 min) did not induce any changes in plasma leptin levels in lean sedentary men and women (Perusse et al., 1997). In these studies, leptin was measured only before, during, and immediately after exercise. Thus, the above studies could not determine any delayed alterations in leptin concentration that could be related to the exercise stimulus *per se*, or the exercise-induced negative energy balance.

A significant decrease (34%) in plasma leptin levels was reported in lean trained males, 44 hours following a 2-hour treadmill session at 75% of their VO_2 max (Tuominen et al., 1997). Similar delayed responses in leptin have been confirmed in a recent study in which lean trained males exercised at 70% of their VO_2 max requiring a caloric expenditure between 800 to 1,500 Kcal (Essig, Alderson, Ferguson, Bartoli, & Durstine, 2000). Finally, in lean sedentary males, leptin concentration decreased 24-48 hours after a 2-hour exercise session (50% Vo_2 max) (van Aggel-Leijssen, van Baak, Tenenbaum, Campfield, & Saris, 1999). These results may highlight a potentially important time delay in the response of leptin following acute exercise.

The effects of chronic exercise on leptin concentrations in humans

It has been previously mentioned that diet-induced fat loss is accompanied by significant reductions in circulating leptin levels in humans. It is not clear whether exercise-induced changes in circulating leptin levels depend on the alterations in fat mass. The effects of 7 consecutive days of exercise training on leptin were examined in lean, sedentary subjects (n=30). Subjects exercised continuously for 60 minutes/day at 70%-75% of their VO_2max and consumed a normal diet during the exercise-training period. Exercise training did not alter body mass or plasma leptin levels (Houmard, Cox, MacLean, & Barakat, 2000). Similarly, in another study in which body weight remained unchanged, 9 weeks of aerobic exercise did not significantly alter serum leptin levels in obese women (Kraemer et al., 1999b). In postmenopausal women, a 36-week endurance training program (Kohrt, Landt, & Birge, Jr., 1996) or a 12-week diet and exercise intervention (Christensen, Svendsen, Hassager, &

Christiansen, 1998) decreased serum leptin levels proportionally to the exercise-induced and/or diet-induced decreases in fat mass. Similarly, in obese men decreases in plasma leptin levels were observed after 12 weeks of diet-induced or exercise-induced weight loss (Thong, Hudson, Ross, Janssen, & Graham, 2000). Although the majority of data regarding the effects of chronic exercise on circulating leptin levels support the notion that there is no profound effect of exercise *per se*, a few studies have demonstrated an independent effect of chronic exercise on systemic leptin levels. Sixteen months of exercise training in obese men resulted in reductions in leptin concentration independent of body fat and insulin levels (Pasman, Westerterp-Plantenga, & Saris, 1998). These independent effects were observed only after the 10th month of the study, indicating that probably the duration of exercise training was important for the observed results. Similarly, other studies have proposed that a gender-dependent effect of exercise training on serum leptin levels may exist. The effects of a 20wk endurance exercise program on leptin levels in 97 men and women were examined (Perusse et al., 1997). Endurance training was associated with a reduction in circulating levels of leptin in men but not in women. After adjustment for fat mass changes, however, the effects of training were no longer significant for the men. In contrast, serum leptin concentrations decreased by 17.5% in lean females after 12 weeks of aerobic exercise training (4 days/week, 30-40min/day) but was not significantly reduced in lean males (Hickey et al., 1997). These data may suggest that exercise training may induce a greater effect on systemic leptin levels in females than in males.

Discussion

The discovery of leptin introduced new insights into the regulation of body mass and the pathophysiology of human obesity. However, the significance of leptin in the regulation of body mass in humans is not fully understood. Circulating leptin increases in response to increased fat mass and, under normal conditions, affects the appetite center in the hypothalamus to reduce food intake. This mechanism ensures that when dramatic increases in fat mass occur, food intake will be reduced to prevent further increases in adiposity and eventually restore the previous set point of body mass.

Exercise training, acute and chronic, has been proposed as one of the regulatory factors that may be involved in leptin metabolism. The effect of a single exercise bout on circulating leptin is controversial. Serum leptin concentration decreased in response to prolonged vigorous exercise in two studies (a marathon and an ultra-marathon race). Nevertheless, no changes in circulating leptin were observed immediately after an acute exercise bout of shorter duration and moderate intensity. This suggests that a substantial negative energy deficit may result in acute changes in serum leptin levels. It has also been suggested that the decreases in leptin levels after a prolonged exercise session could be attributed to the low energy availability caused by the exercise energy expenditure and not to the exercise stress *per se* (Hilton & Loucks, 2000). The lack of change in the circulating leptin concentration immediately following short-term exercise does not preclude the possibility of a delayed effect that may be manifested following exercise. For instance, a decrease in serum leptin levels was manifested 24 to 48 hours after a single exercise bout. These effects were independent of changes in fat

mass or fluctuations in energy intake. Hence, it may be suggested that increased exercise-induced energy expenditure may be the major reason for the observed decrease in leptin concentration during recovery. These changes in leptin may mediate the restoration of energy homeostasis during recovery from periods of increased energy expenditure (i.e. acute exercise). It is of particular note, however, that despite the involvement of leptin metabolism in human obesity no studies, to date, have addressed the effects of an acute exercise bout on systemic leptin concentration in obese individuals.

The data regarding the effects of chronic exercise training on circulating leptin levels are equivocal. It has been suggested that chronic exercise, in the absence of a decrease in body mass, does not alter leptin levels. Therefore, no independent effect of chronic exercise could be demonstrated on circulating leptin levels in obese subjects. It is well documented that chronic exercise training often decreases fat mass in obese individuals (Wilmore et al., 1999). Therefore, when alterations in circulating leptin were observed, it was proposed that they represent the secondary effects of exercise on energy balance and not the exercise stimulus *per se*. In contrast, others have reported independent effects of chronic exercise on leptin levels. For instance, it has been proposed that the effects of chronic exercise on circulating leptin levels may be dose-dependent (i.e. greater energy deficit). The duration and the number of exercise hours, however, may only reflect increased energy expenditure and not necessarily an independent exercise effect. Others have reported that serum leptin concentration decreases after chronic exercise training in females but not in males, suggesting a gender-dependent effect. Caloric intake was not well controlled in this study and therefore these gender

differences may be secondary to fluctuations in energy balance and not the result of the exercise training.

In conclusion, the effects of acute and chronic exercise on systemic leptin levels remain to be determined. Future exercise studies should adequately control energy intake and or expenditure, and therefore results can be interpreted independently of changes in energy balance. Similarly, technologically advanced methods of assessing body composition (i.e. DXA) should be used in order to more accurately evaluate the relationship between fat mass and leptin. Chronic exercise studies should be specifically designed to examine the dose-response relationship between exercise and circulating leptin levels and whether this relationship is gender specific. Moreover, it remains to be elucidated whether acute exercise has an independent effect on leptin levels and the time course of these effects. Finally and more importantly, the effects of acute exercise on systemic leptin levels should be addressed in obese individuals, since these effects may be of particular clinical significance for this population.

METHODS

Subjects

Fifteen adult (age 18-35) obese males (BMI≥30 kg/m^2) voluntarily participated in this project, after signing an informed consent form approved by the Institutional Review Board (IRB) of the University of Central Florida. Participants were randomly assigned to either an experimental or a control group. Qualified participants were sedentary, non-smokers and had no known cardiovascular, pulmonary, metabolic, or chronic diseases.

Screening and qualifying visits

All participants underwent a screening procedure, which included a health/medical questionnaire, anthropometric and body composition measurements, a fasting plasma glucose (FPG) test, a pulmonary function test (PFT), a graded exercise test (GXT), a 3-day self-reported diet record and a maximal aerobic capacity test (ACT) that measured their VO_2max.

The administration of the health/medical questionnaire evaluated current and past medical history, as well as, exercise, dietary and lifestyle habits. Anthropometric measurements included body weight (BW), body height (BH), BMI and waist-to-hip ratio (WHR). BW was measured on a balance-beam scale accurate to 0.1 lb. BH was obtained to the nearest 0.1 cm using a scale-mounted stadiometer. BMI was calculated by dividing body weight by body height squared (Kg/m^2). Waist and hip circumferences were measured at the smallest circumference between the rib cage and iliac crest and at the level of the widest gluteal

protuberance respectively, with the subject in standing position. Body composition included percentage of body fat, fat mass and lean body mass and was determined by dual-energy X-ray absorptiometry (DPX MD+, Lunar Co.) as previously described by others (Jensen et al., 1993). Body composition tests were also repeated one day before the exercise intervention (Day7). Plasma glucose was determined (Day 1), after an overnight fast. Plasma glucose was measured (YSI Glucose/L-Lactate Analyzer 2300 STAT) after centrifugation of a blood sample for 30 minutes at 4^{o}C. The PFT was performed using closed loop spirometry (SuperSpiro Spirometer, Micro Direct Inc.). The criteria for determining possible ventilatory limitations have been previously described by others (Younes, 1984).

All subjects underwent a sub-maximal, 12-lead Graded Exercise Testing (GXT) (Day 2). The GXT was performed on a Quinton Max-1 Exercise Testing System (GE Marquette Electronics) with the presence of an ACSM Program Director$_{SM}$ Certified and other certified personnel. Subjects, after following a warm-up period consisting of light walking at 1.7 mph and 5% grade, were tested employing the Bruce protocol (Bruce, 1971). The test was terminated when subjects reach 85% of their age-predicted heart rate maximum (HR max), and followed by a recovery period until heart rate (HR) and blood pressure (BP) decreased to the baseline values.

Furthermore, subjects were asked to keep a 3-day self-reported diet record (From initial Screening to Day 3), which included a detailed description of the portion size, food groups, and time of the meals. Participants were instructed on how to correctly record and report their food intake. Diet records were analyzed for caloric content and macronutrient composition

(NutriBase 2001 Clinical v.3x, CyberSoft, Inc.).

Finally, subjects underwent a maximal ACT (Day 3) using a MAX-1 Cart (Physio-dyne Instrument Corp). The maximal ACT protocol began with a warm-up period consisting of light walking (2.5 mph, 3%) and proceeded by progressively increasing the workload (1-2 METs) every 3 minutes. The maximum ACT was performed in order to measure peak oxygen consumption (peak VO_2). The test was terminated if requested by the subject, or when at least two of the following criteria were met: i) failure to increase VO_2 more than 150ml/min with an increase in workload, ii) respiratory exchange ratio (RER) over 1.1, and iii) HR above 90% of HR_{max}.

Experimental Design

A randomized pretest-posttest control group design was used in this study. Participants were assigned in groups randomly by alternating participants between the control and experimental group. Both the experimental and the control group were subjected to all screening tests, blood sampling and dietary protocols. Only the experimental group participated in the exercise intervention. A schematic of the design is depicted below:

Table 1: Schematic of the experimental design

Screening	**Day 1-2**	**Day 3**	**Day 4-7**	**Day 8**	**Day 9**	**Day 10**
Health Questionnaire	Blood Sampling 1&2			Blood Sampling 3&4	Blood Sampling 5 (24h-post)	Blood Sampling 6 (48h-post)
Self-reported Diet record						
PFT	FPG test		5-day wash out period	**1-hour Exercise Intervention (experimental)**		
	GXT (Day2)	ACT		**1-hour rest (control)**		
	Anthropometric Measurements	BW		BW		
	Body Composition Measurements			Body Composition Measurements		

Blood Sampling and Analysis

All fasting blood samples (~10ml) were drawn from participants of both groups before 9:00 AM (# 1-3, 5 and 6) except the post-exercise blood sample (# 4), which was taken immediately after the completion of the exercise from the participants of the experimental group. At the day of intervention, blood samples (# 3-4) were taken from the participants of the control group 2 hours apart (i.e. 8:00 a.m. and 10:00 a.m.). This assured that the time of blood sampling of the control group corresponded to the pre/post exercise blood sampling of the experimental group. Blood samples before the day of intervention (# 1-3) were used to test whether there was significant daily variability of the measured variables (i.e. leptin and insulin). Subjects were instructed to abstain from any physical activity the day before the test (except the experimental group for all the post-exercise blood samples) and fast 12h before blood sampling. Hematocrit (Hct) and hemoglobin (Hb) content were measured in pre and post-exercise blood samples and

were used to detect any changes in plasma volume (Dill & Costill, 1974). Plasma blood samples were mixed with EDTA to prevent clotting and were centrifuged immediately. Serum blood was centrifuged after 30 min at room temperature. All blood samples were stored at -80^0 Celsius. Leptin and insulin concentrations determined by ELISA (Linco Research, St. Louis MI).

Diet

The data gathered from the 3-day self-reported dietary record were compared to the predicted total daily energy expenditure (TDEE) adjusted for physical activity. TDEE was calculated as predicted resting energy expenditure (REE) times an activity factor of 1.5. REE was calculated as 370 + [21.6 x (Fat-Free Mass)] (Cunningham, 1991; Warwick, Edmundson, & Thomson, 1988). After estimation of TDEE, participants continued to report a dietary record on a daily basis and until the end of the study. An average energy intake of ± 15% of the predicted TDEE was considered acceptable for participation in the study.

Exercise Protocol

On the day of the exercise intervention (Day 8), all participants arrived at the Exercise Physiology Laboratory no later than 8:00 a.m., after an overnight fast and having abstained from any physical activity for at least 4 days. The experimental group performed a single exercise bout on a motorized treadmill at 50-60% of their measured VO_2 max for 60 minutes with 5-10 min warm-up included. The exercise intensity was progressively increased until the

desired intensity (50-60% VO_2peak) was achieved. The targeted exercise intensity was verified by collecting expiratory gases. Moreover, HR was continuously measured using a HR monitor (Polar Inc.). The net exercise-induced energy expenditure was estimated from expiratory gases collected for 3 minutes in 15-minute intervals, minus the resting energy expenditure (3.5ml/kg/min) during that time. Additionally, their BW was measured pre and post exercise for corrections in blood volume.

Statistical Analysis

Differences in the dependent variable between were examined via analysis of variance (AVOVA) with repeated measures. Significant main effects or interactions were probed using a Tuckey's post-hoc test. Statistical significance for mean differences was set at the $p<0.05$ level. All data analysis was performed using SPSS version 12.0

Definitions of inclusion criteria

Obesity: Individuals with body mass index (BMI) equal or more than 30.0 Kg/m^2.

Sedentary: Individuals who have not engaged in vigorous (>6 METS) physical activity more than one time per week for the prior 6 months.

Non-smoker: Individuals who have never smoked, and/or have quit smoking at least six months prior to the study.

Stable body weight: Individuals who have refrained from any dietary program, that caused or

intended to cause weight reduction, for at least three months prior to the investigation.

Hypertension: Resting blood pressure equal or over 140/90 mmHg that is confirmed by two different measurements in separate days and/or on any antihypertensive drugs.

Type 2 diabetes mellitus: Fasting plasma glucose (FPG) equal or over 126 mg/dl (7.00 mmol/l) confirmed on a subsequent day.

Cardiovascular diseases: Individuals who have been diagnosed with cardiac arrhythmias, stroke, peripheral vascular disease, valvular heart disease, congestive heart failure and/or have had a heart attack, a coronary bypass or other cardiac surgery.

Pulmonary diseases: Individuals who have been diagnosed with asthma, emphysema, bronchitis, or pneumonia.

Metabolic diseases: Individuals who have been diagnosed with diabetes mellitus or a thyroid disease.

Chronic diseases: Individuals who have been diagnosed with renal failure, hepatitis, osteoarthritis, or other orthopedic diseases.

RESULTS

Anthropometric measurements, dietary and exercise data

Sixteen participants completed the study. One subject, however, was excluded from statistical analysis due to elevated fasting plasma glucose levels (see methods). Upon analysis of the data, two of the remaining participants were also found to have baseline plasma leptin concentrations exceeding 100ng/ml. To eliminate the possibility that results are skewed, the data were also statistically analyzed excluding the two aforementioned participants. The results were not different regardless of the presence or absence of the two participants with high leptin levels, so their data are included in the final analysis. No significant differences in body weight, BMI, % fat, and oxygen consumption (Table.2) were detected between the control and experimental group. No changes in body composition, or body weight were observed during the week prior to the intervention. Analysis of the initial 3-day dietary record showed that the daily caloric intake of all participants was appropriate for maintaining their current body weight (see methods), and no significant energy fluctuations were observed thereafter (Table. 5). The experimental group completed a single exercise bout for 1-hour at an average exercise intensity (excluding warm-up period) ranging between 53-66% (58.4%± 4.0) of the measured VO_2max and an average HR intensity (excluding warm-up period) ranging between 126-152 beats/min (140.0± 7.8 beats/min) (Table.3). This intensity resulted in total energy expenditure (including warm-up period) ranging between 480-720 Kcal (567±80 Kcal).

Exercise effects on leptin and insulin concentrations

No significant differences in circulating plasma leptin or insulin were observed across pre-exercise measurements (Time points observed: Baseline1, Baseline 2, and Pre-exercise), or between the two groups (Table.4). These results indicate that both leptin and insulin did not show any significant daily variation and were stable over the measured time points preceding the exercise bout. No significant differences in plasma leptin levels were observed immediately after the exercise bout or on the days following the session (i.e. 24-h, and 48-h post-exercise) in the exercise group. A significant ($p=0.011$) quadratic time by group interaction was observed in plasma insulin concentration which was further evaluated using repeated measures between two sets of consecutive time points [i.e. set A: 1) pre-exercise, 2) post-exercise, 3) 24h post and set B: 1) post-exercise, 2) 24h post, 3) 48h post]. To avoid a Type I error, a Bonfferoni adjustment was carried out to minimize the effects of multiple tests.. Post Hoc analysis showed a quadratic interaction was observed at set A of measurements but it did not reach statistical significance after the Bonfferoni adjustment ($p=0.041$, $alpha<0.025$).

Correlations

Baseline plasma leptin levels were positively correlated with BMI ($r=0.65$; $p<0.01$), Body weight ($r=0.64$; $p<0.01$), % body fat ($r=0.90$; $p<0.01$) and negatively correlated with relative ($r=-0.82$; $p<0.01$) and absolute ($r=-0.59$; $p<0.05$) VO_2max.

Table 2: Baseline VO_2max and anthropometric characteristics (mean±SD).

	Control (n=7)	Exercise (n=8)
Age (years)	22.9± 5.1	22.9± 5.8
Height (m)	1.8± 0.8	1.8± 0.5
Weight (kg)	109.0± 7.4	104.9± 8.6
BMI ($kg•(m^2)^{-1}$)	34.5± 3.3	32.5± 2.3
% Fat	39.9± 5.2	37.1± 6.9
VO_2max ($ml•kg^{-1}•min^{-1}$)	31.0± 5.0	33.6± 5.6
VO_2max ($ml•min^{-1}$)	3737± 471	3579± 504

Table 3: Exercise intensity measurements and energy expenditure estimates (exercise group only) (mean±SD).

Exercise HR ($beats•min^{-1}$)	Exercise Intensity ($ml•kg^{-1}•min^{-1}$)	Exercise Intensity (% of VO_2max)	Total Gross Expenditure (Kcal)	Pre-exercise BW (Kg)	Post-exercise BW (Kg)
140.0± 7.8	19.6± 3.6	58.4± 4.0	567±80	105.1± 9.5	104.9± 9.6

Table 4: Leptin and insulin values (mean±SE) before and after the intervention.

	Leptin ($ng \cdot ml^{-1}$)		Insulin ($\mu U \cdot ml^{-1}$)	
	Control (n=7)	Exercise (n=8)	Control (n=7)	Exercise (n=8)
Baseline 1	47.8± 37.1	30.9± 46.0	14.4± 9.7	11.5± 9.7
Baseline 2	43.8± 35.4	29.2± 41.1	16.4± 10.3	13.6± 9.9
Pre-exercise	44.1± 35.9	23.5± 30.2	14.4± 10.1	16.1± 9.2
Post-exercise	40.8± 37.5	24.3± 34.3	14.8± 11.6	8.09± 9.12
24-h post-exercise	44.7± 36.2	34.9± 66.6	18.4± 8.8	14.3± 9.9
48-h post-exercise	40.5± 35.4	33.8±64.0	13.04± 9.2	13.8± 10.2

Table 5: Predicted total daily energy expenditure (TDEE) and actual average total daily energy intake (TDEI).

Control (n=7)		Exercise (n=8)	
TDEE (Kcal)	TDEI (Kcal)	TDEE (Kcal)	TDEI (Kcal)
4665	4355	3604	3310
3588	3284	3849	3740
3974	3862	3753	3556
3858	3580	3624	3787
4648	4443	3856	3599
3283	3325	4463	4090
4186	-	4109	4314
		3972	4125
4029±515	3808±503	3904±281	3815±337

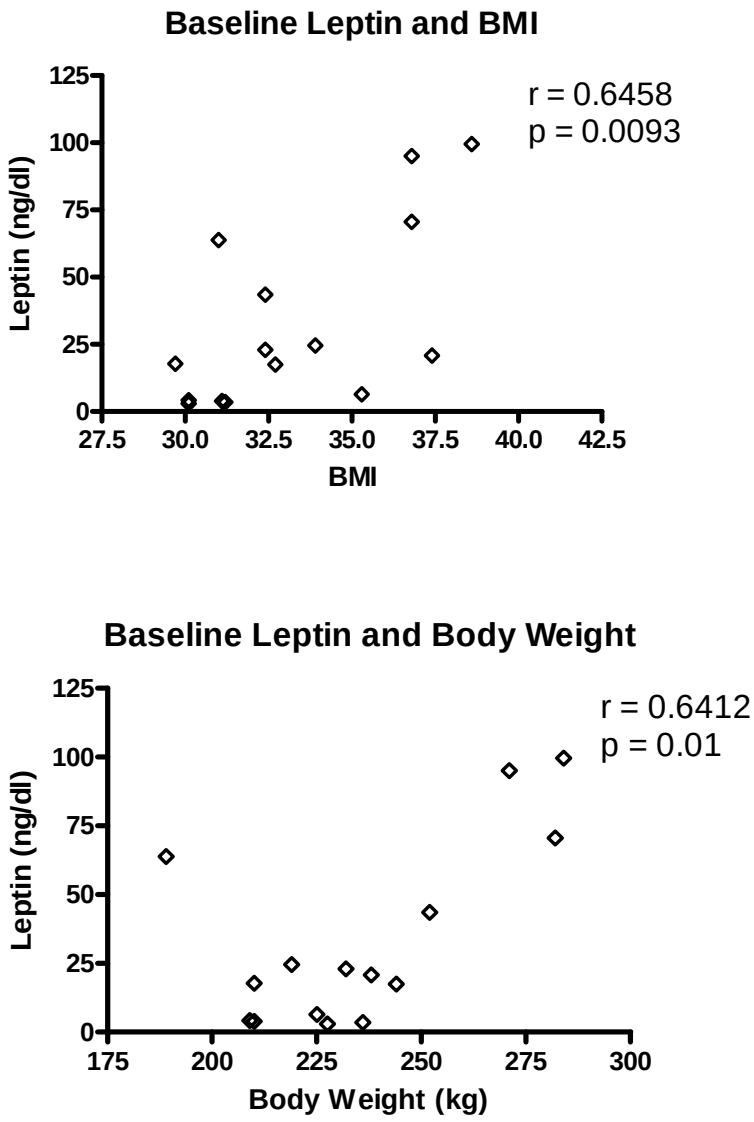

Figure 1. Correlation between baseline plasma leptin, BMI, and Body weight.

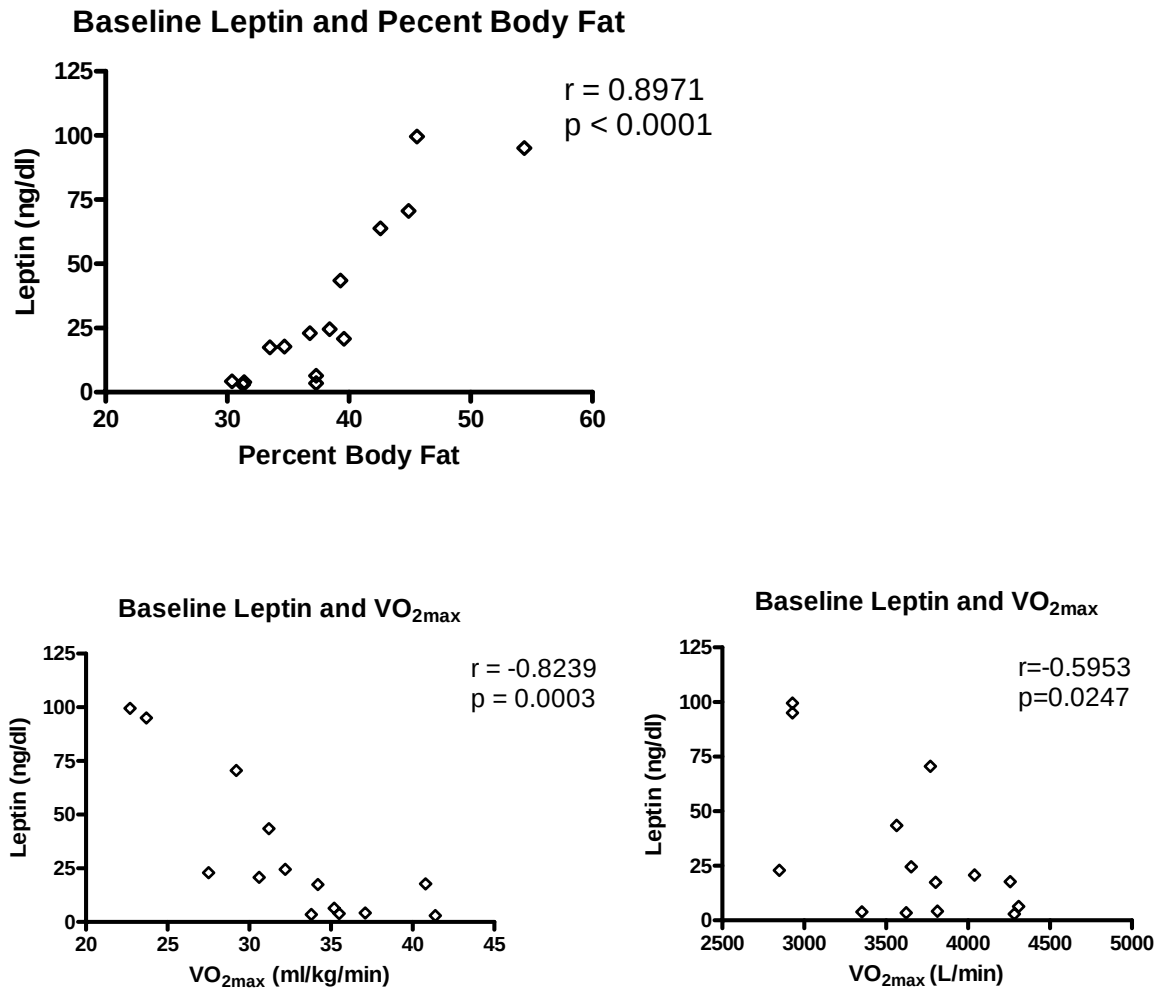

Figure 2. Correlation between baseline plasma leptin, %body fat, and VO_2max.

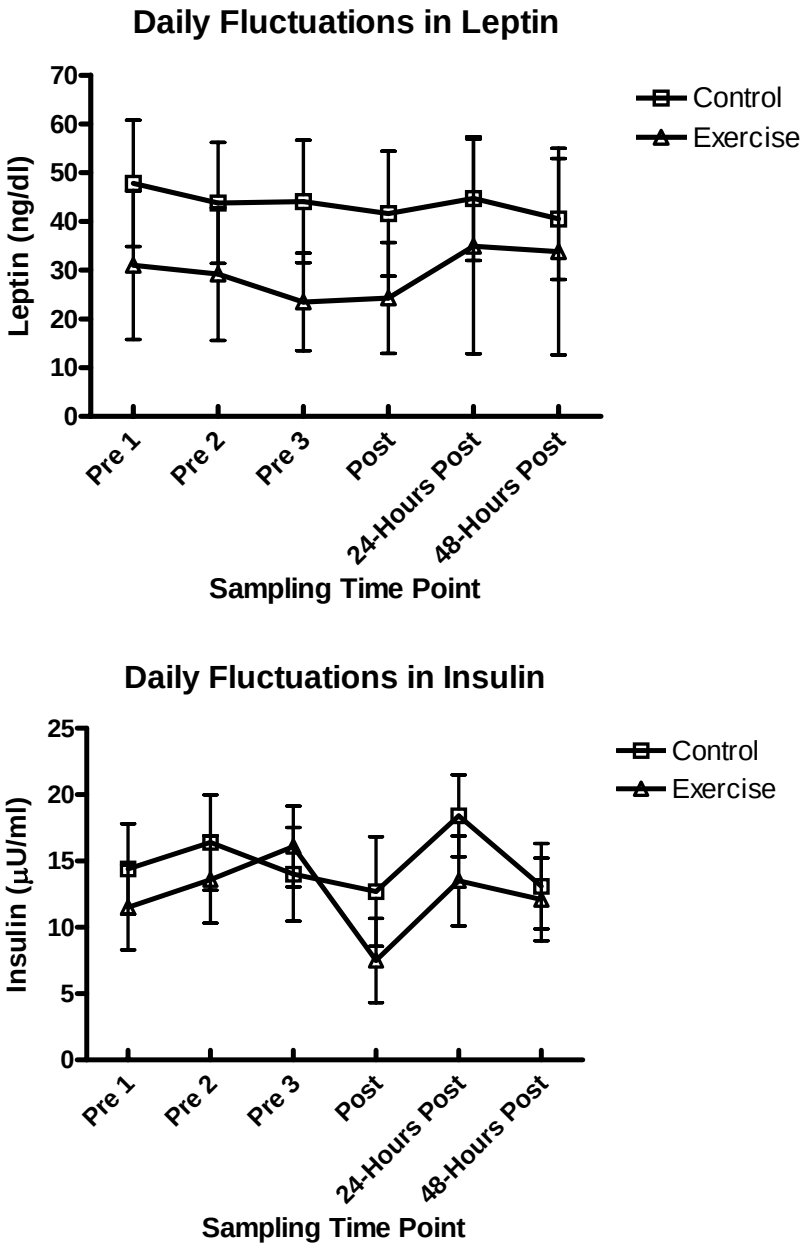

Figure 3. Plasma leptin and insulin fluctuations across time.

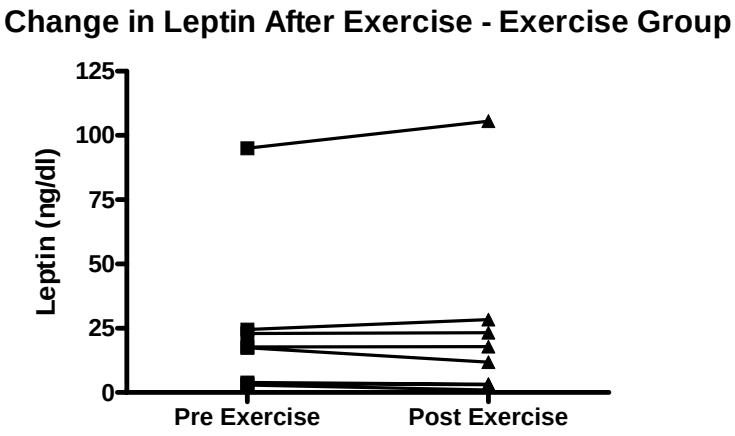

Figure 4. Individual changes in plasma leptin concentration before (pre) and immediately after (post) the exercise intervention (exercise group only).

DISCUSSION

Fasting in humans and animals causes a profound decrease in circulating leptin (Boden, Chen, Mozzoli, & Ryan, 1996; Considine et al., 1996; Kolaczynski, Ohannesian, Considine, Marco, & Caro, 1996; Trayhurn, Thomas, Duncan, & Rayner, 1995), while overfeeding increases leptin over a short period of time (Kolaczynski, Ohannesian, Considine, Marco, & Caro, 1996). These findings suggest that leptin may be acutely regulated in response to abrupt changes in energy intake. Therefore, it is conceivable that other mechanisms that alter energy expenditure (i.e. exercise) may also regulate leptin synthesis and secretion. The purpose of this investigation was to determine whether moderate energy expenditure induced by an acute exercise session is sufficient to alter the levels of circulating leptin immediately after the session, or in the days following the exercise intervention. Thus, we tested the effects of an acute exercise bout of moderate intensity (50-60% of VO_2max), but appropriately long duration (1 hour) to produce sufficient energy expenditure, on circulating leptin levels in obese males. The major findings of this study are: 1) The Exercise bout had no effect on either plasma leptin or insulin levels immediately after, or in 24 and 48 hours post the intervention, and 2) Baseline plasma leptin levels positively correlated with BMI, body weight, % body fat and negatively correlated with VO_2max. No correlations were found between insulin levels and the assessed variables.

Over the past years, several studies have attempted to elucidate the effects of acute exercise on leptin metabolism, but results are inconclusive. Most of the studies have found no significant effects on leptin levels after an acute exercise bout of moderate intensity and duration (Fisher,

2001; Perusse et al., 1997; Racette, Coppack, Landt, & Klein, 1997; Torjman, Zafeiridis, Paolone, Wilkerson, & Considine, 1999; Weltman et al., 2000), while others have detected an immediate decrease (Duclos, Corcuff, Ruffie, Roger, & Manier, 1999; Koistinen et al., 1998; Landt et al., 1997; Leal-Cerro et al., 1998), or a delayed effect that was demonstrated in the days following the intervention (Essig, Alderson, Ferguson, Bartoli, & Durstine, 2000; Olive & Miller, 2001; van Aggel-Leijssen, van Baak, Tenenbaum, Campfield, & Saris, 1999). The present investigation found no significant changes in leptin levels immediately after or in the days following the exercise bout.

Generally, most of the studies that have demonstrated an immediate acute effect of exercise on leptin concentrations have used extremes in duration and/or intensity that resulted in substantial energy expenditure (i.e. >1000 Kcals). Landt et al (1997) found that leptin decreased 32% immediately after an ultramarathon and 16% at 24 hours post. Similar results were demonstrated after 3 hours on an ergometer, or after the completion of a marathon run in fasted trained individuals (Koistinen et al., 1998; Leal-Cerro et al., 1998). These studies demonstrate that when severe energy deficit is achieved, acute deceases in circulating leptin can be observed, at least in lean trained individuals. One hour of cycling at 50% of VO2max did not alter leptin concentrations or subcutaneous adipose tissue leptin production in sedentary subjects (Racette, Coppack, Landt, & Klein, 1997). Moreover, leptin levels were not modified after a submaximal exercise test performed to exhaustion before or after 20 weeks of endurance training (Perusse et al., 1997). The disparity among previous investigations could be due to the factors (i.e. sample population, intensity, duration) that can affect gross energy expenditure, as

well as, factors that affect leptin concentrations including fasting, diurnal effects, and exercise-induced hemoconcetration.

To our knowledge, this is the fist attempt to describe the effects of acute exercise on leptin concentration in obese individuals. Our study was designed to test whether a typical exercise session prescribed for sedentary obese population of moderate intensity, long duration (60 min at 60% of VO_2max) and sufficient energy expenditure can modulate leptin concentrations. As such, our exercise protocol elicited a notable net energy expenditure of approximately 600 Kcal with no observed changes in post circulating leptin levels. Consistent with our findings, similar exercise-induced energy expenditures (200-530Kcal) produced by variable durations and intensities did not alter leptin levels in lean men (Fisher, Van Pelt, Zinder, Landt, & Kohrt, 2001; Olive & Miller, 2001; Weltman et al., 2000). Moreover, Hilton & Loucks, (2000) examined sedentary women when the ~1,400Kcal/day expended by walking was replaced by an equivalent dietary intake suggesting that exercise stress without decreased energy availability does not affect circulating leptin levels. These results, along with the present data, suggest that acute exercise may not affect leptin concentration *per se* immediately after the intervention. It is possible that a threshold for energy deficiency exists that was not reached in the present study.

Despite the absence of an immediate effect of exercise on leptin in most of the studies, some investigators have demonstrated delayed effects that are manifested immediately following the intervention and up to 48 hours post. Two hours of running at 60-75% of VO_2max (~1,200Kcal) decreased leptin levels by 30% at 2 hours post exercise in fed runners (Duclos,

Corcuff, Ruffie, Roger, & Manier, 1999). Exercise-induced energy expenditure of 800 or 1,500 Kcal significantly reduced leptin levels 48 hours after an exercise session at 70% of VO_2max in active men (Essig, Alderson, Ferguson, Bartoli, & Durstine, 2000). Similar decreases in leptin were observed in trained lean men 48 hours after endurance exercise at 70% of VO_2max that induced an energy expenditure of 900 Kcal (Olive & Miller, 2001). Interestingly, in the same study these delayed effects were absent when these men performed a maximum exercise session that allowed an energy expenditure of only 200 Kcal. A possible explanation for this finding is that a threshold level of energy expenditure (i.e.>800 Kcal) may be necessary to stimulate an as yet unknown neural or hormonal signal that depresses leptin synthesis or secretion. We failed to demonstrate a delayed effect on leptin concentration at 24 and 48 hours after the exercise bout, which may reflect an inadequate level of energy expenditure. Despite the importance of exercise-induced energy expenditure in the regulation of leptin levels we cannot exclude the possibility that other factors that regulate leptin homeostasis may have accounted for the observed results.

Plasma leptin concentrations change substantially with brief fluctuations in energy intake (Boden, Chen, Mozzoli, & Ryan, 1996; Grinspoon et al., 1997; Jenkins, Markovic, Fleury, & Campbell, 1997; Kolaczynski, Ohannesian, Considine, Marco, & Caro, 1996), sustained weight loss (Christensen, Svendsen, Hassager, & Christiansen, 1998; Okazaki, Himeno, Nanri, Ogata, & Ikeda, 1999; Sartorio, Agosti, Resnik, & Lafortuna, 2003), and vary throughout the day (Sinha et al., 1996) and between days (Ostlund, Yang, Klein, & Gingerich, 1996). Controlling for the above eliminates factors that may mask any exercise-induced effects. We monitored the

daily dietary intake in both groups for a week prior to the intervention. In addition, body composition was assessed in duplicate (a week before and the day of the intervention) to exclude any acute fluctuations in body weight and fat mass, or body hydration. To further evaluate the reproducibility of plasma leptin and evaluate possible daily fluctuations in our sample population, we used two additional pre-exercise samples assessed during the week preceding the intervention. It has also been suggested that the heterogeneity in plasma leptin after an exercise session may reflect shifts in plasma volume and control of fed or fasting state (Fisher, Van Pelt, Zinder, Landt, & Kohrt, 2001). To account for these factors, all samples were assessed after an overnight fast and at the same time of the day (7-9 am), and post exercise samples of the experimental group were corrected for changes in hemoconcentration.

The major difference between our study and all the previous investigations in this area is the characteristics of the participants. All previous studies have used lean individuals of variable training status, while we used sedentary obese males. Obese individuals have higher levels of the *ob* mRNA than their lean counterparts (Lonnqvist, Arner, Nordfors, & Schalling, 1995) and their leptin concentrations in the peripheral circulation is about four-fold higher compared to lean (Considine et al., 1996). Thus, since the expected response to hyperleptinemia is the decrease in energy intake and an increase in energy expenditure, most obese individuals may be insensitive to the endogenous production of leptin (Caro et al., 1996). These observations coupled with the observation that as the degree of obesity increases the variability of leptin concentration also increases (Caro et al., 1996b; Considine et al., 1996), suggest that in the obese state leptin concentration is affected by factors more than the level of adiposity. For

instance, in our study pre-exercise leptin levels at the experimental group vary from 3.0ng/ml to 95.0ng/ml (mean 23.5±30.2ng/ml), which confirms the heterogeneity of leptin levels among our sample population. Thus, it is possible that the interplay between leptin and other hormones, that are known to be dysregulated in the obese state, may have contributed to the observed variability.

Obesity is frequently accompanied by hyperinsuliemia and insulin resistance (Girard, 1997; Zimmet & Alberti, 1996). Prolonged elevations in insulin under euglycemic conditions increase leptin levels (Boden, Chen, Kolaczynski, & Polansky, 1997; Schmitz et al., 1997; Wellhoener et al., 2000). As such, it is possible that baseline insulin may have affected the leptin response to acute exercise. Participants employed in the present investigation had a normal fasting glucose but were not diabetic. We did not demonstrate an association between baseline leptin and insulin concentrations that has been previously documented in several studies (Boden, Chen, Mozzoli, & Ryan, 1996; Laughlin & Yen, 1997; Malmstrom, Taskinen, Karonen, & Yki-Jarvinen, 1996; Widjaja et al., 1997). Despite the lack of association between insulin and leptin levels in our study, we cannot exclude the possibility that insulin levels may be physiologically implicated in leptin metabolism in the obese population. Insulin appears to act directly at the level of the adipocyte by increasing leptin expression and secretion (Kieffer & Habener, 2000). More importantly, evidence exits that leptin and insulin pathways intersect in skeletal muscle (Muoio, Dohm, Tapscott, & Coleman, 1999), which constitutes one of the major tissues exhibiting insulin resistance (Tomas et al., 2004). This intersection may be regulated according to the relative plasma concentrations of the two hormones and their sensitivity in skeletal

muscle. As such, the relative concentrations of leptin and insulin may be particularly important especially in light of the fact that leptin directly stimulates fatty-acid oxidation in muscle (Minokoshi et al., 2002), an action that is antagonized by insulin (Bryson, Phuyal, Swan, & Caterson, 1999; Ceddia, William, Jr., & Curi, 2001; Muoio et al., 1997; Muoio, Dohm, Tapscott, & Coleman, 1999).

Obesity often correlates with abnormalities in the glucocorticoid metabolism (Aldhahi, Mun, & Goldfine, 2004; Rosmond, 2005). Increased cortisol levels have been observed immediately after acute exercise in humans (Atlaoui et al., 2004; Brandenberger & Follenius, 1975; Duclos et al., 1998; Duclos, Corcuff, Pehourcq, & Tabarin, 2001; Duclos, Corcuff, Rashedi, Fougere, & Manier, 1997), while glucocorticoid administration has shown to stimulate leptin gene expression and secretion independently of effects on food intake (Dagogo-Jack, Selke, Melson, & Newcomer, 1997; Miell, Englaro, & Blum, 1996). As such, it is conceivable to speculate that exercise-induced increases in cortisol may alter leptin concentrations. In fed males, 45 min of intense exercise increased serum cortisol concentration threefold. There was a concomitant increase in leptin levels after exercise, which was recovered at 140 min (Fisher, Van Pelt, Zinder, Landt, & Kohrt, 2001). Others, however, have not observed changes in leptin levels in fed subjects, despite a twofold increase in cortisol levels after 3 hours of exercise. Similarly, elevated cortisol levels have been observed immediately post exercise with either no change (Essig, Alderson, Ferguson, Bartoli, & Durstine, 2000), or a decrease (Duclos, Corcuff, Ruffie, Roger, & Manier, 1999) in leptin levels. The discrepancy of the above results may be attributed to physiological factors that have a restraining effect on leptin secretion. Such factors may

blunt the stimulatory effects of glucocorticoids on the hormone's release. For example, catecholamines have also been implicated in the regulation of leptin since administration of isoprenalin in resting humans significantly reduced basal plasma leptin levels by 81% (Donahoo, Jensen, Yost, & Eckel, 1997; Pinkney, Coppack, & Mohamed-Ali, 1998). Epinephrine and norepinephrine increase linearly during prolonged (>60min) moderate intensity (50-60% of VO2max) exercise (Nybo, Nielsen, Blomstrand, Moller, & Secher, 2003; Roy, Green, Grant, & Tarnopolsky, 2001). During exercise in rats, β3-adrenoreceptor blockage prevented the reduction in leptin mRNA in retroperitoneal fat (Bramlett et al., 1999; Donahoo, Jensen, Yost, & Eckel, 1997), suggesting that sympathetic activity may also regulate leptin levels during physical stress. In humans, the effects of catecholamines on leptin levels during exercise are not clear at the present time, but in one study plasma catecholamines were related to circulating leptin in cyclying women but not in men (Donahoo, Jensen, Yost, & Eckel, 1997; Mills, Ziegler, & Morrison, 1998). In another report, however, a 5-day military course significantly reduced serum leptin in male soldiers, and this decrease was attributed to the exercise-induced sustained elevations in catecholamines (Gomez-Merino, Chennaoui, Drogou, Bonneau, & Guezennec, 2002). Similarly, norepinephrine and leptin were correlated in rowers after 2 hours recovery from a 90 min session at 75% of VO2max (Desgorces, Chennaoui, Gomez-Merino, Drogou, & Guezennec, 2004). Collectively, the aforementioned regulatory mechanisms may explain the conflicting data on the impact of exercise on leptin levels, which may depend on the intensity and duration of the stress, as well as, the energy availability immediately before and during exercise.

Baseline plasma leptin concentration positively correlates will all major indices of obesity confirming the findings of others that body weight and especially the amount of adipose tissue explains most of the variation in fasting leptin levels (Liuzzi et al., 1999; Lonnqvist, Wennlund, & Arner, 1997; Niskanen et al., 1997; Ostlund, Yang, Klein, & Gingerich, 1996; Rosenbaum et al., 1996). Pre-exercise plasma leptin was also negatively correlated with VO_2max, a variable that is known to be affected by body weight and body composition. No correlations were observed between insulin concentration and the aforementioned variables.

This project has certain limitations. First, the relatively small sample size that may have weakened statistical power. A larger sample size (i.e. n>20) also may have eliminated the large individual variability in baseline leptin levels that was present among subjects. Second, despite our successful efforts to monitor and control the dietary intake of our subjects by including a daily dietary record in the design, we recognize that absolute dietary control can only be achieved using individual prescribed meals of particular caloric and nutritional content (inpatient metabolic ward). Third, due to the large variability in baseline leptin among subjects of both groups, it might have been more appropriate if subjects would have served as their own controls in a counterbalanced design. Finally, this project could have been more sensitive if more post-exercise sampling points had been included and if other variables (i.e. glucocorticoids) that may affect the leptin response to exercise had also been assessed.

The potential role of exercise on leptin homeostasis in obese individuals remains elusive. More elaborate studies are needed to elucidate whether a threshold of energy expenditure is necessary to bring about changes in leptin levels after an exercise bout. Moreover, the potential interplay

between leptin and insulin should be further addressed by investigating whether obese insulin-resistant males have different responses in leptin levels than their healthy counterparts after an acute exercise bout or multiple, short-term exercise bouts that are known to improve insulin sensitivity.

In conclusion, we examined the effects of a single exercise session, which induced a notable energy deficit, on leptin concentrations in obese males. Exercise had no effect on leptin levels immediately after the exercise session, or at 24 and 48 hours post. These results are consistent with the notion that a significant decrease in leptin levels can be induced only when a threshold of energy expenditure is met and exercise may not be a regulator of leptin concentration *per se*. However, we do not exclude the possibility that other hormonal changes induced by exercise may have affected the outcomes.

APPENDIX: IRB COMMITTEE APPROVAL FORM

IRB COMMITTEE APPROVAL FORM
FOR UCF/OOR/IRB USE ONLY

PI(s) Name: George Kyriazis
Title: The Effects of a Single Exercise Bout on Serum Leptin Concentration in Obese Males.

Check as applicable:

[ ]Yes [ ]No [ ]N/A	Have sufficient assurances been given to the committee to establish that the potential value of this research exceeds the risks involved?
[ ]Yes [ ]No [ ]N/A	Written and oral presentations must be given to participating subjects (parents or guardians, if minors) informing them of the protocol, possible risks involved, the value of the research, and the right to withdraw at any time. Has such a statement been prepared? Was it attached to the "Assurance" form?
[ ]Yes [ ]No [ ]N/A	A signed written consent must be obtained for each human subject participant. Has such a form been prepared? Was it attached to the "Assurance" form?
[ ]Yes [ ]No [ ]N/A	Are cooperating institutions involved? If yes, was there a sheet attached to the "Assurance" form providing the name of the institutions, the number and status of participants, name of the involved official of the institution, telephone, and other pertinent information.

Date of Review by Institutional Review Board (IRB) for Human Subjects: April 25, 2001

[ ] Contingent Approval
Dated: _____

[X] Final Approval
Dated: 5/10/01

[ ] Expedited
Dated: _____

[ ] Exempt
Dated: _____

Committee Members:

Dr. Theodore Angelopoulos:
Ms. Sandra Browdy:
Dr. Jacqui Byers:
Dr. Ratna Chakrabarti:
Dr. Robert Kennedy:
Dr. Gene Lee:
Ms. Gail McKinney:
Ms. Debra Reinhart:
Dr. Valerie Sims:

Chair, IRB

Signed:
Dr. Glenn Cunningham

Addendum to OSR-21/IRB

Revised 04/24/01

REFERENCE LIST

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