This is to certify that the

thesis entitled

MEASUREMENT OF SERUM LEPTIN CONCENTRATIONS IN SEDENTARY AND EXERCISE TRAINED YOUNG WOMEN

presented by

Juanita Maria Rivera

has been accepted towards fulfillment
of the requirements for

M.S. degree in Physiology

Jama M. Rivera
Major professor

Date 12-4-00

MEASUREMENT OF SERUM LEPTIN CONCENTRATIONS IN SEDENTARY AND EXERCISE TRAINED YOUNG WOMEN

By

Juanita Maria Rivera

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

2000

ABSTRACT

MEASUREMENT OF SERUM LEPTIN CONCENTRATIONS IN SEDENTARY AND EXERCISE TRAINED YOUNG WOMEN

By

Juanita Maria Rivera

The study was designed to determine if a relationship exists between serum leptin and energy expenditure at rest and with exercise (acute and chronic) in young women of varying body sizes during both the follicular (F) and luteal (L) menstrual phases. Aerobic capacity (VO_{2max}), residual lung volume, fat mass (FM), fat free mass (FFM), and resting energy expenditure (REE) were measured in 8 subjects. Venous blood samples were drawn before and after 20 minutes of treadmill exercise, at 60% of each subject's VO_{2max}. Serum leptin was determined via a radioimmunoassay. Small sample size prohibited any inferential statistics to be run on the data collected. There was the appearance of a negative trend between leptin/FM and REE/FFM. Although leptin was higher in the L phase compared to the F phase for 3 of the 4 subjects who completed both menstrual phase visits, the absolute differences were small and the percent differences were variable. No exercise-induced changes in leptin were found.

I would like to dedicate this thesis work to my mother, Zelma Rivera. I truly believe her constant support, as well as her many prayers, have been instrumental to all of my accomplishments!

ACKNOWLEDGEMENTS

First, I would like to acknowledge my advisor, Dr. James Pivarnik who is supportive in too many ways to mention them all. In brief, he has been a great teacher and mentor in the areas of research, exercise, and life in general.

I am thankful for all of the advice and direction of my guidance committee: Dr. Dale Romsos, Dr. Lauryssa Kaufman, Dr. Karl Olson, and Dr. Bob Stephenson. In particular, I really appreciate the role Dr. Stephenson has played in my education. Not only has he always been very supportive of my dual degree status, but he has also very diplomatically handled many of the unique issues related to this status.

This research was supported by a Student Award Program grant from the Blue Cross and Blue Shield of Michigan Foundation. In addition, I received financial support from the graduate school, the Department of Education, and the Human Energy Research Lab.

The data collection would not have been possible without the invaluable help of a number of students, the subjects themselves and students who helped with the project. Cooker Perkins was instrumental in advertising for subjects via class professors. Emily Paxton's

dedication to the project was really demonstrated when she agreed to assist in the nutritional food intake analyses. Chad Paton's key contribution was his willingness to help do whatever needed to be done in a very efficient manner. His ability to anticipate what needed to be done and do it without being asked was also very helpful. All three students assisted with advertising, collecting data and entering data into the computer.

Dr. Chris Womack also played a significant role in the gathering of blood samples. I would also like to thank Dr. Won Song (Food Science and Human Nutrition department) and her graduate students (Kanika Triggs and Jean Kerver) for their important help with respect to the standardized meal and data analysis. My husband, Lorenzo Berlanga, has always been there to reassure me through the rough spots and to celebrate my successes.

Karin Allor, fellow graduate student, was very encouraging mentally and physically. Many times her shared stories about her thesis experience kept me thinking positive. She was also very understanding of my endeavors to balance school and family. In addition, she really motivated me to keep in shape, which has done wonders at lowering my stress levels.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

ACSM - American College of Sports Medicine

Avg - average

BMI - Body Mass Index

BTPS - barometric temperature and pressure, saturated

cm - centimeters

CSF - cerebrospinal fluid

db - diabetic i.e. a db/db mouse is an autosomal recessive, genetically, obese mouse

(E+/EB-) - exercise/negative energy balance

(E+/EB+) - exercise/positive energy balance

(E-/EBO) - no exercise/energy balance

(E+/EBO) - exercise/energy balance

EE - Energy Expenditure

ESRD - End-Stage Renal Disease

fa - fatty i.e. a fa/fa rat is an autosomal recessive, genetically, obese rat

FFM - Fat Free Mass

FFQ - Food Frequency Questionnaire

FM - Fat Mass

HE - High body mass index, Exerciser

hr - hour

HR - heart rate

HS - High body mass index, Sedentary

HRT - hormone replacement therapy

Ht - height

ID - The first two letters of each subject ID are the group designation and the last three letters are the subject initials. The group designations are as follows:
LE = low body mass index, exerciser; symbolized ●
LS = low body mass index, sedentary; symbolized □
HE = high body mass index, exerciser; symbolized ▲

Jak - Janus kinase

kcal - kilocalories

kg - kilogram(s)

L - liter(s)

LE - Low body mass index, Exerciser

LH - Luteinizing Hormone

LS - Low body mass index, Sedentary

m - meter(s)

MET - metabolic equivalent (1 MET is equivalent to resting energy expenditure. Most commonly, 1 MET is accepted to be equivalent to 3.5mL of oxygen$*kg^{-1}*min^{-1}$)

min - minute(s)

ml - milliliter(s)

mRNA - messenger RiboNucleic Acid

n - sample size

N_2 - nitrogen

ng - nanograms

NHANES - National Health and Nutrition Examination Survey

NIDDM - Non-Insulin Dependent Diabetes Mellitus

Ob - obese i.e. an ob/ob mouse is an autosomal recessive, genetically, obese mouse

O_2 - oxygen

P_B - barometric pressure

P_{H20} - pressure of water at T_a

REE - Resting Energy Expenditure

RIA - RadioImmunoAssay

RMR - Resting Metabolic Rate

RV - Residual lung Volume

SD - Standard Deviation

SE - Standard Error

SEM - Standard Error of the Mean

STAT - Signal Transducer and Activator of Transcription

T_a - ambient temperature

TEE - Total Energy Expenditure

TG - triglyceride

TM - TreadMill

VCO_2 - volume of Carbon diOxide produced per unit time

VO_2 - volume of oxygen consumed per unit time

VO_{2max} - maximal oxygen consumption per unit of time

vs - versus

Wt - weight

WT - Wild-Type i.e. WT mice are normal weight mice with no obvious abnormalities

yrs - years

INTRODUCTION

Overweight and obesity are important risk factors associated with many diseases affecting women. Hypertension, atherosclerosis, heart disease, diabetes and some forms of cancer are a few of the more significant health problems where increased body weight plays a role. It is estimated that the cost of treating obesity and its complications is about 68 billion dollars annually (113).

According to the most recent (1988-1994) National Health and Nutrition Examination Survey (NHANES III), the crude prevalence of overweight and obesity for women twenty years and older is 50.7% (29). In addition, the prevalence of overweight women has increased 10-15% from the previous decade (109). The Office of Research on Women's Health (part of the National Institutes of Health) recently compiled recommendations for future research on women's health. Not surprisingly, "Identification of successful interventions for long term weight management" and "effects of endogenous and exogenous hormones" were two of the areas of suggested study (92).

Leptin is a recently identified hormone that may play a role in the regulation of body weight (130). Leptin levels are highly correlated with various measures of adiposity. Although the physiological functions of leptin

in humans have not yet been determined, results from animal studies suggest a relationship between leptin and appetite and energy expenditure (EE) (34, 90). Even though leptin levels appear to be correlated with adiposity in steady state conditions, for a given level of adiposity, concentrations can be quite variable. Age, time of measurement, caloric intake, dietary habits, EE, gender, and menstrual phase are some of the factors thought to explain some of the variation. The present study was designed to control for many of these variables in order to gain a better understanding of the relationship between leptin and EE (both at rest and with exercise) in active and sedentary, young women during both follicular and luteal phases of their menstrual cycles.

SPECIFIC AIMS

Specific Aim 1:

To determine the relationship between leptin and resting EE in young women.

Research Questions

Do serum leptin levels account for a significant amount of variance in resting EE among young women, independent of body weight?

Does exercise training modify the relationship between serum leptin and resting EE in young women?

Specific Aim 2:

To determine the relationship between menstrual phase and serum leptin levels in young women.

Research Question

Do serum leptin levels differ in women in the mid-follicular vs mid-luteal phase of their menstrual cycles when subdivided by exercise training and BMI?

Specific Aim 3:

To determine the relationship between chronic exercise training and serum leptin levels in young women.

Research Questions

Do young women of a given body mass index (BMI) (high or low) differ in serum leptin levels due to exercise status?

Do young women of a given exercise status (exercise trained or sedentary) differ in serum leptin levels due to BMI status?

BACKGROUND AND SIGNIFICANCE

Parabiosis experiments

From the late 1950's through the 1970's, parabiosis experiments were very popular. These experiments involved surgically pairing two animals at the hip and shoulder. Eventually, the two animals shared the same blood supply. Parabiosis experiments were commonly used to provide evidence for blood-borne transport of the particular substance being studied. Many researchers during this time period utilized parabiosis techniques to further their understanding of how body weight is regulated.

In a classic physiology experiment in 1958, Hervey parabiosed two rats (41). Later, he destroyed the ventromedial hypothalamus of one of the rats. The lesioned rat developed obesity and the unlesioned rat starved to death. The results of this study led Hervey to propose a circulating "satiety" factor that interacted with the hypothalamus. He hypothesized that this "satiety" factor was produced in excess in the lesioned rat as it became more obese, and the excess "satiety" factor played a role in the death of the unlesioned rat.

Although one can create obese animals, many animal models of obesity already exist. Three autosomal recessive, genetically, obese rodent models that are

commonly studied are ob/ob mice, db/db mice and fa/fa (also known as Zucker) rats. Ob is short for obese, db is short for diabetic, and fa is short for fatty. These animals have other physiological problems besides obesity. For example, ob/ob mice have abnormal reproduction (sometimes infertility), hypothermia, stunted linear growth, hormonal abnormalities, locomotor retardation, and non-insulin dependent diabetes mellitus (NIDDM) with severe insulin resistance (32).

In 1959, Hausberger parabiosed obese mice with wild-type (WT), normal weight mice (37). Weight gain in the obese mice was suppressed. Therefore, Hausberger hypothesized that there was something missing in the blood of the obese mice that appeared to be transferred from the WT mice. In 1969, Coleman and Hummel parabiosed db/db mice with WT, normal weight mice (18). The db/db mice remained obese and surprisingly, the WT mice died of starvation. The authors postulated that the WT mice died due to a large amount of a "satiety" factor, while db/db mice were resistant to this "satiety" factor. In a review article by Caro, Harris, et al. reported similar results when they parabiosed fa/fa rats with WT, lean mice (11).

Basic characteristics of leptin

In 1994, the "satiety" factor that had been postulated in previous studies was identified and cloned (130). The researchers named it leptin after the Greek root, leptos, meaning thin (30). Leptin is an 167 amino acid, 16-kilodalton, protein product of the ob gene that is primarily produced by adipocytes (130). A large amount of leptin synthesis has also been documented in the placenta (74, 108). In addition, leptin mRNA has been found in human granulosa and cumulus cells of ovarian follicles (77, 96). Human leptin is 84% identical to mouse leptin (130). Both forms of leptin have a secretory signal sequence (130).

Circulating leptin levels are highly correlated with fat mass (FM), percent body fat, and BMI (20, 71, 86, 98). In contrast, leptin is not correlated with visceral fat, waist to hip ratio, abdominal-to-total-body FM ratio (14), or upper or lower body adiposity (110). Using a stepwise multiple regression analysis, Rosenbaum et al. found that percent body fat and BMI did not explain any additional variation beyond that attributable to FM (98). In contrast, Kennedy et al. found that BMI independently accounted for 35% of the variability in serum leptin levels (55). Although many researchers have documented a

relationship between adiposity and leptin, there appears to be a threshold for this relationship, as there is no correlation between leptin and BMI in anorectics with extremely low BMI (70).

When recombinant leptin is given to ob/ob mice, not only do they lose weight and decrease food consumption (9, 34, 90), they also increase thermogenesis (34, 90), and locomotor activity (90). The increased physical activity may be secondary to the increased ability to move around after losing a significant amount of weight.

Since ob/ob animals were found to be deficient in leptin (19), researchers expected that obese individuals would have low leptin levels. However, there are very few documented cases of leptin deficiency in humans. Nevertheless, when individuals are leptin deficient they definitely have a problem with obesity. For example, Montague et al. studied two children (from the same highly consanguineous family of Pakistani origin) with leptin deficiencies that were morbidly obese (80). One child weighed 86kg at age eight (80).

In contrast, the majority of obese individuals studied tend to have high leptin levels. Considine et al. found serum leptin levels in obese individuals to be four times higher than those found in their lean counterparts (31.3 $\pm$

24.1(SD) ng/ml vs 7.5 ± 9.3 ng/ml, respectively) (19). Similarly, Dagogo-Jack's lab also reported a significant difference in plasma leptin levels between obese and lean individuals (37.2 ± 3.6(SE) ng/ml vs 14.2 ± 2.2 ng/ml, respectively) (21). The apparent contradiction between ob/ob mice and humans has been rationalized by hypothesizing that obese individuals are somehow resistant to their leptin.

Potential mechanisms of leptin resistance

Leptin resistance could be secondary to problems with the leptin receptor (i.e., downregulation, defective binding), problems with signaling, inadequate availability (i.e., increased clearance, decreased production), or irregular activity of circulating leptin (50). Furthermore, changes in binding proteins could influence leptin availability or activity.

Sinha et al. showed the majority of total leptin (60-98%) in sixteen lean subjects (<21% fat) is in the bound form (112). On the other hand, thirty obese subjects with leptin levels >35 ng/ml had the majority of their leptin in the free form. Bound and free leptin were both significantly higher in obese vs lean subjects. If the free form of leptin is the active form, then perhaps this provides evidence for leptin resistance in obese

individuals. Saad et al. also documented suppressed diurnal amplitude for leptin in obese vs lean individuals (102).

Houseknecht et al. determined the majority of serum leptin in humans is found attached to binding proteins of approximately 176kDa and 240kDa (50). In a study of seven Caucasian women, they also found free leptin increased with increasing BMI. This finding provides evidence for possible saturation of binding proteins in obese individuals.

Schwartz et al. found leptin transport capacity was decreased in obese humans as evidenced by lower cerebrospinal fluid (CSF)/serum leptin ratios in obese vs normal-weight subjects (107). Therefore, the authors hypothesized a saturable mechanism of CSF transport. Furthermore, they proposed decreased leptin transport in obese individuals could lead to apparent leptin resistance. Karonen et al. also found evidence that uptake of leptin in the choroid plexus is saturable (54). Defects in leptin central nervous system transport have been demonstrated in New Zealand obese mice (77).

Metabolism of leptin

Licinio et al. determined leptin is released in healthy men (n = 6) in a pulsatile fashion with 32.0 $\pm$ 1.5

(SEM) pulses every 24 hours and a pulse duration of 32.8 ± 1.6 minutes (66). In addition, the average interpeak interval was 43.8 ± 1.8 minutes. Sinha et al. also demonstrated leptin pulsatility, but they only measured two to seven oscillations per 12-hour period, with a mean period of 3.5 hours (110).

Licinio et al. also published data from a pilot study comparing pulsatility between one normal-weight woman and one obese woman whose leptin levels were seven times greater (66). The authors did not find any differences in concentration-independent pulsatility parameters including pulse number/24 hours, pulse duration, or interpeak interval. The authors concluded the increased leptin levels in the obese woman were due solely to increased leptin pulse height rather than disruptions in pulsatility.

Klein et al. estimated the human leptin production rate to be 3.2 ± 0.5 ng/100g fat/min and the half-life to be about 25 minutes (129). In a rat study by the same group, the failure of an injection of ^{125}I-leptin to appear immediately in the urine suggests leptin is not eliminated by glomerular filtration alone (129). This group believes their kinetic studies provide evidence for active uptake of leptin by the renal tissues.

Karonen et al., discussed studies by Cumin et al. and Van Heek et al. that showed leptin clearance primarily by the renal system (54). The review article by Sharma also discussed work by Cumin et al. which showed an 81% decrease in leptin clearance after bilateral nephrectomy in rats (109). In a separate rat study, Cumin et al. estimated renal clearance of leptin to be 5.3 ml/min/kg, whereas, whole body clearance was estimated to be 5.4 ml/min/kg (109).

Sharma and Considine investigated the leptin levels of patients with end-stage renal disease (ESRD) (109). The finding that patients with ESRD have 4 fold higher leptin levels supports the hypothesis that the kidney is a primary site of clearance. This effect remained after correction for BMI.

Leptin receptor characteristics

Leptin receptors have been found in animals in the hypothalamus, adipose tissue, gonads, heart, liver, kidney, spleen (39), skeletal muscle (27), adrenal medulla, and pancreatic islets (13). At least 6 splice variants of the leptin receptor have been identified (named Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re) (39). In humans, leptin receptors have been identified in the hypothalamus and gonads (3, 118).

Some forms of the leptin receptor are structurally similar to class I cytokine receptors, with intracellular janus kinase (Jak) and signal transducer and activator of transcription (STAT) motifs (35). The Ob-Rb receptor, also known as the long leptin receptor, contains Jak and STAT motifs (109). The Ob-Ra receptor, also known as the short leptin receptor, contains the Jak, but not the STAT motif (109). The Ob-Ra receptor is thought to play a role in transporting leptin across the blood brain barrier (109). The Ob-Re receptor does not have either a transmembrane or intracellular domain and is thought to function as a soluble receptor or binding protein (109).

As hypothesized db/db mice and fa/fa rats were found to be resistant to their "satiety factor" secondary to mutations in the leptin receptor (19). The mutated receptor in db/db mice does not have the cytoplasmic domain that contains the Jak and STAT signaling motifs (109).

Clement et al. reported a homozygous mutation in the human leptin receptor in three of nine siblings (all female) who were members of a consanguineous family of Kabilian origin with a strong prevalence of morbid obesity at an early age (17). They also found the siblings to have no pubertal development through age 19 and decreased growth hormone and thyrotropin secretions. Other family members

that were heterozygous for the mutation in the leptin receptor were obese, but not morbidly obese, which suggests that one normal allele for the receptor is sufficient for some regulation of body weight.

Age and leptin

Due to conflicting results, the relationship between age and leptin is yet to be determined. Lindroos' and Considine's labs both found a significant positive correlation between age and serum leptin levels (20, 69). Ostlund et al. and Ryan and Elahi found an inverse relationship between age and plasma leptin levels (86, 101). Martin et al., Rosenbaum et al., Roberts et al., and Sumner et al. did not find any significant association between age and leptin levels (73, 97, 98, 115). According to the review article by Sinha, Caprio et al. did not find any significant differences between preadolescent, adolescent, and young adults (110). The diverse results may be due to the fact that only three of the preceding studies had an age range large enough to allow the likelihood of finding a significant relationship with leptin levels (97, 98, 101). Until the relationship between age and leptin is clarified, investigators would be prudent to control for age.

Diurnal variation of leptin

Many investigators have shown a diurnal pattern in leptin levels. Sinha et al. measured serum leptin every 1-2 hours in individuals who were lean (n = 4), obese (n = 11), and obese with NIDDM (n = 9) (111). Lowest leptin values were seen at 2:30 PM, 1 PM, and 2:30 PM, respectively for each group. The peak values were found at 2:30 AM, 12:30 AM, and 4:30 AM, respectively for each group. Sinha et al. suggested that the increase in leptin levels in the early morning hours may represent a delayed response to dinner and may be important for appetite suppression while sleeping (111).

Schoeller et al. investigated variation in leptin with day/night reversal of meal timing in four young men (104). They found the diurnal variation in leptin was shifted 4-7 hours (similar to the meal time shift of 6.5 hours). This study suggests leptin's nocturnal rise may be related to meal timing. This interpretation is supported by the studies of Boden et al. Grinspoon et al. and Bergendahl et al., which found decreased diurnal variation with fasting (5, 7, 33).

Licinio et al. measured plasma leptin levels every seven minutes during mid to late follicular phase (days 8-11) in six, lean young women (67). They found peak leptin

levels at night (between 1 and 2 AM). The lowest leptin values were found ~8 AM. The average nighttime (11 PM-8 AM) leptin concentration was 10.2 $\pm$ 1.7 (SD) ng/ml. The average daytime (8 AM -5 PM) leptin concentration was 7.63 $\pm$ 1.20 ng/ml.

Saad et al. measured plasma leptin levels in 31 moderately obese men and women every 20 minutes for 24 hours (102). This group found a nadir in leptin levels between 8 AM and 2:40 PM (with a median of 10:33 AM) and peaks between 11 PM and 3:00 AM (with a median of 1:20 AM). In all the studies mentioned in the previous few paragraphs, leptin levels were relatively stable from 8:30 AM to 12 PM.

van Aggel-Leijssen et al. measured plasma leptin levels in eight lean, young, previously sedentary males every hour during the day and every two hours during the night (125). They found peak leptin levels around 1 AM with a nadir around 11 AM. Hilton and Loucks documented similar diurnal variation of plasma leptin levels in sixteen, lean, young women (47).

Fasting and leptin

Even though leptin is highly correlated with adiposity, it can be influenced by changes in energy balance. Kolaczynski et al. measured serum leptin after

36- and 60-hour fasts in lean and obese individuals (59). They observed a significant decrease in serum leptin after 12 hours (~20%). After 36 hours of fasting, serum leptin levels decreased 72-84%. Fasting beyond 36 hours did not lead to any further reduction in serum leptin. Furthermore, the decrease in leptin was prevented by an infusion of a small amount of glucose. In addition, the decrease in leptin did not correlate with percent body fat.

Sinha et al. did not find any significant differences in the bound fraction of leptin in lean or obese individuals after a 24-hour fast (112). In contrast, they found a significant decrease in free leptin levels in both lean and obese individuals (10.6 $\pm$ 1.9 (SEM) to 1.3 $\pm$ 0.4 ng/ml; 28.3 $\pm$ 9.8 to 14.7 $\pm$ 14.7 $\pm$ 5.3, respectively). Furthermore, the decrease in the lean individuals was significantly more marked than in the obese individuals.

Boden et al. documented a 60-70% decrease in plasma leptin with a 52-hour fast in normal weight and obese individuals (7). This group also studied four normal subjects who fasted for 72 hours, but with their glucose levels clamped. The serum leptin of these subjects did not change, suggesting a relationship between glucose regulation and/or insulin and leptin.

Grinspoon et al. measured decreased (40%) plasma leptin after a four day fast in normal-weight women (33). The decrease was almost reversed (80%) within 24 hours after glucose infusion. Weigle et al. also showed a suppression of leptin's diurnal variation after a 3 day fast in normal weight women (126). Twelve hours after refeeding, there were no differences in leptin levels compared to the control values. Bergendahl et al. measured leptin levels after a 2.5 day fast in eight, young, lean women during their midluteal phase (5). Serum leptin decreased ~78% from 15.2 $\pm$ 2.3 (SEM) to 3.4 $\pm$ 0.6 ng/ml.

Wisse et al. measured plasma leptin levels in 21 obese, older women after one of three conditions, lasting four weeks: a total fast, a very low energy diet, or a low energy, balanced diet (128). Mean plasma leptin decreased up to 66% by week one, but continued to decrease gradually over the four weeks. The magnitude of these decreases was not consistent with changes in FM over the same time period. In addition, this group did not find any relationship between increased ketone bodies and leptin.

Many researchers have reasoned that since leptin levels and adiposity do not always decrease in parallel, there must be other factors involved. Caro et al. hypothesized a dual role for leptin (11). They believe

leptin plays different roles when the body is in a state of energy balance, compared to when it is in a non-steady state condition. Specifically, this group proposed during steady state, leptin represents adipose stores and when energy balance changes, this relationship is modified.

Acute meal effect and leptin

Results from studies designed to assess acute meal-related changes in leptin are conflicting. Dagogo-Jack et al., Sinha et al., Maffei et al., and van Aggel-Leijssen et al. took venous blood samples from 1-3 hours after a meal (21, 71, 111, 125). None found acute meal-related changes in leptin levels. Considine et al. took a blood sample at time zero, immediately followed by breakfast, then followed by lunch four hours later (20). No meal-related increases were found with eight hourly measurements. Similarly, Weigle et al. found no effect of a high or a low fat meal 4-6 hours after the meal (126). Melanson et al. did not find a meal-related effect on leptin levels during 8-9hours after a low-carbohydrate meal (on the day after glycogen depleting exercise) (76). On the other hand, when Saad et al. and Laughlin et al. took 24hr blood samples, both groups concluded there were meal-related increases in leptin, but the effect was delayed 2-4 hours (63, 102).

Dallongeville et al. performed a study similar to that of Schoeller et al. but with a greater number of subjects (22). This group studied whether time of feeding affects the leptin response to a meal. Thirteen, non-obese male subjects were studied on four occasions. They had blood samples taken every twenty minutes for eight hours following the ingestion of a test meal or water at 12:50 AM or PM. The investigators did not find a significant difference in leptin levels between day and night concentrations, but they did find a meal related increase (after four hours) during both times. The authors suggested that leptin levels may be more acutely affected by food intake compared to a circadian rhythm. As with Schoeller et al., Dallongeville et al. suggests the diurnal effect could really be secondary to a slow and cumulative effect of diurnal meals. They also suggested differences between studies could be secondary to differences in subject pools (lean vs obese), inadequate follow-up/ measurement times, sampling intervals, or lack of test meal standardization. Although these conclusions are conflicting, many studies attempt to control for the potential variation of an acute meal effect by measuring leptin levels in the early morning after a 10-14 hour fast.

Two studies were designed to measure changes in leptin levels with overfeeding. Massive overfeeding (120 Kcal/kg) over twelve hours increased circulating leptin about 40% (60). Increases were seen between the 5^{th} and 10^{th} hours, but leptin levels remained elevated through 24 hours.

Ohannesian et al. measured serum leptin before and after a ten percent weight gain, secondary to overfeeding (84). The weight gain was achieved over a 4-6 week period. The second leptin measurement was not taken until after a two- week maintenance period. Serum leptin significantly increased with overfeeding (3.8 $\pm$ 1.0 (SE) vs 6.4 $\pm$ 1.9 ng/ml).

Diet (chronic meal effect) and leptin

Results among studies reporting on the relationship between diet composition and leptin are also mixed. Although leptin levels increased in rats after 12 weeks on a high fat diet, this effect was not independent of changes in body fat (105). The review article by Remesar reported that Masuzaki et al. also found carbohydrate feeding resulted in increased leptin in rats (95). Most studies with humans that reported dietary effects had subjects complete food records, but this was not their main focus. None showed a relationship between dietary composition (i.e. % fat, % carbohydrate, or % protein) and leptin

levels (52, 63, 73, 86). However, when overall calorie intake was decreased 50%, the change in serum leptin levels did correlate with the change in carbohydrate levels (52). Niskanen et al. also found that after adjustment for age, sex, FM, and fat free mass (FFM), leptin and carbohydrate oxidation rate were negatively correlated ($r = -0.41$, $p<0.01$) (83).

Only a few studies have been designed specifically to look at the effect of diet on leptin levels. Schrauwen et al. measured plasma leptin in twelve lean individuals before and after seven days on a high fat diet (60% of total caloric intake) (105). The investigators manipulated total calories to maintain weight (energy balance) in all subjects. Leptin levels after seven days were not significantly different from baseline. Likewise, Weigle et al. did not find any relationships between plasma leptin and percent fat intake (126). Furthermore, Havel et al. did not find any relationship between plasma leptin levels and dietary fat content (31%, 23%, or 14%) in normal weight and overweight, postmenopausal women after 4-6 weeks on each respective diet (38).

In contrast to human studies, Dyck and Steinberg investigated the effect of high fat diets on leptin levels in rodents (27). They found different effects depending on

whether the fat primarily came from safflower (an N-6 fatty acid) vs fish oil (an N-3 fatty acid). Increasing leptin in rodent muscle usually stimulates fatty acid oxidation and inhibits triglyceride (TG) synthesis. However, the group fed a high safflower diet had increased leptin levels, yet did not have increased fatty acid oxidation or any additional inhibition of TG synthesis compared to control rats. In partial contrast, rodents fed a diet high in fat from fish oil did not have an increase in leptin levels, nor did these animals have an increase in fat oxidation or decreased TG synthesis (vs control).

Hickey et al. also found a differential effect on leptin levels depending on type of fat ingested (43). This group assessed leptin levels in seven obese and nine non-obese individuals after four days of dietary manipulations. After determining each subject's usual caloric intake, the investigators designed two diets for each subject. Both diets consisted of the same total energy and total percent energy from fat. One diet consisted of normal levels of an omega-3 fatty acid, alpha linolenic acid (~1/2% of fat). The remainder of fat came from linoleic acid, an omega-6 fatty acid. The other diet consisted of a 10-fold increase in alpha linolenic acid. The isocaloric diet with increased alpha linolenic acid increased leptin levels 30%

in lean individuals only. The authors felt the timing of this difference in responsiveness might provide information about the mechanisms of how diet may influence leptin levels.

Energy Expenditure (EE) and Leptin

Kennedy et al. measured serum leptin in 116 lean and obese individuals (54 women and 62 men) (55). The authors found no correlation between fasting leptin levels and resting energy expenditure (REE). Moreover, they found that 81% of the variation in leptin among individuals could be explained by percent fat and gender. Rosenbaum et al. measured changes in plasma leptin and EE before, during, and after weight gains and losses (including after maintenance of altered body weights)(99). They did not find any correlation between absolute or relative changes in plasma leptin and changes in EE.

Paolisso et al. did not find any significant correlation between plasma leptin and basal metabolic rate during the follicular, periovulatory, or luteal phases in sixteen, healthy, lean, sedentary, young women (88). Martin et al. measured serum leptin levels in 27 older females. They felt their data suggested a relationship between total energy expenditure (TEE) and leptin (after controlling for percent fat) (73).

Butte et al. studied RMR and serum leptin in 65 pregnant women (8). After adjustment for FM and FFM, they did not find resting metabolic rate (RMR), nor TEE (24 hour) to be significantly correlated with leptin levels. In addition, Highman et al. found a significant positive relationship between serum leptin and EE in ten females before and during pregnancy (46). Many of these subjects were obese prior to pregnancy. When the authors adjusted for FM, the relationship changed to a significant negative correlation. Since leptin levels are strongly influenced by adiposity, the relationship found after adjustment for FM is likely to be more accurate.

Toth et al. studied the relationship between leptin and REE in 59 middle-aged non-obese premenopausal women (121). Although they found serum leptin explained ~8% of REE variation (via stepwise regression analysis), these authors questioned the physiologic significance of this relationship. Toth et al. concluded that leptin's contribution to REE in this study population is minimal.

In contrast, Niskanen et al. found an inverse association ($r = -0.324$) between serum leptin in 45 obese individuals (35 women and 10 men) and REE (after adjusting for FM, age and sex) (83). Campostano et al. measured REE and serum leptin in 43 premenopausal women and twenty men.

Although they found a positive association between REE and leptin, when adiposity was taken into account this relationship disappeared (10).

Though Jorgensen et al. measured leptin levels in men and women, they only found leptin to be significantly correlated with RMR in men (53). Torjman et al. found leptin to be significantly negatively correlated ($r = -0.81$) with REE in young, lean, sedentary men (120).

Nicklas et al. investigated the relationships between plasma leptin and EE in 46 sedentary, African Americans (25 women and 21 men) (81). After adjustment for FFM, these authors found a significant relationship between leptin and REE only in females. In addition, they found a trend towards significance between TEE and leptin (again only in females).

All of the above mentioned studies either measured REE in sedentary individuals or did not report the activity levels of their subjects. Currently, there are no studies that have determined the relationship between REE and leptin levels in exercise trained women.

Gender and leptin

Early studies did not find a gender difference in leptin levels in fetal amniotic fluid or in adult serum (20, 106). Despite these results, many studies have

documented a gender difference in plasma or serum leptin independent of adiposity (21, 40, 44, 83, 97-99, 102, 115). The review article by Macut reported that Clayton et al. measured decreased leptin levels during puberty in boys, while they remained constant in girls throughout puberty (70). Schwartz et al. also documented a gender difference in CSF leptin levels independent of BMI (107). Ostlund et al. found leptin levels to be three times higher in women than in men (17.1 $\pm$ 14.8(SD) ng/ml vs 5.8 $\pm$ 4.4 ng/ml, respectively) (86). So far, gender differences have been documented in Caucasians, Mexican Americans, Samoans (115), Pima Indians (31), and African Americans (81).

Some authors have found gender differences only with a certain body habitus. Maffei et al. found a gender difference after controlling for BMI, but this difference disappeared after controlling for percent body fat (71). Kennedy et al. found serum leptin levels to be higher in females compared to males when the percent body fat of both groups was greater than 25% or BMI was greater than twenty (55). According to the article by Rosenbaum et al., Lonnqvist et al. also found greater leptin mRNA in obese females compared to obese males, but no difference in lean individuals (98).

The female reproductive system and leptin

Female mice injected with leptin show earlier signs of puberty than normal (77). According to the review article by Messinis et al., using food-deprived female rats (a delayed puberty model) Cheung et al. reported leptin is not the first signal of puberty onset, but it probably plays a permissive role (70, 77).

The article by Teirmaa reported Lalou et al. found an association between female puberty and increased serum leptin in lean and obese children (118). The review article by Messinis, reported that Matkovic et al. prospectively studied serum leptin levels in 343 prepubertal girls over 4 years (77). Matkovic et al. found that for every 1ng/ml increase in serum leptin, age of menarche decreased by one month.

Andreelli et al. evaluated reproductive function and plasma leptin in two women with lipoatrophic diabetes (2). Secondary to their disease status, the women had complete atrophy of subcutaneous and visceral adipose tissue. Although both women had leptin levels <1 ng/ml, they had normal menstrual function and one successfully delivered three children. The authors concluded that leptin is not the sole trigger of puberty. In addition, factors other

than leptin must also be involved with obesity related infertility problems.

Clapp and Kiess found leptin levels increased linearly through 36 $\pm$ 1 (SEM) weeks (from prepregnancy values of 8.2 $\pm$ 0.9 ng/ml to 21.4 $\pm$ 1.8 ng/ml near term) (16). Sattar et al. documented decreased leptin levels by six months after delivery compared to near term measurements (~35 weeks gestation) (103). No earlier time points after delivery were studied.

Results from studies designed to assess the effect of menopausal status on leptin levels are somewhat conflicting. Kohrt et al., Castracane et al., and Hickey et al. found no statistically significant difference in leptin levels between premenopausal and postmenopausal women (12, 45, 57). In contrast, Rosenbaum et al. found plasma leptin levels were significantly higher in premenopausal women than postmenopausal women (98).

Castracane et al. found no difference in serum leptin levels when comparing young women who were or were not on standard low-dose estrogen-progesterone oral contraceptives (12). Similarly, Thong et al. did not find any differences in plasma leptin levels of recreational athletes, when comparing samples taken during the active birth control pill phase with the placebo pill phase (119). Leptin

levels were similar despite a 400% difference between 17αethinylestradiol between active and placebo pill phases.

Castracane et al. also did not find any differences in postmenopausal women who were or were not receiving hormone replacement therapy (HRT). The studies of Kohrt et al. and Kraemer et al. supported these results of no effect of HRT on leptin levels (57, 61). Taken together, these findings provide evidence against a relationship between estrogen and leptin.

In contrast, Paolisso et al. found a positive relationship between leptin and sex hormones in 39 non-obese females during the follicular phase of the menstrual cycle (87). After controlling for age, body fat, and waist-to-hip ratio, they found a positive correlation between leptin and fasting plasma estradiol.

Licinio et al. observed a significant change in the pulsatile profile of luteinizing hormone (LH) when peak leptin levels were present (66). LH pulses were fewer, longer in duration, higher in amplitude, and had a four-fold larger area at night than during the day. This relationship between leptin and LH was observed in all six subjects studied during the follicular phase.

Stock et al. measured serum leptin in thirteen, lean women on days 1-3, 6-8, 13-15, and 22-25 of the menstrual

cycle (114). Although, the authors found a small overall variation in leptin levels during the menstrual cycle, there were no statistical differences when the phases were compared.

Teirmaa et al. also did not find any differences in serum leptin during the menstrual cycle in eight, lean, normally, menstruating, young women (118). However, they did note a small insignificant trend (~1.25-fold increase) to higher leptin levels at the end of the cycle vs the beginning of the cycle. Perhaps this lack of significant difference was because they did not take their samples during the middle of the various menstrual phases. Blood samples were obtained between days 2 and 5, days 14 or 15, and between days 24 and 26. However, the authors propose the difference may simply be random variation. In support of their opinion they cite other data collected (by them) that showed an approximately 1.25-fold difference in leptin levels was found in women who were taking oral contraception, at two physiologically identical time points, one month apart.

Hardie et al. has shown that leptin levels in lean women are significantly higher in the luteal phase (36.7 $\pm$ 4.7 (SEM) ng/ml) vs the follicular menstrual phase (22.9 $\pm$ 3.5 ng/ml) (36). According to the article by Teirmaa et

al., Schimizu et al. also found a statistically significant (51 ± 14% (SEM)) increase in the luteal phase vs the follicular phase (12.2 and 9.2 ng/ml respectively) leptin levels (118).

Riad-Gabriel et al. measured significantly higher plasma leptin levels in nine women during the luteal vs follicular phase (20.4 ± 4.2 (SEM) ng/ml vs 14.9 ± 2.9 ng/ml, respectively) (96). In addition, they did not find any relationship between the changes in leptin and changes in sex hormones. These authors hypothesized the increased leptin may have been secondary to leptin release from mature ovarian follicles.

Quinton et al. found significantly higher serum leptin levels in the luteal vs the follicular phase (median 11.4 ng/ml vs 10.0 ng/ml, respectively) in seventeen middle-aged women of varying BMI (93). But, there was a wide range of leptin values (4.9-66.7 vs 4.3-32.2, luteal vs follicular, respectively). Furthermore, three women failed to show a difference between the follicular and luteal phases. Messinis et al. found increased serum leptin levels at the end of the follicular phase compared to the early follicular phase and the day of the LH surge in nine, lean women with unexplained infertility of 5-8 years (78).

Mannucci et al. measured serum leptin in 18 lean women on days 3, 10, 17, and 24 of one menstrual cycle (72). These authors found a 23 $\pm$ 7% (SEM) significant increase in the late follicular phase (day 10). Leptin continued to rise through the 24^{th} day. The increase was not related to BMI.

Thong et al. documented average increases (40-46%) in the luteal phase compared to the follicular phase of the menstrual cycle in normally cycling elite (n = 8) and recreational athletes (n = 13)((119). However, there was interindividual variation, including three individuals whose leptin levels were lower in the luteal phase compared to the follicular phase and several athletes whose plasma leptin only increased 4-7% in the luteal phase. These indiscrepancies may have been secondary to the way the luteal phase was estimated (using a date that was three-quarters of the average cycle length). Despite the large average difference between menstrual phases, no correlation between leptin and sex hormones or changes in leptin and changes in sex hormones were found in either menstrual phase.

Exercise and leptin

My primary interest is the relationship between exercise and leptin. Therefore the exercise section is

considerably longer than the preceding ones. I have subdivided the sections by gender and exercise type. There are five instances where human studies involved both male and female subjects. These studies were put in one section, but the gender of all subjects is detailed with each study. Cross-sectional studies, acute exercise (in previously sedentary and chronically active individuals), and training programs are discussed separately. Within each subheading the individual studies are grouped with similar studies, with respect to similar intensity of exercise, sampling methods, or publication date.

Cross-sectional studies of sedentary and physically active men

Two labs have done cross-sectional studies comparing leptin levels of sedentary to physically active men. Leal-Cerro et al. compared leptin levels between 22 sedentary, non-obese males and 29 nonprofessional, male athletes trained for marathon running. When levels were expressed as leptin per kg body fat, the authors did not find any difference between the subject groups (64). Berman et al. found decreased leptin levels in older, male masters athletes compared to age matched lean, sedentary males, but the differences did not persist after controlling for

percent fat (6). In summary, no cross-sectional study has shown differences in leptin levels between sedentary and physically active men after adjustment for adiposity.

Acute exercise and leptin in men

Racette et al. looked at plasma leptin levels in five sedentary men, before and after 60 minutes of moderate exercise at 50% maximum heart rate (94). This group did not find any change in leptin production in the abdominal adipose tissue using an arteriovenous balance technique.

Hickey et al. measured serum leptin before and after a 20 mile treadmill (TM) run at 70% VO_{2max} in thirteen male subjects (42). These authors found no acute effect of exercise on leptin levels. Because leptin levels in these subjects were initially low, the ability to measure a difference after exercise may have been impaired. Melanson et al. designed a study to assess appetite and glucose profiles after glycogen depleting exercise (76). In addition they measured plasma leptin levels. The authors did not find any differences in plasma leptin levels in ten men after one hour of moderate (50% VO_{2max}) bicycle exercise, the day after glycogen depleting bicycle exercise.

Koistinen et al. investigated the effect of exercise on serum leptin in nine healthy, young, lean men and eight type one diabetic, young, lean men (58). Each subject pedaled on a cycle ergometer for 3 hours at 450-600 kpm/min. Serum leptin significantly decreased 42% in the healthy subjects and by 23% in the diabetic subjects. The decrease in serum leptin (12%) in separate control groups (who did not exercise) was significantly different from the healthy subjects, but not the diabetic subjects. The authors hypothesized the difference between the normal and diabetic subjects could have been secondary to insulin differences between the groups. However, the authors also considered the possibility that type I diabetic patients may be more resistant to exercise-induced falls in leptin.

Landt et al. examined the relationship between serum leptin and exercise in two different ways (62). One part of their study involved measuring leptin before and after a one-hour bike ride at 75% VO_2 max in twelve males. There was no mention of previous physical activity in these subjects. Under these conditions, the authors did not find changes in leptin levels. In the second part of their study, Landt et al. found that leptin levels decreased in fourteen men after an ultramarathon run (101 miles). The average decrease in leptin concentration was 32%. Leptin

before the race was 2.64 ± 0.94(SD) ng/ml vs 1.8 ± 0.45 ng/ml after the race.

Hilton et al. investigated the effect of carbohydrate availability on leptin levels during acute exercise in nine, young, sedentary men (48). Each subject underwent a control session and four exercise bouts. Two exercise bouts were at 50% VO_{2max} and two were at 70% VO_{2max}. At both exercise intensities, there were two levels of energy availability. During the high carbohydrate availability bout, subjects consumed a dextrose solution equivalent to the exercise expenditure amount (30kcal/kg of lean body mass). During the low carbohydrate availability bout subjects consumed a placebo solution. Leptin decreased with exercise compared to the control session, and the authors concluded carbohydrate availability predicts the leptin response to acute exercise. Specifically, for every 1 kcal/kg of lean body mass of carbohydrate availability increase, leptin decreased 0.07 ng/ml. Although this relationship was found to be significant, it was pretty short-lived, as the authors found leptin levels increased towards baseline after about two hours post exercise.

Three investigative groups measured leptin levels before and after a marathon race. Leal-Cerro et al. found leptin to be significantly decreased in young, lean males

(2.9 ng/ml vs 2.6 ng/ml) (64). The authors felt this decrease was underestimated because they did not adjust for the hemoconcentration that occurred. In contrast, Tuominen et al. found similar baseline serum leptin levels before and after a marathon in twelve men (123). However, they found leptin levels increased 35-45% during an euglycemic, hyperinsulinemic clamp study. Therefore, these authors concluded that leptin was correlated with the increased EE under maintained insulin conditions.

Similarly, Koistinen et al. assessed leptin levels in fourteen healthy, lean males, and seven (one female, six males) type one diabetic patients before and after a marathon (58). No differences in leptin levels were found after the marathon compared to baseline values in either group. The authors hypothesized any small changes in leptin with exercise may have been masked by other variables. For example, increased cortisol, which in animals has been shown to increase ob mRNA and leptin secretion, could counterbalance an exercise-induced decrease in leptin (58). Kolaczynski et al. hypothesized the lack of change could have been due to food intake during the marathon, since small amounts of glucose appear to prevent the decrease in leptin with fasting (59).

Five groups measured leptin levels for extended time periods after an acute exercise bout to assess any delayed response secondary to exercise. Torjman et al. measured serum leptin levels in six, lean, untrained males during a control session and before and after prolonged TM exercise (one hour at 50% of VO_{2max}) (120). Blood samples for the two sessions were taken every hour, for four hours, at the same times on the two different days (SE $\pm$ 6-7 minutes). Although they found a decrease in leptin levels four hours after the exercise, the decrease was not significantly different from the control group.

Duclos et al. found leptin levels in lean men were significantly decreased after exercise (120 minutes of running 65-75% of VO_{2max}) and a rest period (two hours) compared to a control day under similar conditions minus the exercise (1.7 $\pm$ 0.1 (SE) vs 2.5 $\pm$ 0.2 ng/ml) (25). This decrease corresponded to an average 30% decrease (range 9.5-45.8). The authors believed these results provide evidence for exercise influencing leptin levels on a more long-term basis as opposed to the other short-term assessments when studied by others.

Tuominen et al. measured serum leptin before and after an euglycemic, hyperinsulinemic clamp on two different days

(124). About 44 hours prior to one day, fourteen, lean men performed glycogen depleting exercise. The glycogen depleting exercise consisted of about two hours of TM exercise at 75% of VO_{2max}. The authors found decreased (~32%) basal (preclamp) leptin levels after the glycogen depleting exercise. In addition, they found increased leptin levels with hyperinsulinemia, regardless of initial (preclamp) leptin.

Essig et al. measured plasma leptin before and after TM exercise at 70% VO_{2max} in eleven, lean, moderately trained male subjects (28). Significant decreases (~30%) in leptin were not observed until 48 hours after the exercise.

van Aggel-Leijssen et al. assessed the relationship between leptin levels, EE, and exercise (125). Eight, lean, young, previously sedentary men were studied under four different conditions: no exercise/energy balance (E-/EBO), exercise/energy balance (E+/EBO), exercise/negative energy balance (E+/EB-), and exercise/positive energy balance (E+/EB+). The exercise consisted of biking on a cycle ergometer for a total of two hours (4, 30 minute bouts) at 50% VO_{2max}. Plasma leptin samples were obtained every hour during the day and every two hours during the

night for 24 hours. The authors found weighted 24 hour and peak plasma leptin were significantly lower (~20%) in the E+/EBO trial than the E-/EBO trial. Their main conclusion was exercise has a delayed effect on plasma leptin levels, evident in a 24-hour measurement.

Exercise training and leptin in men

Pasman et al. found significantly decreased plasma leptin levels in seven weight-reduced obese, older males after ten months of a 16-month training program compared to a control group that did not exercise (5.6 $\pm$ 2.5 (SD) vs 8.9 $\pm$ 3.8 ng/ml) (89). The two groups did not differ with respect to body weight or BMI. The differences in leptin persisted to the end of the program (16 months). When a multiple regression analysis was performed, the authors found changes in insulin and changes in percent body fat explained 44% of the leptin variance. With addition of number of training hours into the regression model, 61% of the variance in leptin could be explained.

Summary of the relationship between exercise in men and leptin

Even though some studies show an effect of exercise on plasma leptin levels, the majority, do not. Many studies that found a relationship had exercise bouts that were either prolonged or very intense. It is possible the

studies did not show an effect of exercise due to study design limitations i.e., small sample sizes, inadequate methods of sampling, or because they only studied men. However, the study by van Aggel-Leijssen, et al. was very powerful because they measured leptin over 24 hours and found an effect when the subjects were in energy balance (125). Nevertheless, it is currently unknown if a 30% difference in leptin levels is physiologically significant.

Cross-sectional studies of sedentary and physically active women

De Silva et al. assessed the relationship between physical activity and leptin in women (23). Their 359 subjects were a subgroup of the Geelong Osteoporosis study. Subjects were subdivided into three activity groups (low, medium, and high) after completing a self-administered questionnaire. After adjustment for age and BMI, physical activity levels did not appear to significantly alter leptin levels.

Clapp and Kiess measured serum leptin in 31 lean women before and during pregnancy (16). They subdivided the women into three groups. The first group consisted of an intermittently physically active group (with no regular weight-bearing exercise). The second group was called the "stopped exercise group" because subjects decreased their

pre-pregnancy activity by greater than 60% by 30 weeks of pregnancy. The third group consisted of a continued physically active group who exercised $\geq$ 4 times/week, for $\geq$ 40 minutes a session, at an intensity of $\geq$ 55% of VO_{2max}. The authors found preconceptional leptin levels were lower in the two weight bearing exercise subgroups compared to the intermittently active subgroup, but the differences were not significant after controlling for FM. They also found the magnitude of leptin increase with increasing gestation was significantly reduced in the continued physically active group compared to the intermittently active group. Clapp and Kiess hypothesized this was due to an exercise-induced decrease in fat accumulation.

Thong et al. compared plasma leptin in female athletes (119). The authors found no difference between leptin in regularly cycling elite athletes (n = 8) compared to regularly cycling recreationally active women (n = 13) after controlling for body fat. However, they found decreased leptin in amenorrheic, elite athletes (n = 5) compared to the regularly cycling athletes. This decrease persisted after adjusting for adiposity.

Four studies compared baseline leptin levels between exercise trained and sedentary women. Tataranni et al. studied leptin in four groups: eumenorrheic sedentary

(n = 7), eumenorrheic trained (n = 14), anovulatory trained (n = 7), and amenorrheic trained women (n = 6) (117). After adjustment for percent body fat, there were no independent effects of exercise training on leptin.

In contrast, Laughlin and Yen found leptin levels in sedentary women to be 3-4 times as high compared to those of athletes (63). These results could not be fully explained by differences in body fat. Clapp et al. investigated leptin levels in sixteen trained and sedentary women prior to pregnancy (15). They found leptin levels to be about doubled in the sedentary women (average 6.2 $\pm$ 1.0(SEM) ng/ml) compared to exercise trained women (average 2.5 $\pm$ 0.5 ng/ml). The authors did not report if the difference was independent of adiposity. Ryan and Elahi measured baseline plasma leptin in 42 trained female athletes (average 4.0 ng/ml) and twelve sedentary female controls (average 12.6 ng/ml) (101). They found plasma leptin to be higher in controls compared to the athletes even after adjusting for percent body fat.

Acute exercise and leptin in women

Only four studies have assessed the relationship between acute exercise and leptin in women. Dirlewanger et al. measured leptin levels in eleven, healthy, lean, previously sedentary, subjects (4 men and 7 women) three

different times (24). Each session lasted three days and samples were collected on the fourth morning. The first session was considered the control, which consisted of an isoenergetic diet and no physical activity. The second session was designed to achieve a moderate negative energy balance. Each subject maintained the same diet and biked two times a day for 30 minutes at 60 watts. The third session consisted of increasing food intake to cover the estimated cost of the exercise. Although leptin levels decreased after the second session (moderate negative energy balance), the change was not statistically significant. In fact, there were no significant differences among any of the three sessions (8.64 $\pm$ 2.22 (SEM), 7.17 $\pm$ 1.66, 7.33 $\pm$ 1.72 respectively).

Kraemer et al. compared the effect of exercise on serum leptin levels in fifteen non-obese, postmenopausal women (61). Subjects were studied on two occasions. At the first study session, subjects performed 30 minutes of TM exercise at 80% VO_{2max}. A month later, the subjects had their blood drawn at similar times as on the first day, but did not exercise. Kraemer et al. found leptin decreased in both sessions to the same degree. Therefore, they concluded there was no exercise-associated decrease in serum leptin levels.

Hilton and Loucks evaluated the relationship between exercise, energy availability, and leptin in sixteen previously sedentary women (47). These authors subdivided the subjects into sedentary and exercising groups. Women in both groups were studied on two occasions: under conditions of energy balance and under conditions of low energy availability. The results did not show a decrease in the 24-hour mean nor the amplitude of the diurnal leptin rhythm due to exercise alone. However, there appeared to be an interaction effect between physical activity status and energy availability, because only sedentary women showed a significant decrease in diurnal amplitude under conditions of low energy availability.

In contrast to the results of Hilton and Loucks (47), Kern et al. found serum leptin levels were significantly decreased after a 30 minute cycle exercise at 70% VO_{2max} in eight, young, lean, minimally active women (56). Furthermore, they found no significant differences in the magnitude of the decrease with either high fat (-12.2%) or a high carbohydrate diet (-11.3%).

Exercise training and leptin in women

Six studies have measured leptin after an aerobic, exercise training program performed by previously sedentary women. Kohrt et al. was the first group to study the

relationship between exercise training and leptin (57). Sedentary women were divided into four groups: sixteen sedentary women, seventeen exercising women, fifteen sedentary women on HRT, and thirteen exercising women on HRT. The eleven-month exercise program consisted of two months of flexibility training, followed by nine months of exercise. The subjects exercised three times per week, working up to 40-45 minutes at 80-85% max heart rate. Kohrt, et al. did not find any effect of HRT on leptin levels, so they combined the women into two larger groups of sedentary women and exercising women. Initially, it appeared as if training did not affect leptin levels. However, after subdividing the exercisers into tertiles based on fat content, the authors found a significant difference in the ratio of leptin/FM before and after exercise in the tertile with the most fat.

Hickey et al. examined serum leptin levels before and after a 12-week exercise program in nine, sedentary females and nine, sedentary males (44). The exercise program consisted of supervised exercise four days per week, with subjects working up to 85% of their VO_{2max} for 45 minutes by the end of the twelve weeks. This group found serum leptin significantly decreased (17.5%), but only in females. Since FM was not altered with this training program, it

cannot explain the significant change in leptin concentration.

Perusse et al. examined the effect of acute exercise and training on plasma leptin levels in sedentary women (91). This group studied 97 previously sedentary individuals (46 women and 51 men) before and after a 20-week training program. The training program consisted of cycling on a computerized cycle ergometer three times a week increasing towards 75% VO_{2max} by the end of the 20 weeks. Leptin was measured before and after training: at rest, with acute exercise (50 watt cycle exercise), and after an aerobic capacity test with the cycle ergometer. The authors said they found "no meaningful acute or chronic effects of exercise, independent of body fat." Although they did mention they found a gender difference, the authors did not elaborate on the significance of the results in women. By analyzing their table of results, it appears as if after controlling for FM, there was a main effect of training on leptin values during an acute bout of exercise and at VO_{2max}.

Christensen et al. assessed the relationship between serum leptin and one of three interventions in 121 overweight, postmenopausal women (14). The women were

randomized into one of the following three groups for twelve weeks: low energy diet, low energy diet and exercise program, or no intervention. The authors did not find any additional effect of exercise on serum leptin compared to those induced by diet alone (probably secondary to weight losses).

Kraemer et al. measured serum leptin in sixteen older, obese women before and after a nine-week aerobic training program (61). Despite increases in aerobic fitness, the authors found no differences in serum leptin.

Okazaki et al. measured plasma leptin in 41 middle-aged, sedentary, Japanese lean and obese women before and after a 12-week intervention that included diet counseling and aerobic exercise (85). Plasma leptin decreased significantly from 14.7 $\pm$ 5.3 (SD) to 8.9 $\pm$ 3.6 ng/ml in the obese group and from 7.6 $\pm$ 3.9 to 5.6 $\pm$ 2.2 ng/ml in the lean group. This effect was still significant after controlling for BMI. Due to the design of the study it cannot be determined if the changes in leptin were secondary to the exercise training program.

Houmard et al. measured plasma leptin levels in sixteen, lean young subjects (nine women and seven men) and fourteen relatively lean, older subjects (eight women and six men) before and after a seven-day exercise training

program (49). Seven days was chosen in order to increase insulin sensitivity without changing body mass or composition. The subjects exercised for one hour, for seven consecutive days on a cycle ergometer at 70-75% of VO_{2max}. The authors reported no differences in plasma leptin with short-term training in either group. Furthermore, when subdivided by gender, similar results were obtained.

At present, there has only been one training study assessing the effect of resistance training on plasma leptin levels. Ryan et al. did not find any differences in plasma leptin after sixteen weeks of resistance training in eight obese postmenopausal women who did not lose any weight with the training (100). In contrast the authors found decreased (36%) leptin levels in seven other obese postmenopausal females who did lose weight with the resistance training. The authors concluded there was no independent effect of resistance training on leptin levels in this study population.

Summary of the relationship between exercise in women and leptin

Results from studies in women are more mixed than the results from those performed with men. Some studies found a relationship between leptin and exercise training status,

while others did not. Even though there were no positive relationships found between acute exercise and leptin, all studies were done in apparently sedentary women.

Many studies have shown leptin levels in lean, sedentary women are significantly higher in the luteal vs follicular menstrual phase. Only five of the above mentioned exercise studies reported the menstrual phase in which leptin was measured. In three of four studies, leptin was measured at different times during the menstrual cycle. Recently, Thong et al. compared plasma leptin levels in lean athletes during both phases of the menstrual cycle (119). These authors also found leptin was greater during the luteal phase compared to the follicular phase. Study results would likely be biased towards the null hypothesis due to lack of control for menstrual status. It would be interesting to know if the relationship between menstrual phase and leptin also exists in overweight or obese women and if the relationship is modified by training status.

Clinical Significance

Many health problems are negatively affected by underlying overweight or obesity. Investigators have high hopes that recombinant leptin given to humans might result in body weight reductions. Although information from

clinical trials will shed light on the weight regulation issue, it is also necessary to learn more about what factors interact with leptin under normal physiological circumstances. In the long run, understanding more about the factors involved in weight regulation may eventually lead to decreased incidence of overweight and obesity in women.

PRELIMINARY STUDIES

I acquired blood samples from four young women of varying adiposity, before and after a 24-hour fast. These samples were used as a positive control to estimate my ability to measure a change in leptin levels in young women. I also gathered venous blood samples from four women in both phases of their menstrual cycles. These samples provide evidence of my ability to measure a change in leptin levels in two different menstrual phases. In addition, I acquired venous blood samples from two women who ate a standardized meal, and then a double quantity of the same standardized meal (on two consecutive days). These samples provide evidence of my ability to measure an increase in leptin levels after an increase in food intake. Minimal pilot data were also collected to assess the effect of acute exercise, pregnancy, timing of blood draws, and

meal composition. (Appendix A provides more details of these preliminary studies.)

RESEARCH DESIGN AND METHODS

Subjects

Subjects were obtained from the female students attending Michigan State University, as well as members of the surrounding communities via fliers, word of mouth, and e-mail. The protocol was approved by the University Committee for Research Involving Human Subjects. Each subject received a detailed oral and written description of the study before providing written informed consent. (See Appendix B for more details.) Subjects were a) apparently healthy, b) between the ages of 18-30 years, and c) had self-reported regular menstrual periods (22-32 days/cycle). Subjects were excluded if: a) they had taken oral contraceptives during the past 6 months, b) they were current smokers or had smoked a significant amount in the past year, c) their weight had changed more than two kilograms in the three months prior to the study or d) they had any known thyroid, renal, cardiac, or hepatic disease, diabetes, or eating disorder. In addition, subjects were asked about family history of thyroid problems. The initial plan was to recruit twenty subjects into each of four study groups. Groups were defined as follows:

Group 1 Low BMI and Exercise trained (LE)
Group 2 High BMI and Exercise trained (HE)
Group 3 Low BMI and Sedentary (LS)
Group 4 High BMI and Sedentary (HS)

For the purposes of this study, the following definitions were used to classify subjects:

Low BMI: BMI 18-22 (kg/m^2)

High BMI: BMI 25-29

Exercise trained: activity $\geq$ 5 days per week, $\geq$ 30 min/day, $\geq$ 6 MET level (for the past year), OR activity $\geq$ 3 days per week, $\geq$ 1 hour/day, $\geq$ 6 MET level (for the past year)

Sedentary: activity $\leq$ 3 days/week, < 20 min/day, < 4 MET level (for the past year)

Note: 1 MET is equivalent to REE. Most commonly, 1 MET is accepted to be equivalent to 3.5 ml of $O_2*kg^{-1}*min^{-1}$. For example, for a 59kg woman, backpacking, basketball, cycling (10mph), medium intensity aerobics, field hockey, rowing, running, soccer, or swimming are some activities that are greater than 6 METs. Cooking, slow bicycling (5mph), food shopping, housework, or walking (2.5mph) are examples of activities that are less than 4 METs (75).

Brief overview of protocol

Lab Visit #1:

- Aerobic capacity test
- Discussion of other study details:

- ovulation kits
- food records and food frequency questionnaires (FFQ's)

Lab Visits #2 and #3 (Menstrual phase visits):

Visits #2 and #3 involved identical protocols but at two different times in the subject's menstrual cycle. One visit was mid-follicular (4-6 days post start of menses) and one visit was mid-luteal (6-8 days post-ovulation). The order of completion of the mid-follicular and mid-luteal visits was by convenience.

- Pre-test standardized meal, followed by a 12-hour fast
- Anthropometric measurements
- Resting Energy Expenditure (REE) measurement
- Measurement of Residual lung Volume (RV)
- Estimate of body density via underwater weighing
- Venous blood draw #1
- Acute exercise bout
- Venous blood draw #2

Aerobic capacity test

Each subject performed an aerobic capacity (VO_{2max}) test on a motorized TM (SensorMedics; Yorba Linda, CA). This test is recognized as the criterion measure for determining an individual's cardio-pulmonary (aerobic) fitness, and is highly correlated with physical activity in most adults.

This test was done for two reasons. First, it helped confirm each subject's physical activity status (sedentary vs exercise trained). Second, the maximal oxygen consumption values were used to standardize future acute exercise bouts among the subjects.

The protocol for the aerobic capacity test began with subjects walking 2.0 miles*hr^{-1}. Workload was increased by 1.0 mile*hr^{-1} at two minute intervals until TM speed reached 6.0 miles*hr^{-1}. If the subject was still running, speed remained constant while elevation was increased by 3% per minute until she could no longer continue and terminated her effort due to volitional exhaustion.

During the treadmill test, each subject had her expired respiratory gases measured continuously by the open-circuit method. Oxygen consumption (VO_2) and carbon

dioxide (VCO_2) production were calculated according to standard equations via the Sensormedics metabolic cart. In addition, each woman's heart rate (HR) was measured using a telemetry system (Polar Vantage; Gays Mills, WI). Since there is little to no effect of menstrual cycle on women's VO_{2max} values (4), each subject only completed one VO_{2max} test at her convenience.

Ovulation kits

Day of ovulation was estimated from evidence of LH surge in the urine (OvuQuick; Quidel Corporation). This urine test has been shown to be a valid indicator of ovulation when used at home by normal women with regular menstrual cycles and when it is compared to serum hormone levels (LH and progesterone) (79). (See Appendix C for the instructions given to the subjects on how to use the OvuQuick kit.)

Food records and Food Frequency Questionnaire (FFQ)

Before each of the menstrual phase visits, subjects were asked to complete a 3-day food record (including two weekdays and one weekend day) and a FFQ. The food records were completed in order to document if there were any differences in the amount or types of foods eaten in the different groups around the time of the study. The FFQ was

completed in order to assess any differences in amount or types of foods eaten in the different groups during the year prior to the first menstrual phase visit. (Appendix D contains the written instructions given for how to complete a food record, a size approximation sheet, a sample food record, and information about the FFQ.)

Pretest standardized meal, followed by 12-hour fast

On the night before the menstrual phase visits, the subjects were asked to eat a standardized, nutritionally balanced meal provided by the investigator. The meal was standardized by each individual's weight and physical activity level. Each meal was designed to have very similar composition with respect to percent carbohydrates, protein, and fats. The mean percents and ranges were 55.07% (52-61%), 17.79% (15-21%), and 28.79% (24-33%) respectively. (See appendix E for details of the standardized, nutritionally balanced meals.) After completion of the meal, subjects were asked to fast for the next 12 hours prior to the study. Subjects were allowed to drink water ad libitum.

Subjects began all other test procedures at either 8 AM or 8:30 AM. This test time was chosen to help control for diurnal variation in plasma leptin levels (102, 111).

Anthropometrics

Upon arrival at the laboratory, each subject had her height (to the nearest 0.1 centimeter) measured via a wall mounted stadiometer. In addition, each subject had her weight (to the nearest 0.1 kg) determined via a beam balance scale.

Resting Energy Expenditure (REE)

Each subject rested in a semi-recumbent supine position in a comfortable room (ambient temperature (T_a) ~24°C and relative humidity ~35%). REE was determined via indirect calorimetry (SensorMedics; Yorba Linda, CA). Briefly, the subject breathed into a clear, unobtrusive, plastic face mask. Air flow was set at ~40-60 $L \cdot min^{-1}$, and the subject "diluted" the air passing over her face. The subject performed the REE test for 30 minutes. However, the first 20 minutes of data were excluded to allow for stability of the woman's metabolic rate. Previous experience with this technique has yielded minute to minute coefficients of variation of <5% (unpublished observations of Dr. James Pivarnik).

Body composition

Following the REE test, each subject donned a bathing suit and had her body composition estimated via

hydrodensitometry. This technique involved submersion and measurement of weight after the subject was underwater and had completely exhaled (1). Each subject completed a variable number of trials until they had three measurements that were within 1/8 of a pound. The three highest values were averaged and used for her underwater weight. This weight was then entered into an equation to estimate body density. Body density was then converted to percent body fat, using a modified Siri equation for women (51).

The largest potential source of error with the hydrodensitometry technique is inaccurate estimation of an individual's residual lung volume (RV). However, measuring each woman's RV via the nitrogen washout technique minimized this error (127). To allow for a learning effect, multiple trials were performed, with RV being considered the average of the two highest values recorded.

The nitrogen washout technique involves expiring completely, taking six breaths of approximately 100% oxygen from a bag, then maximally expiring again. (See Appendix F for the body composition equations and a sample calculation of residual volume and percent body fat.)

Blood sampling and assays

Following body composition estimates, the subject dried off and put on her exercise clothes and shoes. She

then had a venous blood sample drawn from an antecubital vein. Sufficient blood (~ 10ml) was obtained to perform the various study assays. A second blood sample (~ 10ml) was also obtained immediately after the submaximal exercise test. Serum leptin levels were determined in duplicate from both resting and post-exercise samples. The analysis was performed via radioimmunoassay (RIA)(Linco Research Inc. St. Charles, MO) (68). (See Appendix G for a brief overview of the RIA.) In addition, hematocrit was determined from both resting and post-exercise samples. This was done so that changes in leptin could be corrected for plasma water shifts that may have occurred during the exercise.

Acute Exercise

Following the first venous blood draw, each subject performed twenty minutes of TM exercise at a workload 60% of her previously determined VO_{2max} (1). This level of acute exercise was chosen since it represents a session typically recommended for the maintenance and/or development of aerobic fitness in adults (1). Depending on her aerobic fitness and level of comfort, the woman was either walking or running during the exercise test.

Brief statistical methodology

Attention to detail (time of day, standardized test meal followed by fasting conditions, and separate menstrual phase cycle testing) all helped reduce within group variability in the study. But, due to the limited number of subjects, only basic statistical tests of mean and standard deviation were performed on this pilot data.

RESULTS

Subjects

E-mail advertising via classroom announcements was the most effective method of recruitment. Three hundred forty-five women responded to my advertisements for subjects. The majority of the respondents did not fit the study criteria. There were four main reasons why many women did not fit the inclusion criteria. Despite advertisements asking for non-smokers and women who were not on oral contraceptives or Depo Provera, many respondents were either smokers or were currently taking these medications. However, the bulk of the ineligible women fell in-between either the body mass index or the physical activity level categories.

Forty-two women were identified as being eligible to participate in the study. Fourteen came in for the first lab visit (the VO_{2max} test). Eight women completed one

menstrual phase visit. Six subjects completed the luteal menstrual phase visit and seven subjects completed the follicular phase visit. Five women completed the entire protocol.

Five women who only completed the VO_{2max} test were Caucasian and one was Hispanic. Of the three women who only completed one menstrual phase visit, two were Caucasian and one was Asian. Of the five women who completed the whole protocol, three were Caucasian Americans, one was Romanian, and one was African American.

VO_{2max} test

Demographic information of the fourteen women who completed the VO_{2max} test can be found in Table 1. The fourteen women were subdivided by group, seven in the LE group, three in the LS group, and four in the HE group. Two women that fit the criteria for the HS group were recruited, but they did not participate in the study due to scheduling problems. The mean age of subjects at the VO_{2max} visit was 22.5 $\pm$ 3.35 (SD) (range 19-27). There were no changes in classification or eligibility of subjects after measurement of BMI (recruitment was primarily based on reported heights and weights). The average BMI for the LE and LS groups was about in the middle of the desired range.

Table 1: VO_{2Max} results

	LE	LS	HE
n	7	3	4
Age (yrs) mean ± SD	21.7 ± 3.9	22.7 ± 1.2	23.8 ± 3.8
Range	(19-29)	(22-24)	(20-27)
Ht (cm)	171.3 ± 3.1	166.7 ± 6.5	169.2 ± 6.1
Range	(167.7-176.2)	(159.2-170.9)	(161-175.4)
Wt (kg)	61.1 ± 4.9	58.5 ± 5.8	73.4 ± 4.1
Range	(51.1-66.4)	(51.8-62.3)	(68.5-77.3)
BMI (kg/m^2)	20.8 ± 1.4	21.0 ± 0.6	25.6 ± 0.9
Range	(18-22.4)	(20.4-21.6)	(24.7-26.4)
VO_2 (ml/kg/min)	46.4 ± 1.6	46.9 ± 6.0	46.1 ± 5.8
Range	(44.8-48.7)	(40.6-52.6)	(38.9-52.7)

Note: LE = Low body mass index, Exercisers, LS = Low body mass index, Sedentary, HE = High body mass index, Exercisers

However, the average BMI of the HE group was at the low end of the desired range.

The mean fitness levels between groups were similar, ranging from good to superior, according to the American College of Sports Medicine classification system (1). Despite the fact that the women in the LS group did not exercise on a regular basis, they were still physically fit.

Other demographic data

The remainder of the data was collected during separate menstrual phases. Due to small sample sizes, Tables 2 and 3 present primarily individual demographic information for the respective menstrual phases. As expected, age, height, weight, and BMI were similar at the various time points.

Percent fat, FM, and FFM were also similar within individual subjects between menstrual phase visits. Percent fat for all individuals in the LE group ranged from 14.0-25.2%. Percent fat for all individuals in the LS group ranged from 11.4-26.4%. Percent fat for all individuals in the HE group ranged from 22.5-28.8%.

Figure 1 shows the relationship between leptin and fat mass during the follicular menstrual phase. As fat mass increased in five individuals their leptin levels tended to

Table 2: Follicular phase data

ID	Age (yrs)	Height (cm)	Weight (kg)	Body Mass Index (kg/m^2)	% Fat	Fat Mass (kg)	Fat Free Mass (kg)	Resting Energy Expenditure (kcals/day)
LEEAK	19	168.2	64.5	22.8	25.2	16.2	48.3	1315
LEMIP	25	173.1	59.9	20.0	19.4	11.6	48.3	1457
LEJMD	19	174.5	63.5	20.9	21.0	13.3	50.2	1480
AVG LE (n=3)	21.0	171.9	62.6	21.2	21.8	13.7	48.9	1417
LSACH	23	174.1	60.9	20.1	13.0	7.9	53.0	1267
HESRT	27	169.3	76.2	26.6	28.8	21.9	54.3	1778
HEKMA	27	173.7	76.7	25.4	24.1	18.5	58.2	1673
HECDP	22	167.2	70.7	25.3	22.4	15.9	54.8	1541
AVG HE (n=3)	25.3	170.1	74.5	25.8	25.1	18.8	55.8	1664

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers, LS = Low body mass index, Sedentary, HE = High body mass index, Exercisers

Table 3: Luteal phase data

ID	Age (yrs)	Height (cm)	Weight (kg)	Body Mass Index (kg/m^2)	% Fat	Fat Mass (kg)	Fat Free Mass (kg)	Resting Energy Expenditure (kcals/day)
LEMIP	26	172.9	59.4	19.9	14.0	8.3	51.1	1390
LEJMD	19	175.1	62	20.2	20.3	12.6	49.4	1488
AVG LE (n=2)	22.5	174.0	60.7	20.0	17.2	10.5	50.2	1439
LSACH	23	170.9	59.8	20.5	11.4	6.8	53.0	1285
LSTAP	24	160.3	52.1	20.3	26.4	13.8	38.3	1335
AVG LS (n=2)	24.0	160.3	52.1	20.3	26.4	13.8	38.3	1335
HEKMA	27	173.8	76	25.2	22.9	17.4	58.6	1736
HECDP	21	167.4	70.5	25.2	23.8	16.8	53.7	1662
AVG HE (n=2)	24.0	170.6	73.3	25.2	23.3	17.1	56.2	1699

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers, LS = Low body mass index, Sedentary, HE = High body mass index, Exercisers

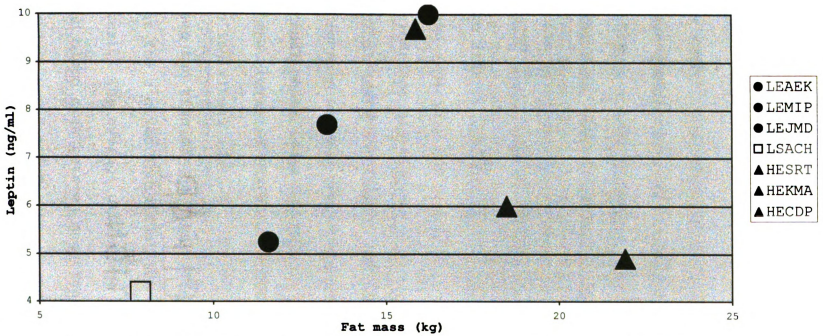


Figure 1: Relationship between leptin and fat mass during the follicular menstrual phase

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers (●), LS = Low body mass index, Sedentary (□), HE = High body mass index, Exercisers (▲)

increase. However, values from two individuals did not fit this relationship. No comparison can be made between sedentary and exercising women as there was only one sedentary subject who completed the follicular menstrual phase visit. The relationship between leptin and percent fat was almost identical (data not shown).

Figure 2 also shows the relationship between leptin and fat mass, but in the luteal menstrual phase. The positive relationship between leptin and fat mass was more distinct during the luteal menstrual phase. Although the sample size is limited, the relationship may be different between sedentary and exercising women. These relationships between leptin and percent fat were almost identical (data not shown). As expected there was no relationship between leptin and fat free mass in either menstrual phase (data not shown).

The timing of the blood draws was consistent for a given individual (data not shown). The average difference between the time of the first blood draw between menstrual phases for a given individual was 2 $\pm$ 2 minutes (range 2-5 minutes). The average difference between the time of the second blood draw between menstrual phases for a given individual was 8 $\pm$ 6 minutes (range 5-17 minutes).

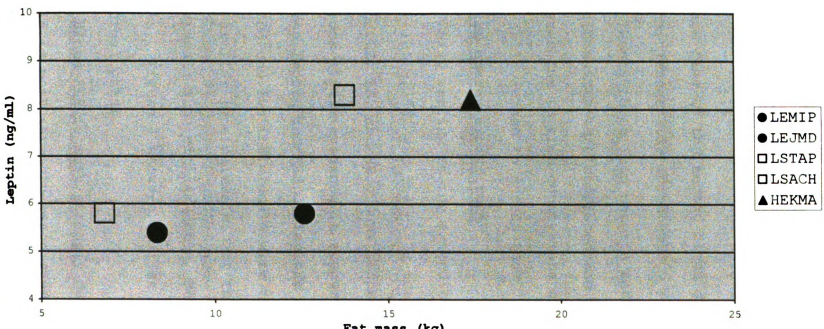


Figure 2: Relationship between leptin and fat mass during the luteal menstrual phase

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers (●), LS = Low body mass index, Sedentary (□), HE = High body mass index, Exercisers (▲)

The timing of the blood draws between individuals was still similar, although not as tight as within individuals (data not shown). The average time of the first blood draw was 9:46 AM ± 23 minutes (range 9:12-10:19 AM). The average time of the second blood draw was 10:25 AM ± 20 minutes (range 9:53-10:52 AM).

Twenty-three food records and six food frequency questionnaires were completed and returned. These were not analyzed due to time constraints of the individuals who originally agreed to assess them.

Resting energy expenditure

Daily resting energy expenditure ranged from 1315-1488, 1267-1335, and 1541-1778 kcal/day for all individuals in the LE, LS, and HE groups respectively. Figure 3 provides little evidence in support of a relationship between leptin and REE during the follicular phase. Not only do the two individuals with the highest and lowest REE have similar leptin levels, but there are also three individuals with similar REE's, yet very diverse leptin levels. Figure 4 shows a similar result during the luteal menstrual phase.

Since REE is most strongly determined by FFM and leptin is most strongly associated with FM, I chose to reevaluate the relationship between REE and leptin after

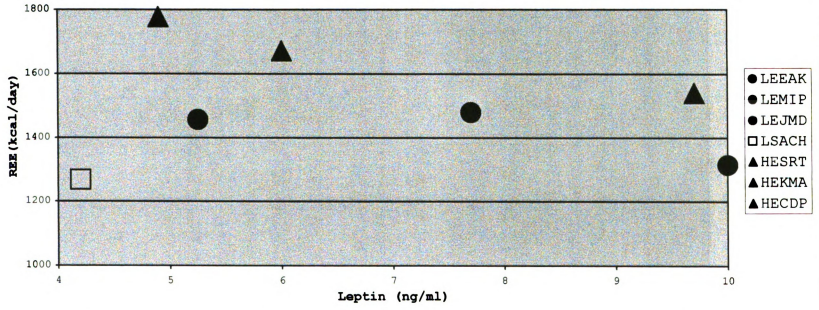


Figure 3: Relationship between leptin and resting energy expenditure (REE) during the follicular menstrual phase

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers (●), LS = Low body mass index, Sedentary (□), HE = High body mass index, Exercisers (▲)

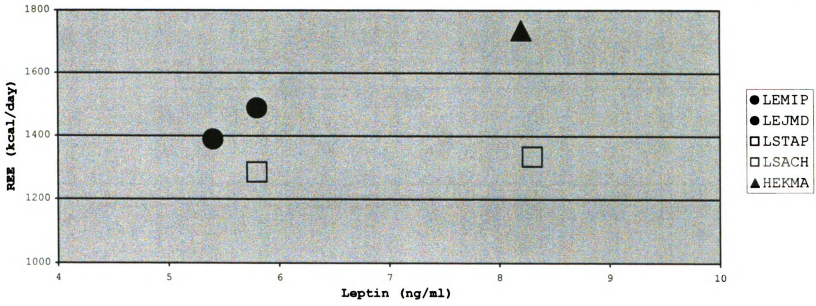


Figure 4: Relationship between leptin and resting energy expenditure (REE) during the luteal menstrual phase

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers (●), LS = Low body mass index, Sedentary (□), HE = High body mass index, Exercisers (▲)

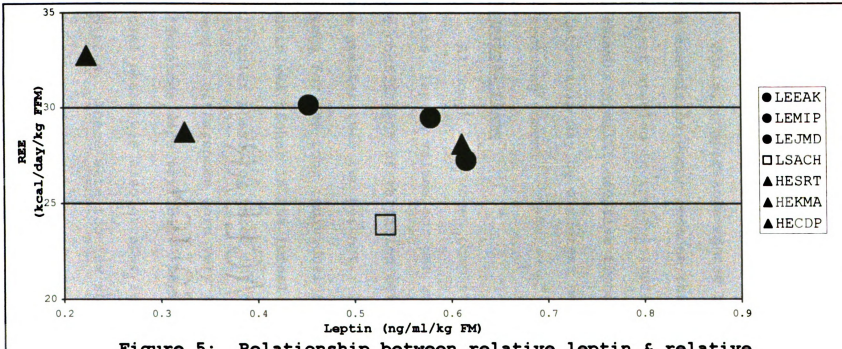


Figure 5: Relationship between relative leptin & relative resting energy expenditure (REE) during the follicular menstrual phase

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers (●), LS = Low body mass index, Sedentary (□), HE = High body mass index, Exercisers (▲)

controlling for these variables. Figure 5 shows this relationship during the follicular menstrual phase. After controlling for body composition, there appears to be a negative relationship between L/FM and REE/FFM.

Again, comparisons cannot be made between sedentary and exercising women because there was only one sedentary subject studied in the follicular menstrual phase. Figure 6 shows a similar negative trend. Once again, the relationship may be different between sedentary and exercising women.

Menstrual phase

Although five women completed both menstrual phase visits, leptin values for one woman was discarded because the coefficient of variation between the duplicate samples was greater than ten percent. Table 4 shows serum leptin levels for the four individual subjects. Although serum leptin was higher in the luteal phase compared to the follicular phase for three out of the four subjects, the absolute differences were very small and the percent differences were variable. Leptin/FM measured during the luteal phase was also greater in three out of four subjects. There were too few subjects in each group to make any comparisons between groups.

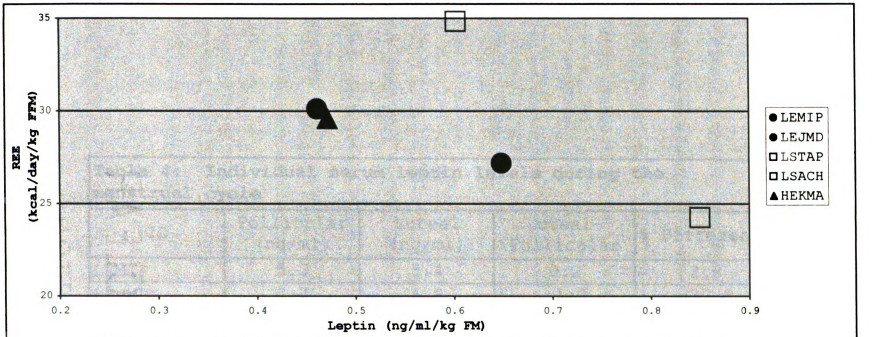


Figure 6: Relationship between relative leptin & relative energy expenditure (REE) during the luteal menstrual phase

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers (●), LS = Low body mass index, Sedentary (□), HE = High body mass index, Exercisers (▲)

Table 4: Individual serum leptin levels during the menstrual cycle

ID	Follicular (ng/ml)	Luteal (ng/ml)	Luteal-Follicular	% Difference
LEMIP	5.3	5.4	0.2	2.8
LEJMD	7.7	5.8	-1.9	-32.8
LSACH	4.2	5.8	1.6	27.6
HEKMA	6.0	8.2	2.2	26.8

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers, LS = Low body mass index, Sedentary, HE = High body mass index, Exercisers

Exercise

Individual serum leptin levels before and after exercise during the follicular menstrual phase are shown in Table 5. Leptin slightly decreased in four of seven subjects (range 0.2-1.55 ng/ml). In the LE group (n = 3) there was an average decrease in serum leptin of 0.35 ng/ml after exercise. In the HE group (n = 3) there was no average change in leptin levels after exercise.

Individual serum leptin levels before and after exercise during the luteal menstrual phase are shown in Table 6. Leptin decreased a small amount in four of five subjects (range 0.2-2 ng/ml). In the LE group (n = 2) there was an average decrease in serum leptin of 0.2 ng/ml after exercise. In the LS group (n = 2) there was an average decrease in serum leptin of 0.6 ng/ml after exercise.

The effect of chronic exercise on leptin levels could only be assessed in the luteal menstrual phase because there was only one sedentary subject who completed the follicular phase visit. Pre-exercise and post-exercise serum leptin levels were higher in LS subjects (n = 2) compared to LE subjects (n=2).

Table 5: Individual serum leptin levels before and after exercise during the follicular menstrual phase

ID	Pre-Exercise (ng/ml)	Post-Exercise (ng/ml)	Post-Pre Exercise	% Difference
LEAEK	10.0	11.0	1.0	10.0
LEMIP	5.3	3.7	-1.6	-29.5
LEJMD	7.7	7.2	-0.5	-6.5
LSACH	4.2	5.0	0.8	19.0
HESRT	4.9	5.4	0.5	10.2
HEKMA	6.0	5.8	-0.2	-3.3
HECDP	9.7	9.4	-0.3	-3.1

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers, LS = Low body mass index, Sedentary, HE = High body mass index, Exercisers

Table 6: Individual serum leptin levels before and after exercise during the luteal menstrual phase

ID	Pre-Exercise (ng/ml)	Post-Exercise (ng/ml)	Post-Pre Exercise	% Difference
LEMIP	5.4	5.2	-0.2	-3.7
LEJMD	5.8	5.6	-0.2	-3.4
LSTAP	8.3	9.1	0.8	9.6
LSACH	5.8	3.8	-2.0	-34.5
HEKMA	8.2	8.0	-0.2	-2.4

Note: The first two letters of each subject ID are the group designation and the last three letters are the subject's initials. The group designations are: LE = Low body mass index, Exercisers, LS = Low body mass index, Sedentary, HE = High body mass index, Exercisers

DISCUSSION

General

Scheduling problems were the main reason why women did not complete the protocol. The short window of opportunity for the menstrual phase visits (days 4-6 and days 7-9 post-ovulation for the follicular and luteal visits respectively) made it difficult to schedule the visits in a timely fashion. Scheduling was also limited by morning kinesiology classes in our lab and school vacations. If the window of opportunity was missed, then we had to wait another month for the next potential test date.

Subjects needed to inform me of the first day of their menses or the day they had a positive ovulation test in order for me to schedule the visit on time. Despite the fact that I stressed the importance of keeping me up to date on where they were in their cycles, most subjects had trouble remembering to either let me know when they had started their period or they forgot to begin the urine tests on the appropriate days. For all but one subject who completed the protocol, I had to keep track of the dates for them and remind them almost immediately ahead of time that we needed to schedule the visits soon.

The short turn around time between notification and the potential study date made it hard to schedule the

actual visit. This was primarily because I had to arrange for four people to be present at one time or another during any menstrual phase visit (the subject, two study assistants, and me). The second assistant was needed initially to handle the venous blood draws, but later to supervise my venipunctures. The original plan was for me to eventually be able to take the blood samples myself, but due to the small sample size this did not occur.

Studies have shown dissociation between leptin and FM when subjects were not in energy balance i.e. with fasting, weight loss, or overfeeding (45). Perhaps the two subjects, who appeared to be outliers in Figure 1, showing the well-established positive relationship between leptin and fat mass, were not in a similar state of energy balance as the other subjects. Furthermore, Hamann and Matthaei reported that a small subset of individuals in which FM and plasma leptin are not correlated exist (35).

Fox, et al. cited many studies that have not found ethnic variation in leptin levels (31). Differences were not found between 1) White, Afro-Caribbean and Asian subjects with type II diabetes, 2) African American and white children, and 3) Mexican American and non-Hispanic white adults. In contrast, they cited a study by Lu, et al., which found a difference between Indians and Creole or

Chinese population of Mauritius. Nicklas, et al. also found differences in plasma leptin between Caucasian and African American obese postmenopausal women (82). It is conceivable that any differences in leptin levels among individuals of diverse ethnic backgrounds may be secondary to ethnic diversity of leptin concentrations.

Since the study of Schoeller et al. provided evidence that the diurnal variation of leptin could be secondary to meal timing, it was important to control for this potential effect. However, the standardized meal may have modified leptin levels if the meal was not representative of each woman's normal diet i.e., if the quantity was more or less than usual. Nevertheless, Paolisso et al. did not find any significant relationships between plasma leptin and food intake during the follicular, periovulatory or luteal phases of the menstrual cycle in sixteen, healthy, lean, sedentary, young women (88).

Energy expenditure

The data suggest a negative trend between L/FM and REE/FFM. As L/FM increases, REE/FFM tends to decrease. Flier et al. has suggested that leptin is not really a molecule that signals satiety, but rather one that plays a role in the prevention of starvation (30). Based on the limited understanding of leptin's function, such a

relationship appears to be opposite of what is expected (34, 90).

The relationship between leptin and REE may be influenced by exercise training status. However, limited sample size prevented this assessment in the present study.

Menstrual phase

Similar to Thong et al., our study results demonstrated interindividual variation of leptin levels from different menstrual phases (119). Differences in the four subjects ranged from a 2.8% decrease to a 32.8% increase. Together, these data support potential differences in how leptin levels vary between exercise trained and sedentary individuals with respect to the menstrual cycle.

Since leptin levels vary with short term fasting and acute overfeeding, variation in leptin levels during the menstrual cycle could be secondary to differences in caloric intake. Gathering data about each subject's normal nutritional habits (via food records and food frequency questionnaires) was part of the original study design to assess this possibility. Future analysis of the food records from the two time points could demonstrate whether differences exist.

Mechanistically, increased leptin could be secondary to increased production or decreased elimination. Increased production could be secondary to release of leptin from the ovary during the luteal menstrual phase. Furthermore, exercise training could modify this production of leptin by the ovaries.

Although there appears to be a relationship between menstrual phase and leptin levels, the physiological significance of such a difference is not known. Many studies have not shown a relationship between leptin and sex hormones (12, 72, 93, 96, 122). Current thinking is that leptin is not solely responsible for pubertal development or reproductive function (2). Rather, leptin is thought to play some permissive role, perhaps by providing signals of adequate energy levels.

Exercise

This study did not show significant exercise-induced changes in leptin levels. Although four of seven women during the follicular phase and four of five women during the luteal phase had decreased leptin levels after exercise compared to before exercise, most changes were minimal. It is probable the intensity and duration of the exercise in this study was insufficient to induce large changes in leptin levels in an acute setting.

Similar to many of the previous exercise studies, single fasting samples were used. However, the relationship between leptin and exercise may not be recognized acutely. Perhaps if measurements were made serially over extended times, larger decreases in leptin could have been observed. Others have documented this type of delayed effect (25, 28, 125). Essig et al. hypothesized the delay may correspond to the time required to induce changes in gene expression (28). Ideally, subjects would enter a clinical research center and have leptin levels monitored over 24-hours or longer.

It is conceivable that there is no relationship between leptin and acute exercise in young women. Hickey et al. pointed out that documented responses to exercise have been very small when compared to the responses with similar caloric deficits in fasting studies (43). No relationship between leptin and acute exercise would support the hypothesis that leptin is more of an antistarvation hormone than an antiobesity hormone. However, further research with proper controls is still needed, particularly involving females.

Recommendations for future studies

Since some studies have shown the leptin decreases after exercise are equivalent to the decreases seen with an

extended fast, it is important to have control subjects who undergo similar timing of blood sampling, but no exercise. In addition, future studies should include repeated measurements of a subsample of subjects under similar conditions to assess for intra-individual and day-to-day variation.

Secondly, few acute exercise studies have determined if the subject was in positive or negative energy balance at the time of measurement. It would be beneficial to either have this information or control for energy balance as van Aggel-Leijssen et al. did in their study with male subjects (125). In their study subjects were studied under four different conditions: no exercise/energy balance (E-/EBO), exercise/energy balance (E+/EBO), exercise/negative energy balance (E+/EB-), and exercise/positive energy balance (E+/EB+). By using this experimental design, the authors were able to truly assess the effect of exercise independent of energy balance.

In addition, sometimes the TM speed and grade (at 60% VO_{2max}) calculated from the ACSM (American College of Sports Medicine) metabolic equations underpredicted the 60% VO_{2max} work intensity. Such observations have recently led ACSM to modify their metabolic equations to use percent of VO_{2max}

reserve rather than simply percent VO_{2max} when estimating submaximal workloads (116). Therefore, I would recommend that future studies use these more recent metabolic equations.

APPENDICES

APPENDIX A: Results of preliminary studies

I acquired blood samples from four young women of varying adiposity, before and after a 24-hour fast. These samples are a type of positive control to estimate my ability to measure a change in leptin levels in young women. All four subjects had their blood samples drawn around the time of dinner on the same two days, but individually staggered approximately fifteen minutes. All subjects had their blood drawn, ate dinner within one hour and then fasted for the remainder of the 24 hours. All four women had markedly decreased leptin levels after the approximate 24-hour fast. The absolute decreases ranged from 1.4-7.9 ng/ml. The percent decreases ranged from 62.5-89.3%. The average percent decrease was 77.3%

I also gathered venous blood samples from four women in both phases of their menstrual cycles. These samples provide evidence of my ability to measure a change in leptin levels in two different menstrual phases. The majority of samples were drawn in the early morning. Attempts were made to acquire the samples around the same target days as in the actual study (follicular days 4-6 and luteal days 6-8). Luteal phase dates were estimated based on average cycle length rather than actual determination of ovulation with a kit. All four women had higher leptin

levels in the luteal phase compared to the follicular phase. The absolute differences ranged from 1.3-2.1 ng/ml. The percent increase ranged from 50-301%. The average percent increase was 77%.

In addition, I acquired venous blood samples from two women after eating the same standardized meal and a double quantity of the same standardized meal (on two consecutive days). These samples provide evidence of my ability to measure an increase in leptin levels after an increase in food intake. Leptin levels in both women increased. Of note, the leptin levels only increased about 60% in the women with a high body mass index. In contrast, leptin levels increased by 143% in the woman who had a low body mass index. This suggests the increase in leptin levels seen with acute overfeeding may be relative to the amount the individual normally eats.

No difference in leptin levels was observed after an acute exercise bout in two different women with high body mass index. Menstrual phase was not well documented. Despite this lack of difference in two subjects, I decided to continue to assess the relationship between leptin and exercise, thinking that perhaps there was an interaction effect between exercise status or menstrual phase.

Leptin levels were also measured in one woman before pregnancy in the follicular and luteal phases and at about 9 weeks of pregnancy. As expected leptin levels were markedly increased during pregnancy as compared to prepregnancy.

Equivocal results were obtained when trying to assess the effect of changing the time of measurement between the hours of 7 AM and 11 AM and when trying to assess the composition of a meal. These equivocal results led to tight controls for both of these factors.

Some samples gathered for pilot data were rerun with the second assay for comparison. This was important because the second assay utilized the normal leptin assay, whereas the first assay utilized a sensitive leptin assay. All samples that were repeated had different calculated leptin levels compared to the values from the first assay. But, all trends in how leptin was changed were the same. The differences in calculated values may have been due to error in the way the assay was performed. This is more likely to have happened with the first assay, since it was the first time I had ever performed a radioimmunoassay. Even though error did occur in the second assay, it was of a type that was easily corrected because it was detected early.

APPENDIX B: UCRIHS information and consent form

MICHIGAN STATE
UNIVERSITY

September 21, 1998

TO: James M. Pivarnik
3 I.M. Sports Circle

RE: IRB#: 98-516
TITLE: ASSESSMENT OF LEPTIN AT REST AND WITH EXERCISE, IN SEDENTARY AND TRAINED YOUNG WOMEN OF VARYING BODY SIZES DURING BOTH THE FOLLICULAR AND LUTEAL MENSTRUAL PHASES
REVISION REQUESTED: N/A
CATEGORY: 2-C,D,G
APPROVAL DATE: 09/21/98

The University Committee on Research Involving Human Subjects'(UCRIHS) review of this project is complete. I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the UCRIHS approved this project and any revisions listed above.

RENEWAL: UCRIHS approval is valid for one calendar year, beginning with the approval date shown above. Investigators planning to continue a project beyond one year must use the green renewal form (enclosed with the original approval letter or when a project is renewed) to seek updated certification. There is a maximum of four such expedited renewals possible. Investigators wishing to continue a project beyond that time need to submit it again for complete review.

REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please use the green renewal form. To revise an approved protocol at any other time during the year, send your written request to the UCRIHS Chair, requesting revised approval and referencing the project's IRB # and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.

PROBLEMS/ CHANGES: Should either of the following arise during the course of the work, investigators must notify UCRIHS promptly: (1) problems (unexpected side effects, complaints, etc.) involving human subjects or (2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

If we can be of any future help, please do not hesitate to contact us at (517)355-2180 or FAX (517)432-1171.

Sincerely,

David E. Wright

David E. Wright, Ph.D.
UCRIHS Chair

DEW:bed

cc: Juanita M. Rivera

OFFICE OF
RESEARCH
AND
GRADUATE
STUDIES

University Committee on Research Involving Human Subjects (UCRIHS)

Michigan State University
246 Administration Building
East Lansing, Michigan
48824-1046

517/355-2180
FAX: 517/432-1171

MICHIGAN STATE
UNIVERSITY

February 19, 1999

TO: Dr. James Pivarnik
3 I.M. Sports Circle

RE: IRB # 98516 CATEGORY: 2-C,D,G

TITLE: AN ASSESSMENT OF LEPTIN AT REST AND WITH EXERCISE, IN SEDENTARY AND TRAINED YOUNG WOMEN OF VARYING BODY SIZES DURING BOTH THE FOLLICULAR AND LUTEAL MENSTRUAL PHASES

ANNUAL APPROVAL DATE: September 21, 1998
REVISION REQUESTED: January 28, 1999
REVISION APPROVAL DATE February 19, 1999

The University Committee on Research Involving Human Subjects' (UCRIHS) review of this project is complete and I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the UCRIHS APPROVED THIS PROJECT'S REVISION.

This letter approves the revision to recruit additional subjects via e-mail.

RENEWALS: UCRIHS approval is valid for one calendar year, beginning with the approval date shown above. Projects continuing beyond one year must be renewed with the green renewal form. A maximum of four such expedited renewal are possible. Investigators wishing to continue a project beyond that time need to submit it again for a complete review.

REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please use the green renewal form. To revise an approved protocol at any other time during the year, send your written request to the UCRIHS Chair, requesting revised approval and referencing the project's IRB# and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.

PROBLEMS/CHANGES: Should either of the following arise during the course of the work, notify UCRIHS promptly: 1) problems (unexpected side effects, c...xplaints, etc.) involving human subjects or 2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

If we can be of further assistance, please contact us at 517 355-2180 or via email: UCRIHS@pilot.msu.edu.

Sincerely,

David E. Wright

David E. Wright, Ph.D.
UCRIHS Chair

DEW: bd
cc: Juanita Rivera

All UCRIHS forms are located via the web: http://www.msu.edu/unit/vprgs/UCRIHS/

OFFICE OF
RESEARCH
AND
GRADUATE
STUDIES

University Committee on
Research Involving
Human Subjects
(UCRIHS)

Michigan State University
246 Administration Building
East Lansing, Michigan
48824-1046

517/355-2180
FAX: 517/353-2976

MICHIGAN STATE
UNIVERSITY

May 4, 1999

TO: Dr. James PIVARNIK
3 I.M. Sports Circle

RE: **IRB # 98516 CATEGORY: 2-B,D**

TITLE: ASSESSMENT OF LEPTIN AT REST AND WITH EXERCISE, IN SEDENTARY AND TRAINED YOUNG WOMEN OF VARYING BODY SIZES DURING BOTH THE FOLLICULAR AND LUTEAL MENSTRUAL PHASES.

ANNUAL APPROVAL DATE: September 21, 1998
REVISION REQUESTED: April 13, 1999
REVISION APPROVAL DATE: May 3, 1999

The University Committee on Research Involving Human Subjects' (UCRIHS) review of this project is complete and I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the UCRIHS APPROVED THIS PROJECT'S REVISION.

RENEWALS: UCRIHS approval is valid for one calendar year, beginning with the approval date shown above. Projects continuing beyond one year must be renewed with the green renewal form. A maximum of four such expedited renewal are possible. Investigators wishing to continue a project beyond that time need to submit it again for a complete review.
REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please use the green renewal form. To revise an approved protocol at any other time during the year, send your written request to the UCRIHS Chair, requesting revised approval and referencing the project's IRB# and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.
PROBLEMS/CHANGES: Should either of the following arise during the course of the work, notify UCRIHS promptly: 1) problems (unexpected side effects, complaints, etc.) involving human subjects or 2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.
If we can be of further assistance, please contact us at 517 355-2180 or via email: UCRIHS@pilot.msu.edu.

Sincerely,

David E. Wright, Ph.D.
UCRIHS Chair

DEW: ah
cc: Juanita Rivera

All UCRIHS forms are located via the web: http://www.msu.edu/unit/vprgs/UCRIHS/

OFFICE OF RESEARCH AND GRADUATE STUDIES
University Committee on Research Involving Human Subjects (UCRIHS)
Michigan State University
246 Administration Building
East Lansing, Michigan
48824-1046
517/355-2180
FAX: 517/353-2976

MICHIGAN STATE
UNIVERSITY

October 4, 1999

TO: James Pivarnik
3 I.m. Sports Circle

RE: IRB # 98516 CATEGORY: 2-C,D,G
RENEWAL APPROVAL DATE: September 29, 1999

TITLE: ASSESSMENT OF LEPTIN AT REST AND WITH EXERCISE, IN SEDENTARY AND TRAINED YOUNG WOMEN OF VARYING BODY SIZES DURING BOTH THE FOLLICULAR AND LUTEAL MESNTRUAL PHASES

The University Committee on Research Involving Human Subjects' (UCRIHS) review of this project is complete and I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the UCRIHS APPROVED THIS PROJECT'S RENEWAL.

RENEWALS: UCRIHS approval is valid for one calendar year, beginning with the approval date shown above. Projects continuing beyond one year must be renewed with the green renewal form. A maximum of four such expedited renewal are possible. Investigators wishing to continue a project beyond that time need to submit it again for complete review.
REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please use the green renewal form. To revise an approved protocol at any other time during the year, send your written request to the UCRIHS Chair, requesting revised approval and referencing the project's IRB# and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.
PROBLEMS/CHANGES: Should either of the following arise during the course of the work, notify UCRIHS promptly: 1) problems (unexpected side effects, complaints, etc.) involving human subjects or 2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

If we can be of further assistance, please contact us at 517 355-2180 or via email: UCRIHS@pilot.msu.edu.

Sincerely,

David E. Wright
Chair, UCRIHS

DEW: bd
cc: Juanita Rivera

OFFICE OF
RESEARCH
AND
GRADUATE
STUDIES

University Committee on Research Involving Human Subjects (UCRIHS)
Michigan State University
246 Administration Building
East Lansing, Michigan
48824-1046
517/355-2180
FAX: 517/353-2976

Principal Investigator: James M. Pivarnik, Ph.D. (517)355-3520
Co-Investigator: Juanita M. Rivera, MD/Ph.D. student (517)355-4734

STATEMENT OF INFORMED CONSENT

SUBJECT'S NAME _____

A. Title of the study

Assessment of leptin at rest and with exercise, in sedentary and trained young women of varying body sizes.

B. Purpose of the study

Leptin is a chemical produced by your fat cells. Leptin is thought to affect the amount of energy a person uses. We would like to determine the relationship between leptin and exercise behavior in young women. In addition, since leptin levels are altered with menstrual cycle phase, we would like to study the relationship of exercise and leptin during both follicular and luteal phases of the menstrual cycle.

C. Description

You are invited to participate in this project which involves coming to the Human Energy Research Lab at Michigan State University on three different occasions.

During the first visit, you will have your height and weight measured. Then you will perform an aerobic fitness test on a motorized treadmill. This test involves measuring the air you breathe (through a valve and mouthpiece), while increasing exercise intensity every 1-2 minutes until you are no longer able to continue. For the first two minutes you will be walking 2 miles per hour. Then, the speed will increase 1 mile per hour every 2 minutes until the treadmill speed reaches 6 miles per hour. If you are able to continue past ten minutes (end of the 6 mph timepoint), the elevation will be increased 3% every minute, until you cannot run any longer and voluntarily choose to stop. At the end of the test, we will slow the treadmill down and you will walk to cool off. It is expected that this test will last approximately 10-15 minutes. During this test, your heart rate will be measured with a lightweight monitor that fits around your lower chest with an elastic belt. This visit will also include an explanation of how to use the family planning kit (discussed below) and how to complete the food records and the food frequency questionnaire. Total time in the lab on the first visit should not exceed 50 minutes.

The other two visits will be during different parts of your menstrual cycle, during the follicular phase and during the luteal phase. The follicular phase visit will be done approximately 4-6 days after onset of your menstrual period. To determine luteal phase, we will need to have you use a family planning kit. This kit helps determine when you ovulate (and are therefore most likely to become pregnant). Using this kit involves urinating in a cup on a daily basis (for about 5-6 days), after completion of menstruation. The kit includes a plastic eye dropper for you to put a small amount of urine on a plastic test pad. The test pad turns purple when you reach the day just prior to when you ovulate. We will need to verify the color change before we can schedule the luteal phase visit. Your luteal phase visit will be approximately 6-8 days after ovulation.

Before each of the last two visits (one during your follicular menstrual phase and one during your luteal menstrual phase) you will complete a 3 day food record (including two weekdays and one weekend day). On the night before the last two visits, you will be expected to eat a standardized, nutritionally balanced, meal (which will be provided). Then, we would like you to fast for the ensuing 12 hours prior to the study (although drinking of water is permitted). When you come to the lab in the morning, after the 12 hour fast, we will first measure your height and weight. Next,

we will have you lay down and breathe room air through a clear plastic face mask that is attached to a machine in order to measure oxygen in the air you breathe out. This will tell us how much energy you use while you are resting. Following this measurement, we will obtain 10 ml of blood (about 1 tablespoon) from a vein in your arm.

Next, you will have your residual lung volume measured. Residual lung volume is the amount of air that is still in your lungs when you exhale as completely as possible. The test involves breathing 100% oxygen for a few seconds so it can mix with the air in your lungs. This process will be repeated a number of times to be sure the measure is consistent.

Then, we will have you change into your bathing suit, so we can estimate your percent body fat with a technique known as underwater weighing. The technique involves a few seconds of complete submersion (after you exhale) in a shallow water tank. This process will also be repeated a number of times.
Finally, we will measure the air that you breathe while you walk and/or run on the treadmill for 20 minutes at 60% of your previously determined aerobic capacity. This amount of exercise is similar to current recommendations for healthy adults to become more aerobically fit. Your heart rate and amount of oxygen used will also be measured during this test as it was in the aerobic fitness test. Immediately after you finish the exercise, we will take another blood sample (another tablespoon). Total time in the lab on each of the second and third visits should not exceed 2.5 hours.

D. Side effects and risks
There are minimal risks to exercise testing under the conditions specified above. The potential risks associated with tests include muscle soreness. The other risk is remote, but no one can be absolutely certain that you will not suffer a heart attack during the exercise tests. A recent national survey of exercise testing centers indicated an average of 3.6 heart attacks, 4.8 irregular heart beats, and 0.5 deaths per 10,000 tests in procedures performed for medical reasons. For a healthy, but inactive OR physically active population, there should be minimal risk to exercise testing for you under the specified conditions. There is also a slight chance of a bruise (hematoma) during the blood draws.

If you feel that you might have any medical condition (i.e. asthma, orthopedic problems, heart condition, etc.) which might be a reason not to perform the test, you should inform the investigators prior to any testing.

E. Benefits
For your participation, you will learn your current level of physical fitness, an estimate of your body composition, and a free blood glucose and cholesterol screen. Also, the information obtained in this research may help future studies dealing with women, their energy expenditure, and risk of some chronic problems like heart disease.
After completion of all tests, you will be given $60 for your participation in the study. You should understand that your participation is voluntary, and you may decline to participate in given activities if you so choose, or withdraw from the study at any time without penalty. If for any reason you do not complete the study, you will receive a percentage of the cash compensation, based on the amount of participation before leaving the study.

F. Confidentiality
All of your records from this study will be considered confidential and your name will not be identified in any publication or report resulting from this study. Confidentiality of data will be maintained within legal limits.

G. Cost
There will be no direct cost to you during your participation in this study.

In the event of injury resulting from this research, Michigan State University is not able to offer financial compensation nor to absorb the costs of medical treatment. However, necessary facilities, emergency treatment and professional services will be available to research subjects, just as they are to the community generally. Your signature below acknowledges your voluntary participation in this research project. Such participation does not release the investigators, institutions, sponsor, or granting agency from their professional and ethical responsibility to you.

_____ _____
Signature of Study Participant Date

_____

_____ _____ _____
Address Phone(home) Phone (work)

_____
Signature of investigator

APPENDIX C: Ovulation kit instructions

Instructions for determining day of ovulation using Ovu Quick® urine kits

Important!

* Samples must be collected between 10 a.m. and 8 p.m.
* Test samples should be taken at about the same time each day (within an hour)
* The amount of liquid that you drink will affect the test results. You should reduce your liquid intake for about 2 hours before you collect your urine. A diluted urine sample could prevent you from seeing your results.

1. Collect urine in the urine collection cup provided
2. You will need a watch or a clock before you begin the test
3. Remove the Test Cassette from the foil pouch and place it on a flat, dry surface.
4. Place the dropper in the urine sample. Draw urine into the dropper.
5. **Hold the urine dropper upright** about 1/2 inch above the Urine Well at the bottom of the Test Cassette. **Add only 3 DROPS** of urine to the Urine Well.
6. **WAIT 3-5 Minutes** and then read your Test Result.

*** Timing is very important when doing your test! Use your watch or clock and read your results between 3 and 5 minutes.***

7. Label each test cassette with your **ID#** (_____) and the date

Interpretation of Results

Positive Result (P): a pink-to-purple Test Line (next to the letter "T") that is **DARKER** than the Reference Line (next to the letter "R") indicates a *positive* result.

Negative Result (N): a pink-to-purple Test Line that is either **LIGHTER** than the Reference Line (or no Test Line at all), or **THE SAME COLOR** as the Reference Line.

Invalid Result (I): you MUST see a pink-to-purple Reference Line within 3 minutes after adding your urine. If you do not see a Reference Line, the test is considered **INVALID**, and you cannot use your Test Result.

When your test results are positive:

Please call the lab at 355-4734 to report which day your Ovu Quick® result is positive.

Day 1:_____	Time:_____	Result:___	Day 9:_____	Time:_____	Result:___
Day 2:_____	Time:_____	Result:___	Day 10:_____	Time:_____	Result:___
Day 3 _____	Time:_____	Result:___	Day 11:_____	Time:_____	Result:___
Day 4:_____	Time:_____	Result:___	Day 12:_____	Time:_____	Result:___
Day 5:_____	Time:_____	Result:___	Day 13:_____	Time:_____	Result:___
Day 6:_____	Time:_____	Result:___	Day 14:_____	Time:_____	Result:___
Day 7:_____	Time:_____	Result:___	Day 15:_____	Time:_____	Result:___
Day 8:_____	Time:_____	Result:___	Day 16:_____	Time:_____	Result:___

APPENDIX D: Food record and FFQ information

Currently, no one single method for assessing an individual's normal eating habits is available (65). However, 24-hour food records and food frequency questionnaires are commonly used.

I developed the food record that was used by pulling bits and pieces from many sample food records. Dr. Won Song, from the Food Science and Human Nutrition Department, provided the FFQ. It was the Block 98.1 version designed by Block Dietary Data Systems, Berkeley, CA.

Jean Kerver taught me how to give proper directions to the subjects with respect to correctly filling out the food records and FFQs. She also taught me how to recognize common errors that could be easily corrected when the forms were returned. The instructions given to the subjects included many visual and written examples of how to estimate portion sizes. When the subjects returned the food records and FFQs, I checked them for completeness and clarity.

The remaining pages of appendix D include all of the paperwork given to each subject with respect to assessing their nutritional habits except the FFQ. This includes the instructions given for how to complete a food record, the size approximation sheet, and a sample food record.

Directions for Completing a 3-day Food Record

1. Please refer to the sample food record to see how food is recorded.

2. List **ALL FOOD** and **BEVERAGES FOR ALL MEALS** and **SNACKS** for 3 days. (2 weekdays and 1 weekend day). Record your intake immediately after you eat. Be sure to measure your food.

Do not change your usual eating habits!

3. To measure solid food, use standard dry measuring cups or spoons. For meat or cheese, estimate the size of a portion by measuring with a ruler. For example, do not record the weight or size of a raw pork chop: instead, record the amount of a fried pork chop i.e. 2 x 4 x 1 inch.

*Please refer to the handout ***Size It Up!*** (on back) for a guide to portion sizes. For items that are round, i.e. some fruit, cakes, or pies, record the fraction of the whole consumed i.e. 1/4 of a 6 inch diameter cantaloupe or 1/8 of a 9 in. diam. pie.

4. To measure liquids, use a standard measuring cup (8fl.oz.) to measure the amount of liquid consumed. i.e. 12oz. Coke, 4oz. red wine, 2oz. liquor (include all mixers).

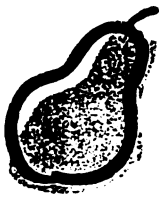
5. You will need to indicate individual ingredients in mixed dishes. An example of a mixed dish is a tuna casserole. The casserole may contain noodles, canned water packed tuna, cream of mushroom soup, and mozzarella cheese. If a recipe is used to make composite products such as casseroles, baked goods, sauces, etc., please provide a copy of the recipe on the index cards provided. Please provide the measured amount of each ingredient

6. List all condiments and their amounts separately. Don't forget things like added butter/ margarine, coffee creamer, salad dressing, spreads (i.e. jelly), sauces (i.e. Ketchup or mustard), sugar and oils. For example a hamburger might be:

3 oz. of ground beef (ground round, ground chuck, etc.)
2 Tbsp. ketchup (regular, low sodium, etc.)
2 Tbsp. yellow mustard
1 tsp. pickle relish
1 oz. of cheese (for example a Kraft American cheese single)
1 bun (whole wheat, white, etc.)

Size approximation sheet

Size It Up!

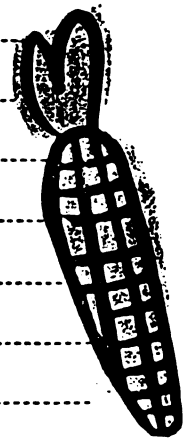


A Guide to Portion Sizes

To put your healthful eating plan into action, you need to know what one serving looks like. The portion or amount of a food that you choose to eat may be more or less than one serving. If you choose a large portion, it may count as 2 servings, a small portion may only be half a serving.

Learning to judge servings sizes takes a little practice. And, since carrying around measuring cups and a scale just isn't practical, here are some visual examples to help you make quick estimates.

3 ounces of meat, poultry or fish	Deck of playing cards, cassette tape or the palm of a woman's hand
1 ounce of meat, poultry or fish	Matchbook
1 cup of fruit or yogurt	Baseball
1/2 cup of chopped vegetables	Three regular ice cubes
1 medium potato	Computer mouse
1 cup of potatoes, rice or pasta	Size of a fist or a tennis ball
1 medium orange or apple	Baseball
1 standard bagel	Hockey puck
1 cup chopped fresh leafy greens	4 lettuce leaves
2 Tablespoons peanut butter	Golf ball
1 ounce of cheese	Four dice or a tube of lipstick
1 slice of cheese	3.5 inch computer disk



1/2 cup of cooked vegetables equals:
6 asparagus spears
7-8 baby carrots or carrot sticks
1 ear of corn
3 spears of broccoli

Date: 5/6/99		Day of Week: Thursday	Subject ID No.: LEJMR	
First Name: Juanita		Age: 28	USUAL/LESS/MORE	
Time	**Place Eaten**	**Description: Method/Type/Brand Name**	**Amount**	**Label Info/Other/Comments**
		roasted/fried/stewed/boiled/steamed; raw/cooked;		low salt, low fat, cut of
		peeled/unpeeled; 2%/whole/skim,white/whole wheat;		meat, with/without skin or bone,
		dried/fresh/homemade/can/frozen/instant		diet, fat free, regular, lite
7:45 AM	Home	Welch's grape juice	1/2 cup	sweetened
		Quaker, instant, boiled, cinnamon apple oatmeal	1 pouch (35g)	
		Aunt Millie's cracked wheat toast	1 slice	
		Land O Lakes butter	1 pat	
		Smucker's grape jelly	1 Tbsp	
10:23 AM	Class	fresh green grapes	15	
11:30 AM	School café.	salad: romaine lettuce	1 cup	
		tomato	1/4 cup	
		shredded cheddar cheese	1/8 cup	fat free
		Kraft French dressing	2 Tbsp	
		2% milk	8 oz	
		Tombstone frozen pizza (14inch diameter), baked	1/2 of total	
		pepperoni		
		onions		
		black olives		
5:15 PM	Car	Mr. Goodbar candy bar	2.25 oz	
8:00 PM	Home	tuna casserole, baked	1/6 of total	
		Starkist canned water packed tuna	6.5 oz	
		Campbell's cream mushroom soup	10.5 oz	
		Mueller's wide egg noodles, boiled then baked	4 cups	
		Kroger grated mozzarella cheese	1/2 cup	
		Coca-Cola	12 oz	Diet, caffeine free

APPENDIX E: Details of standardized, nutritionally balanced meals

Due to the potential variation of weights within a BMI category it was decided to create a number of meals for the range of potential weights, in 20-pound increments. Separate meals were created for sedentary and active individuals.

In nutrition circles, it is standard practice for dinner calories to be about 40% of TEE (26). Since meals had to be determined before the subjects were enrolled (and actual weights were known), Total Energy Expenditure (TEE) was estimated using the following equation: total energy expenditure (TEE) = REE + Activity calories + Digestive calories (26). REE was estimated as weight (in pounds) * 10. Activity calories were estimated as REE * 0.2 for sedentary subjects and REE * 0.4 for exercising subjects. Digestive calories were estimated as (REE + Activity calories) * 0.1.

After TEE was estimated for the BMI ranges of each group, an average, dinner meal calorie value was obtained for each 20-pound increment weight range. Table 7 shows the estimated dinner meal calorie values. Fourteen meals were designed using these estimates.

Table 7: Dinner meal calorie value estimates

Study Groups	Average Weight	REE (kcals)	Activity (kcals)	Digestive (kcals)	TEE (kcals)	Dinner (kcals)	Average Dinner (kcals)
Sedentary	81	810	162	97	1069	428	
	100	1000	200	120	1320	528	478 (average 81&100)
	101	1010	202	121	1333	533	
	120	1200	240	144	1584	634	583 (average 101&120)
	121	1210	242	145	1597	639	
	140	1400	280	168	1848	739	689 (average 121&140)
	141	1410	282	169	1861	744	
	160	1600	320	192	2112	845	795 (average 141&160)
	161	1610	322	193	2125	850	
	180	1800	360	216	2376	950	900 (average 161&180)
	181	1810	362	217	2389	956	
	200	2000	400	240	2640	1056	1006 (average 181&200)
	201	2010	402	241	2653	1061	
	220	2200	440	264	2904	1162	1111 (average 201&220)
Active	81	810	405	122	1337	535	
	100	1000	500	150	1650	660	597 (average 81&100)
	101	1010	505	152	1667	667	
	120	1200	600	180	1980	792	729 (average 101&120)
	121	1210	605	182	1997	799	
	140	1400	700	210	2310	924	861 (average 121&140)
	141	1410	705	212	2327	931	
	160	1600	800	240	2640	1056	993 (average 141&160)
	161	1610	805	242	2657	1063	
	180	1800	900	270	2970	1188	1125 (average 161&180)
	181	1810	905	272	2987	1195	
	200	2000	1000	300	3300	1320	1257 (average 181&200)
	201	2010	1005	302	3317	1327	
	220	2200	1100	330	3630	1452	1389 (average 201&220)

Note: REE = Resting Energy Expediture, kcals = kilocalories, TEE = Total Energy Expenditure

Kanika Triggs, graduate student in the Food Science and Human Nutrition department, (under the direction of Dr. Song) started to develop the standardized meals. Jean Kerver, another graduate student in the Food Science and Human Nutrition department, (also under the direction of Dr. Song), continued the work. I completed the development of the standardized meals under the direction of Jean Kerver and Dr. Song. Table 8 shows the food choices and the individual meals that were created. In addition, the amounts of carbohydrate, protein, and fat are shown. The desired calorie value is in parentheses next to each meal.

The actual meal consumed by each subject, was determined by their weight and exercise status on the day of the VO_{2max} test. Emily Paxton and Juanita Rivera prepared all of the meals.

Table 8: Individualized, Nutritionally Balanced Meals

Overview of Food Choices for Leptin Study

	Portion Size	Kilocalories	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
Wishbone Fat Free Ranch	2 Tbsp	40	9	0	0
Newman's Own Light Italian	2 Tbsp	45	3	0	4
Kraft Fat Free French	2 Tbsp	45	11	0	0
Kraft Zesty Italian	2 Tbsp	110	2	0	11
Kraft Classic Caesar	2 Tbsp	110	1	1	11
Henri's French	2 Tbsp	120	6	0	11
average values for full fat dressings	2 Tbsp	113	3	0	11
average values for reduced fat dressings	2 Tbsp	43	8	0	1
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Promise Ultra, Fat-Free Margarine	1 Tbsp	5	0	0	0
Apple	1 Item	81	21	0	1
Nabisco Ritz Crackers	1 cracker	16	2	0	1
Quaker Chewy Granola Bars, Choc. Chip	1 bar	120	21	2	6
Chips Ahoy Cookies	1 Cookie	53	7	1	3

Items for Ad Lib consumption will include: water, diet soda, and iced tea with no sugar.

Meal 1: Sedentary 81-100 (~478 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for reduced fat dressings	2 Tbsp	43	8	0	1
Nabisco Ritz Crackers	2 cracker	32	4	0	2
Chips Ahoy Cookies	1 Cookie	53	7	1	3
Total		478	62	25	15
Percent of Total Kcals			52%	21%	28%

	Portion Size	Kilocalories	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Meal 2: Active 81-100 (~597 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for reduced fat dressings	2 Tbsp	43	8	0	1
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Nabisco Ritz Crackers	3 cracker	48	6	0	3
Total		596	82	29	16.5
Percent of Total Kcals			55%	19%	25%
Meal 3: Sedentary 101-120 (~583 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for reduced fat dressings	2 Tbsp	43	8	0	1
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Nabisco Ritz Crackers	2 cracker	32	4	0	2
Total		580	80	29	15.5
Percent of Total Kcals			55%	20%	24%
Meal 4: Active 101-120 (~729 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for reduced fat dressings	2 Tbsp	43	8	0	1
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Apple	1 Item	81	21	0	1
Chips Ahoy Cookies	2 Cookie	106	14	2	6
Total		735	111	31	20.5
Percent of Total Kcals			60%	17%	25%

	Portion Size	Kilocalories	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Meal 5: Sedentary 121-140 (~689 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for reduced fat dressings	2 Tbsp	43	8	0	1
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Nabisco Ritz Crackers	2 cracker	32	4	0	2
Chips Ahoy Cookies	2 Cookie	106	14	2	6
Total		686	94	31	21.5
Percent of Total Kcals			55%	18%	28%
Meal 6: Active 121-140 (~861 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Promise Ultra, Fat-Free Margarine	1 Tbsp	5	0	0	0
Apple	1 Item	81	21	0	1
Nabisco Ritz Crackers	1 cracker	16	2	0	1
Chips Ahoy Cookies	1 Cookie	53	7	1	3
Quaker Chewy Granola Bars, Choc. Chip	1 bar	120	21	2	6
Total		868	122	32	31.5
Percent of Total Kcals			56%	15%	33%

	Portion Size	Kilocalories	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Meal 7: Sedentary 141-160 (~795 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Promise Ultra, Fat-Free Margarine	1 Tbsp	5	0	0	0
Apple	1 Item	81	21	0	1
Nabisco Ritz Crackers	1 cracker	16	2	0	1
Chips Ahoy Cookies	2 Cookie	106	14	2	6
Total		801	108	31	28.5
Percent of Total Kcals			54%	15%	32%
Meal 8: Active 141-160 (~993 kcals)					
Lean Cuisine Cheese Lasagna	2 Item	580	76	40	12
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Apple	1 Item	81	21	0	1
Total		989	130	49	30.5
Percent of Total Kcals			53%	20%	28%
Meal 9: Sedentary 181-200 (~1006 kcals)					
Lean Cuisine Cheese Lasagna	2 Item	580	76	40	12
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Nabisco Ritz Crackers	1 cracker	16	2	0	1
Apple	1 Item	81	21	0	1
Total		1005	132	49	31.5
Percent of Total Kcals			53%	20%	28%

	Portion Size	Kilocalories	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Meal 10: Sedentary 161-180 (~900 kcals)					
Lean Cuisine Cheese Lasagna	1 Item	290	38	20	6
Wendy's side salad	1 Item	60	5	4	3
average values for reduced fat dressings	2 Tbsp	43	8	0	1
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Apple	1 Item	81	21	0	1
Nabisco Ritz Crackers	3 cracker	48	6	1	2
Quaker Chewy Granola Bars, Choc. Chip	1 bar	120	21	2	6
Chips Ahoy Cookies	2 Cookie	106	14	2	6
Total		903	138	34	28.5
Percent of Total Kcals			61%	15%	28%
Meal 11: Active 161-180 (~1125 kcals)					
Lean Cuisine Cheese Lasagna	2 Item	580	76	40	12
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Apple	1 Item	81	21	0	1
Nabisco Ritz Crackers	1 cracker	16	2	0	1
Quaker Chewy Granola Bars, Choc. Chip	1 bar	120	21	2	6
Total		1125	153	51	37.5
Percent of Total Kcals			54%	18%	30%

	Portion Size	Kilocalories	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Meal 12: Sedentary 201-220 (~1111 kcals)					
Lean Cuisine Cheese Lasagna	2 Item	580	76	40	12
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	1 tsp	25	0	0	3
Apple	1 Item	81	21	0	1
Quaker Chewy Granola Bars, Choc. Chip	1 bar	120	21	2	6
Total		1109	151	51	36.5
Percent of Total Kcals			54%	18%	30%
Meal 13: Active 181-200 (~1257 kcals)					
Lean Cuisine Cheese Lasagna	2 Item	580	76	40	12
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Fleishman's Margarine, Regular, Stick	2 tsp	50	0	0	6
Apple	1 Item	81	21	0	1
Quaker Chewy Granola Bars, Choc. Chip	1 bar	120	21	2	6
Nabisco Ritz Crackers	1 cracker	16	2	0	1
Chips Ahoy Cookies	2 Cookie	106	14	2	6
Total		1256	167	53	46.5
Percent of Total Kcals			53%	17%	33%

	Portion Size	Kilocalories	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Meal 14: Active 201-220 (~1389 kcals)					
Lean Cuisine Cheese Lasagna	2 Item	580	76	40	12
Wendy's side salad	1 Item	60	5	4	3
average values for full fat dressings	2 Tbsp	113	3	0	11
Healthy Choice Italian Semolina Rolls	1 Item	130	25	5	0.5
Promise Ultra, Fat-Free Margarine	2 Tbsp	10	0	0	0
Apple	1 Item	81	21	0	1
Quaker Chewy Granola Bars, Choc. Chip	2 bar	240	42	4	12
Nabisco Ritz Crackers	1 cracker	16	2	0	1
Chips Ahoy Cookies	3 Cookie	160	21	2	8
Total		1390	195	55	48.5
Percent of Total Kcals			56%	16%	31%

APPENDIX F: Body composition equations & sample calculation

Equations:

RV Equation

$RV = [(A * B)/79.8 - B] * C$

A = volume(L) of O_2 injected into the bag

B = Post-test N_2 (nitrogen) reading - Pre-test N_2 reading

C = BTPS correction factor (see below)

Barometric temperature and pressure, saturated (BTPS) correction factor equation

$BTPS\ correction\ factor = [310/(273+T_a)]*[(P_B - P_{H2O})/(P_B - 47)]$

T_a = ambient temperature (in Kelvin)

P_B = barometric pressure (mm Hg)

P_{H2O} = pressure of water at T_a (mm Hg)

Body Density Equation

$$\text{Body Density} = \frac{\text{Weight in air (in kg)}}{[(\text{Weight in air} - \text{Weight in water})/\text{Density of water}] - RV^*}$$

*This RV should also include 100 ml to account for intestinal gas

Modified Siri equation for women

$\%\ fat = ((5.05/\text{body density}) - 4.62) * 100$

Sample calculation

RV calculation

A = 2L; Average B (from two highest values of six) = 23.6; C = 1.068

$RV = [(2*23.6)/79.8-23.6]*1.068 = 0.89$ L

BTPS correction factor calculation

$T_a = 26°C$; $P_B = 768$ mmHg; $P_{H2O} = 25.2$ mmHg

$BTPS\ correction\ factor = [310/(273 + 26)]*[(768-25.2)/(768 - 47)]=[310/299]*[742.8/721]=1.037*1.030 = 1.068$

Body density calculation

Weight in air = 47kg; Average weight in water = 1.36kg (3 pounds ÷ 2.2 pounds/kg); density of water at 25.2°C = 0.9941; RV =0.89 + 0.100L (for intestinal gas)=0.99 L

$\text{Body density} = 47/(((47-1.36)/0.9941)-0.99) = 1.0463$

% fat calculation

$\%\ fat = ((5.05/1.0463)-4.62)*100=20.7\%$

APPENDIX G: Brief overview of leptin RIA

Table 9: Leptin radioimmunoassay flow chart

Tube #	Buffer (ul)	Standard Quality Control/Sample	Label (ul)	Leptin Antibody (ul)	Precipitating Reagent (ml)
1,2	"-"	"-"	100	"-"	"-"
3,4	300	"-"	100	"-"	1
5,6	200	"-"	100	100	1
7,8	100	100 ul of 0.5 ng/ml	100	100	1
9,10	100	100 ul of 1 ng/ml	100	100	1
11,12	100	100 ul of 2 ng/ml	100	100	1
13,14	100	100 ul of 5 ng/ml	100	100	1
15,16	100	100 ul of 10 ng/ml	100	100	1
17,18	100	100 ul of 20 ng/ml	100	100	1
19,20	100	100 ul of 50 ng/ml	100	100	1
21,22	100	100 ul of 100 ng/ml	100	100	1
23,24	100	100 ul of QC 1	100	100	1
25,26	100	100 ul of QC 2	100	100	1
27,28	100	100 ul of unknown	100	100	1
29-n	100	100 ul of unknown	100	100	1
Vortex and incubate 20-24 hrs at 4°C					
Vortex and incubate 20 min at 4°C. Centrifuge, decant and count pellet.					

REFERENCES

REFERENCES

1. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription.* Baltimore: Williams and Wilkins, 1995. p. 373.

2. Andreelli, F., H. Hanaire-Broutin, M. Laville, J. P. Tauber, J. P. Riou, and C. Thivolet. Normal Reproductive Function in Leptin-Deficient Patients with Lipoatrophic Diabetes. *The Journal of Clinical Endocrinology and Metabolism* 85: 715-719, 2000.

3. Basdevant, A., J. Bringer, and P. Lefebvre. *Weight, nutrition, and hormonal events in women,* 1997. p. v-vii.

4. Bemben, D., P. Salm, and A. Salm. Ventilatory and blood lactate responses to maximal treadmill exercise during the menstrual cycle. *J Sports Med Phys Fitness* 35: 257-62, 1995.

5. Bergendahl, M., A. Iranmanesh, W. S. Evans, and J. D. Veldhuis. Short-Term Fasting Selectively Suppresses Leptin Pulse Mass and 24-Hour Rhythmic Leptin Release in Healthy Midluteal Phase Women without Disturbing Leptin Pulse Frequency or Its Entropy Control (Pattern Orderliness). *The Journal of Clinical Endocrinology and Metabolism* 85: 207-213, 2000.

6. Berman, D. M., E. M. Rogus, M. J. Busby-Whitehead, L. I. Katzel, and A. P. Goldberg. Predictors of Adipose Tissue Lipoprotein Lipase in Middle-Aged and Older Men: Relationship to Leptin and Obesity, But Not Cardiovascular Fitness. *Metabolism* 48: 183-189, 1999.

7. Boden, G., X. Chen, M. Mozzoli, and I. Ryan. Effect of Fasting on Serum Leptin in Normal Human Subjects. *Journal of Clinical Endocrinology and Metabolism* 81: 3419-2423, 1996.

8. Butte, N., J. Hopkinson, and M. Nicolson. Leptin in human reproduction: serum leptin levels in pregnant and lactating women. *J Clin Endo Metab* 82: 585-589, 1997.

9. Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos, and P. Burn. Recombinant Mouse OB Protein: Evidence for a Peripheral Signal Linking Adiposity and Central Neural Networks. *Science* 269: 546-549, 1995.

10. Campostano, A., G. Grillo, D. Bessarione, R. De Grandi, and G. F. Adami. Relationships of Serum Leptin to Body Composition and Resting Energy Expenditure. *Hormone and Metabolism Research* 30: 646-647, 1998.

11. Caro, J., M. K. Sinha, J. Kolaczynski, P. L. Zhang, and R. V. Considine. Leptin: The Tale of an Obesity Gene. *Diabetes* 45: 1455-1462, 1996.

12. Castracane, V., R. Kraemer, M. Franken, G. Kraemer, and T. Gimpel. Serum leptin concentration in women: effect of age, obesity, and estrogen administration. *Fertility and Sterility* 70: 472-7, 1998.

13. Ceddia, R. B., J. William, W.N., F. B. Lima, A. R. Carpinelli, and R. Curi. Pivotal role of leptin in insulin effects. *Brazilian Journal of Medical and Biological Research* 31: 715-722, 1998.

14. Christensen, J., O. Svendsen, C. Hassager, and C. Christiansen. Leptin in Overweight Postmenopausal Women: No relationship with Metabolic Syndrome X or Effect of Exercise in addition to diet. *International Journal of Obesity Related Metabolic Disorders* 22: 195-9, 1998.

15. Clapp, J. F., W. F. Blum, and W. Kiess. Effect of exercise during pregnancy on two metabolic markers - tumor necrosis factor alpha and leptin. *ACSM National Conference*, Orlando, FL, 1998.

16. Clapp, J. F., and W. Kiess. Effects of pregnancy and exercise on concentrations of the metabolic markers tumor necrosis factor alpha and leptin. *American Journal of Obstetrics and Gynecology* 182: 300-306, 2000.

17. Clement, K., C. Vaisse, N. Lahlou, S. Cabrol, V. Pelloux, D. Cassuto, M. Gourmelen, C. Dina, J. Chambaz, J.-M. Lacorte, A. Basdevant, P. Bougneres, Y. Lebouc, P. Froguel, and B. Guy-Grande. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392: 398-401, 1998.

18. Coleman, D., and K. Hummel. Effects of Parabiosis of normal with genetically diabetic mice. *American J of Physiol* 217: 1298-1304, 1969.

19. Considine, R. V., and J. F. Caro. Leptin: Genes, Concepts and Clinical Perspective. *Hormone Research* 46: 249-256, 1996.

20. Considine, R. V., M. K. Sinha, M. L. Heiman, A. Kriauciunas, T. W. Stephens, M. R. Nyce, J. P. Ohanessian, C. C. Marco, L. J. McKee, T. L. Bauer, and J. F. Caro. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *NEJM* 334: 292-295, 1996.

21. Dagogo-Jack, S., C. Fanelli, D. Paramore, J. Brothers, and M. Landt. Plasma Leptin and Insulin Relationships in Obese and Nonobese Humans. *Diabetes* 45: 695-698, 1996.

22. Dallongeville, J., B. Hecquer, P. Lebel, J.-L. Edme, C. LeFur, J.-C. Fruchart, J. Auwerx, and M. Romon. Short term response of circulating leptin to feeding and fasting in man: influence of circadian cycle. *International Journal of Obesity* 22: 728-733, 1998.

23. De Silva, A., M. De Courten, P. Zimmet, G. Nicholson, M. Kotowicz, J. Pasco, and G. R. Collier. Lifestyle Factors Fail to Explain the Variation in Plasma Leptin Concentrations in Women. *Nutrition* 14: 653-657, 1998.

24. Dirlewanger, M., V. Di Vetta, V. Giusti, P. Schneiter, E. Jequier, and L. Tappy. Effect of moderate physical activity on plasma leptin concentration in humans. *European Journal of Applied Physiology* 79: 331-335, 1999.

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

26. Duyff, R. L. *The American Dietetic Association's Complete Food and Nutrition Guide*. Minneapolis: Chronimed Publishing, 1998.

27. Dyck, D. J., and G. Steinberg. N-3 and N-6 High-Fat Diets Reduce Leptin Sensitivity in Rodent Skeletal

Muscle. *American College of Sports Medicine 47th Annual Meeting*, Indianapolis, IN, 2000.

28. Essig, D. A., N. L. Alderson, M. A. Ferguson, W. P. Bartoli, and J. L. Durstine. Delayed Effects of Exercise on the Plasma Leptin Concentration. *Metabolism* 49: 395-399, 2000.

29. Flegal, K., M. Carroll, R. Kuczmarski, and C. Johnson. Overweight and obesity in the United States: prevalence and trends, 1960-1994. *Int J Obes Relat Metab Disord* 22: 39-47, 1998.

30. Flier, J. S. Clinical Review 94 What's in a Name? In search of Leptin's Physiologic Role. *Journal of Clinical Endocrinology and Metabolism* 83: 1407-1413, 1998.

31. Fox, C. S., J. Esparza, M. Nicolson, P. H. Bennett, L. O. Schulz, M. E. Valencia, and E. Ravussin. Is a low leptin concentration, a low resting metabolic rate, or both the expression of the "thrifty genotype"? Results from Mexican Pima Indians. *American Journal of Clinical Nutrition* 68: 1053-7, 1998.

32. Friedman, J. M. Leptin, Leptin Receptors, and the Control of Body Weight. *Nutrition Reviews* 56: S38-S46, 1998.

33. Grinspoon, S., H. Askari, M. Landt, D. Nathan, D. Schoenfeld, D. Hayden, M. Laposata, J. Hubbard, and A. Klibanski. Effects of Fasting and glucose infusion on basal and overweight leptin concentrations in normal-weight women. *Am J Clin Nutr* 66: 1352-1356, 1997.

34. Halaas, J., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley, and J. M. Friedman. Weight-Reducing Effects of the Plasma Protein Encoded by the obese Gene. *Science* 269: 543-546, 1995.

35. Hamann, A., and S. Matthaei. Regulation of energy balance by leptin. *Exp Clin Endocrinol Diabetes* 104: 293-300, 1996.

36. Hardie, L., P. Trayhurn, D. Abramovich, and P. Fowler. Circulating Leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clinical Endocrinology* 47: 101-106, 1997.

37. Hausberger, F. Parabiosis and transplantation experiments in hereditarily obese mice. *Anat Rec* 130: 313, 1959.

38. Havel, P. J., S. Kasim-Karakas, W. Mueller, P. R. Johnson, R. L. Gingerich, and J. S. Stern. Relationship of Plasma Leptin to Plasma Insulin and Adiposity in Normal Weight and Overweight Women: Effects of Dietary Fat Content and Sustained Weight Loss. *J Clin Endo Metab* 81: 4406-4413, 1996.

39. Haynes, W., D. Morgan, S. Walsh, S. WI, and A. Mark. Cardiovascular consequences of obesity: role of leptin. *Clin Exp Pharmacol Physiol* 25: 65-69, 1998.

40. Helland, I. B., J. E. Reseland, O. D. Saugstad, and C. A. Drevon. Leptin Levels in Pregnant Women and Newborn Infants: Gender Differences and Reduction During the Neonatal Period. *Pediatrics* 101: e 1-8, 1998.

41. Hervey, G. The Effects of Lesions in the Hypothalamus in Parabiotic Rats. *J Physiol* 145: 336-352, 1959.

42. Hickey, M. S., R. V. Considine, R. G. Israel, T. L. Mahar, M. R. McCammon, G. L. Tyndall, J. A. Houmard, and J. F. Caro. Leptin is related to body fat content in male distance runners. *JAP* 271: E938-E940, 1996.

43. Hickey, M. S., C. Cooke, R. Seebohar, S. Franco, G. Alvarez, P. Curry, and A. Wagner. Plasma Leptin Responses To Isocaloric And Hypercaloric Manipulations Of Dietary Fat. *American College of Sports Medicine 47th Annual Meeting*, Indianapolis, IN, 2000.

44. Hickey, M. S., J. A. Houmaard, R. V. Considine, G. L. Tyndall, J. B. Midgette, K. E. Gavigan, M. L. Weidner, M. R. McCammon, R. G. Israel, and J. F. Caro. Gender-dependent effects of exercise training on serum leptin levels in humans. *JAP* 272: E562-E566, 1997.

45. Hickey, M. S., R. G. Israel, and R. V. Considine. The Adipocyte Hormone Leptin: Relation to Obesity, Diet, and Exercise. *American College of Sports Medicine 46th Annual Meeting*, Seattle, Washington. Medicine and Science in Sports and Exercise, 1999.

46. Highman, T. J., J. E. Friedman, L. P. Huston, W. W. Wong, and P. W. Catalano. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy. *Am J Obstet Gynecol* 178: 1010-1015, 1998.

47. Hilton, L. K., and A. B. Loucks. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. *American Journal of Physiology, Endocrinology, and Metabolism* 278: E43-49, 2000.

48. Hilton, L. K., M. Verdun, R. A. Brown, J. R. Thuma, and A. B. Loucks. Leptin Responses To Acute Exercise Depend on Carbohydrate Availability. *American College of Sports Medicine 47th Annual Meeting*, Indianapolis, IN, 2000.

49. Houmard, J. A., J. H. Cox, P. S. MacLean, and H. A. Barakat. Effect of Short-Term Exercise Training on Leptin and Insulin Action. *Metabolism* 49: 858-861, 2000.

50. Houseknecht, K., C. Mantzoros, R. Kuliawat, E. Hadro, J. Flier, and B. Kahn. Evidence for leptin binding to proteins in serum of rodents and humans: modulation with obesity. *Diabetes* 45: 1638-1643, 1996.

51. Houtkooper, L., and S. Going. Guidelines for body composition assessment. *Sports Science Exchange* 7: 1-6, 1994.

52. Jenkins, A., T. Markovic, A. Fleury, and L. Campbell. Carbohydrate intake and short-term regulation of leptin in humans. *Diabetologia* 40: 348-351, 1997.

53. Jorgensen, J. O. L., N. Vahl, R. Dall, and J. S. Christiansen. Resting Metabolic Rate in Healthy Adults: Relation to Growth Hormone Status and Leptin Levels. *Metabolism* 47: 1134-1139, 1998.

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

55. Kennedy, A., T. W. Gettys, P. Watson, P. Wallace, E. Ganaway, Q. Pan, and W. T. Garvey. The Metabolic Significance of Leptin in Humans: Gender-Based Differences in Relationship to Adiposity, Insulin Sensitivity, and Energy Expenditure. *J Clin Endo Metab* 82: 1293-1300, 1997.

56. Kern, M. J., J. I. Edmondson, and H. Broder. Serum Leptin Is Altered By Acute Fat And Carbohydrate. *American College of Sports Medicine 47th Annual Meeting*, Indianapolis, IN, 2000.

57. Kohrt, W. M., M. Landt, and S. Birge. Serum Leptin Levels Are Reduced in Response to Exercise Training, But Not Hormone Replacement Therapy, in Older Women. *J Clin Endo Metab* 81: 3980-3985, 1996.

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

59. Kolaczynski, J. W., R. V. Considine, J. Ohannesian, C. Marco, I. Opentanova, M. R. Nyce, M. Myint, and J. F. Caro. Responses of Leptin to Short-Term Fasting and Refeeding in Humans: A Link With Ketogenesis but Not Ketones Themselves. *Diabetes* 45: 1511-1515, 1996.

60. Kolaczynski, J. W., J. P. Ohannesian, R. V. Considine, C. C. Marco, and J. F. Caro. Response of Leptin to Short-Term and Prolonged Overfeeding in Humans. *J Clin Endo Metab* 81: 4162-4165, 1996.

61. Kraemer, R. R., L. G. Johnson, R. Haltom, G. R. Kraemer, E. P. Hebert, T. Gimpel, and V. D. Castracane. Serum Leptin Concentrations in Response to Acute Exercise in Postmenopausal Women With and Without Hormone Replacement Therapy.: 171-177, 1999.

62. Landt, M., G. M. Lawson, J. M. Helgeson, D.-R. V. G., J. H. Ladenson, A. S. Jaffe, and R. C. Hickner.

Prolonged Exercise Decreases Serum Leptin Concentrations. *Metabolism* 46: 1109-1112, 1997.

63. Laughlin, G., and S. Yen. Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. *J Clin Endo Metab* 82: 318-321, 1997.

64. Leal-Cerro, A., P. Garcia-Luna, R. Astorga, J. Parejo, R. Peino, C. Dieguez, and F. Casanueva. Serum leptin levels in male marathon athletes before and after the marathon run. *J Clin Endocrinol Metab* 83: 2376-2379, 1998.

65. Lee-Han, H., M. V., and N. Boyd. *J Clin Epidemiol* 42: 269-273, 1989.

66. Licinio, J., C. Mantzoros, A. B. Negrao, G. Cizza, M.-L. Wong, P. B. Bongiorno, G. P. Chrousos, B. Karp, C. Allen, J. S. Flier, and P. W. Gold. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nature Medicine* 3: 575-579, 1997.

67. Licinio, J., A. B. Negrao, C. Mantzoros, V. Kaklamani, M.-L. Wong, P. B. Bongiorno, A. Mulla, L. Cearnal, J. D. Veldhuis, J. S. Flier, S. M. McCann, and P. W. Gold. Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. *Proc. Natl. Acad. Sci USA* 95: 2541-2546, 1998.

68. Linco Research, I. Human Leptin RIA Kit Cat. # HL-81K. St. Louis, MO: Linco Research, Inc., 1998.

69. Lindroos, A. K., L. Lissner, B. Carlsson, L. M. Carlsson, J. Torgerson, C. Karlsson, K. Stenlof, and L. Sjostrom. Familial predisposition for obesity may modify the predictive value of serum leptin concentrations for long-term weight change in obese women. *Am J Clin Nutr* 67: 1119-1123, 1998.

70. Macut, D., D. Micic, F. P. Pralong, P. Bischof, and A. Campana. Is there a role for leptin in human reproduction. *Gynecological Endocrinology* 12: 321-326, 1998.

71. Maffei, M., J. Halass, E. Ravussin, R. Pratley, G. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S.

Ranganathan, P. Kern, and J. Friedman. Leptin levels in human and rodent: Measurements of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Medicine* 1: 1155-1161, 1995.

72. Mannucci, E., A. Ognibene, A. Becorpi, F. Cremasco, S. Pelligrini, S. Ottanelli, S. M. Rizzello, G. Massi, G. Messeri, and C. M. Rotella. Relationship between leptin and oestrogens in healthy women. *European Journal of Endocrinology* 139: 198-201, 1998.

73. Martin, L. J., P. J. H. Jones, R. V. Considine, W. Su, N. F. Boyd, and J. F. Caro. Serum leptin levels and energy expenditure in normal weight women. *Can J Physiol Pharmacol* 76: 237-241, 1998.

74. Masuzaki, H., Y. Ogawa, N. Sagawa, K. Hosoda, T. Matsumoto, H. Mise, H. Nishimura, Y. Yoshimasa, I. Tanaka, T. Mori, and K. Nakao. Nonadipose tissue production of leptin: Leptin as a novel placenta-derived hormone in humans. *Nature Medicine* 3: 1029-1033, 1997.

75. McArdle, W. D., F. I. Katch, and V. L. Katch. *Exercise Physiology: Energy, Nutrition, and Human Performance*. Baltimore: Williams & Wilkins, 1996. p. 849.

76. Melanson, K., M. Westerterp-Plantenga, L. A. Campfield, and W. H. M. Saris. Appetite and blood glucose profiles in humans after glycogen-depleting exercise. *Journal of Applied Physiology*: 947-954, 1999.

77. Messinis, I., E., and S. D. Milingos. Leptin in human reproduction. *Human Reproduction* 5: 52-63, 1999.

78. Messinis, I. E., S. Milingos, K. Zikopoulos, G. Kollios, K. Seferiadis, and D. Lolis. Leptin concentrations in the follicular phase of spontaneous cycles and cycles superovulated with follicle stimulating hormone. *Human Reproduction* 13: 1152-1156, 1998.

79. Miller, P., and M. Soules. The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. *Obstet Gynecol* 87: 13-17, 1996.

80. Montague, C. T., I. S. Farooqi, J. P. Whitehead, M. A. Soos, H. Rau, N. J. Wareham, C. Sewter, J. E. Digby, S. N. Mohammed, J. A. Hurst, C. H. Cheetham, A. R. Earley,

A. H. Barnett, J. B. Prins, and S. O'Rahilly. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387: 903-908, 1997.

81. Nicklas, B., M. J. Toth, and E. T. Poehlman. Daily Energy Expenditure Is Related to Plasma Leptin Concentrations in Older African-American Women but Not Men. *Diabetes* 46: 1389-1392, 1997.

82. Nicklas, B. J., M. J. Toth, A. P. Goldberg, and E. T. Poehlman. Racial Differences in Plasma Leptin Concentrations in Obese Postmenopausal Women. *Journal of Clinical Endocrinology and Metabolism* 82: 315-317, 1997.

83. Niskanen, L., S. Haffner, L. Karhunen, A. Turpeinen, H. Miettinen, and M. Uusitupa. Serum leptin in relation to resting energy expenditure and fuel metabolism in obese subjects. *International Journal of Obesity* 21: 309-313, 1997.

84. Ohannesian, J., C. Marco, P. Najm, B. Goldstein, J. Caro, and J. Kolaczynski. Small Weight Gain is not Associated with Development of Insulin Resistance in Healthy, Physically Active Individuals. *Hormone and Metabolism Research* 31: 323-325, 1999.

85. Okazaki, T., E. Himeno, H. Nanri, H. Ogata, and M. Ikeda. 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, 1999.

86. Ostlund, R. E., J. W. Yang, and S. Klein. Relation between Plasma Leptin Concentration and Body Fat, Gender, Diet, Age and Metabolic Covariates. *J Clin Endo Metab* 81: 3909-3913, 1996.

87. Paolisso, G., M. Rizzo, C. Mone, M. Tagliamonte, A. Gambardella, M. Riondino, C. Carella, M. Varricchio, and F. D'Onofrio. Plasma sex hormones are significantly associated with plasma leptin concentration in healthy subjects. *Clin Endocrinol* 48: 291-297, 1998.

88. Paolisso, G., M. R. Rizzo, G. Mazziotti, M. Rotondi, M. R. Tagliamonte, G. Varricchio, C. Carella, and M. Varricchio. Lack of association between changes in plasma leptin concentration and in food intake during the

menstrual cycle. *European Journal of Clinical Investigation* 29: 490-495, 1999.

89. Pasman, W. J., M. S. Westerterp-Platenga, and W. H. M. Saris. The effect of exercise training on leptin levels in obese males. *American Journal of Physiology* 274: E280-6, 1998.

90. Pelleymounter, M. A., C. M. Jane, M. B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. Effects of obese Gene Product on Body Weight Regulation in ob/ob Mice. *Science* 269: 540-543, 1995.

91. Perusse, L., G. Collier, J. Gagnon, A. S. Leon, D. C. Rao, J. S. Skinner, J. H. Wilmore, A. Nadeau, P. Z. Zimmet, and C. Bouchard. Acute and chronic effects of exercise on leptin levels in humans. *JAP* 83: 5-10, 1997.

92. Pinn, V. The NIH Office of Research on Women's Health: Goals and Recent Activities. *JAMWA* 53: 35-37, 1998.

93. Quinton, N. D., S. M. Laird, M. A. Okon, T. C. Li, R. F. Smith, R. J. M. Ross, and A. I. F. Blakemoore. Serum leptin levels during the menstrual cycle of healthy fertile women. *British Journal of Biomedical Science* 56: 16-19, 1999.

94. Racette, S. B., S. W. Coppack, M. Landt, and S. Klein. Leptin Production during Moderate-Intensity Aerobic Exercise. *J Clin Endo Metab* 82: 2275-2277, 1997.

95. Remesar, X., I. Refecas, J. Fernandez-Lopez, and M. Alemany. Is leptin an insulin counter-regulatory hormone? *FEBS Lett* 402: 9-11, 1997.

96. Riad-Gabriel, M. G., S. D. Jinagouda, A. Sharma, R. Boyadjian, and M. F. Saad. Changes in plasma leptin during the menstrual cycle. *European Journal of Endocrinology* 139: 528-531, 1998.

97. Roberts, S. B., M. Nicholson, M. Staten, G. E. Dallal, A. L. Sawaya, M. B. Heyman, P. Fuss, and A. S. Greenberg. Relationship Between Circulating Leptin and Energy Expenditure in Adult Men and Women Aged 18 to 81 Years. *Obesity Research* 5: 459-463, 1997.

98. Rosenbaum, M., M. Nicolson, J. Hirsch, S. B. Heymsfield, D. Gallagher, F. Chu, and R. L. Leibel. Effects of Gender, Body Composition, and Menopause on Plasma Concentrations of Leptin. *J Clin Endo Metab* 81: 3424-3427, 1996.

99. Rosenbaum, M., M. Nicolson, J. Hirsch, E. Murphy, F. Chu, and R. L. Leibel. Effects of Weight Change on Plasma Leptin Concentrations and Energy Expenditure. *J Clin Endo Metab* 82: 3647-3654, 1997.

100. Ryan, A., R. Pratley, D. Elahi, and A. Goldberg. Changes in plasma leptin and insulin action with resistive training in postmenopausal women. *International Journal of Obesity* 24: 27-32, 2000.

101. Ryan, A. S., and D. Elahi. The Effects of Acute Hyperglycemia and Hyperinsulinemia on Plasma Leptin Levels: Its Relationships with Body Fat, Visceral Adiposity, and Age in Women. *J Clin Endo Metab* 81: 4433-4438, 1996.

102. Saad, M. F., M. G. Riad-Gabriel, A. Khan, A. Sharma, R. Michael, S. D. Jinagouda, R. Boyadjian, and G. M. Steil. Diurnal and Ultradian Rhythmicity of Plasma Leptin: Effects of Gender and Adiposity. *J Clin Endo Metab* 83: 453-459, 1998.

103. Sattar, N., I. A. Greer, I. Pirwani, J. Gibson, and A. M. Wallace. Leptin levels in pregnancy: marker for fat accumulation and mobilization. *Acta Obstet Gynecol Scand* 77: 278-283, 1998.

104. Schoeller, D. A., L. K. Cella, M. Sinha, and J. Caro. Entrainment of the Diurnal Variation in Plasma Leptin Levels. *Obesity Research* 4: 38S, 1996.

105. Schrauwen, P., W. van Marken Lichtenbelt, K. Westerterp, and W. Saris. Effect of diet composition on leptin concentration in lean subjects. *Metabolism* 46: 420-424, 1997.

106. Schubring, C., W. Kiess, P. Englaro, W. Rascher, J. Dotsch, S. Hanitsch, A. Attanasio, and W. F. Blum. Levels of Leptin in Maternal Serum, Amniotic Fluid, and Arterial and Venous Cord Blood: Relation to Neonatal and Placental Weight. *J Clin Endo Metab* 82: 1480-1483, 1997.

107. Schwartz, M. W., E. Peskind, M. Raskind, E. J. Boyko, and J. Porte, Daniel. Cerebrospinal Fluid leptin levels: Relationship to plasma levels and to adiposity in humans. *Nature Medicine* 2: 589-593, 1996.

108. Senaris, R., T. Garcia-Caballero, X. Casabiell, R. Gallego, R. Castro, R. V. Considine, C. Diequez, and F. F. Casanueva. Synthesis of Leptin in Human Placenta. *Endocrinology* 138: 4501-4504, 1997.

109. Sharma, K., and R. V. Considine. The Ob protein (leptin) and the kidney. *Kidney International* 53: 1483-1487, 1998.

110. Sinha, M., and J. F. Caro. Clinical Aspects of Leptin. *Vitamins and Hormones* 54: 1-30, 1998.

111. Sinha, M. K., J. P. Ohannesian, M. L. Heiman, A. Kriauciunas, T. W. Stephens, S. Magosin, C. Marco, and J. F. Caro. Nocturnal Rise of Leptin in Lean, Obese, and Non-Insulin-dependent Diabetes Mellitus Subjects. *J Clin Invest* 97: 1344-1347, 1996.

112. Sinha, M. K., I. Opentanova, J. P. Ohannesian, J. W. Kolaczynski, M. L. Heiman, J. Hale, G. W. Becker, R. R. Bowsher, T. W. Stephens, and J. F. Caro. Evidence of Free and Bound Leptin in Human Circulation: Studies in Lean and Obese Subjects and During Short-Term Fasting. *J.Clin.Invest* 98: 1277-1282, 1996.

113. Sorensen, T. I., S. M. Echwald, and J.-C. Holm. Leptin in Obesity. *British Medical Journal* Cotober 19: 953-954, 1996.

114. Stock, S. M., E. M. Sande, and K. A. Bremme. Leptin levels vary significantly during the menstrual cycle, pregnancy, and in vitro fertilization treatment: possible relation to estradiol. *Fertility and Sterility* 72: 657-662, 1999.

115. Sumner, A. E., B. Falkner, H. Kushner, and R. Considine. Relationship of Leptin Concentration to Gender, Menopause, Age, Diabetes, and Fat Mass in African Americans. *Obesity Research* 6: 128-133, 1998.

116. Swain, D. P. VO_2 Reserve: The New Standard for Prescribing Cardiorespiratory Exercise. *ACSM Annual Meeting*, Indianapolis, IN. ACSM, 2000.

117. Tataranni, P., M. Monroe, C. Dueck, S. Traub, M. Nicolson, M. Manore, K. Matt, and E. Ravussin. Adiposity, plasma leptin concentration and reproductive function in active and sedentary females. *International Journal of Obesity* 21: 818-821, 1997.

118. Teirmaa, T., V. Luukkaa, J. Rouru, M. Koulu, and R. Huupponen. Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. *European Journal of Endocrinology* 139: 190-194, 1998.

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

120. Torjman, M. C., A. Zafeiridis, A. M. Paolone, C. Wilkerson, and R. V. Considine. Serum Leptin During Recovery Following Maximal Incremental and Prolonged Exercise. *International Journal of Sports Medicine* 20: 444-450, 1999.

121. Toth, M. J., C. K. Sites, and E. T. Poehlman. Hormonal and Physiological Correlates of Energy Expenditure and Substrate Oxidation in Middle-Aged Premenopausal Women. *The Journal of Clinical Endocrinology and Metabolism* 84: 2771-2775, 1999.

122. tukaszuk, K., J. Liss, E. Kusiak, and C. Wojcikowski. Serum Leptin Concentration Increase During Luteal Phase in Healthy Premenopausal Women. *Hormone and Metabolism Research* 30: 172-174, 1998.

123. Tuominen, J. A., P. Ebeling, M. L. Heiman, T. Stephens, and V. A. Koivisto. Leptin and thermogenesis in humans. *Acta Physiol Scand* 160: 83-87, 1997.

124. Tuominen, J. A., P. Ebeling, F. W. Laquier, M. L. Heiman, T. Stephens, and V. A. Koivisto. Serum leptin concentration and fuel homeostasis in healthy man. *European Journal of Clinical Investigation* 27: 206-211, 1997.

125. van Aggel-Leijssen, D., M. van Baak, R. Tenenbaum, L. Campfield, and W. Saris. Regulation of average 24 h human plasma leptin level; the influence of exercise and physiological changes in energy balance. *International Journal of Obesity* 23: 151-158, 1999.

126. Weigle, D., P. Duell, W. Connor, R. Steiner, M. Soules, and J. Kuijper. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endo Metab* 82: 561-565, 1997.

127. Wilmore, J. H., P. A. Vodak, R. B. Parr, R. N. Girandola, and J. E. Billing. Further simplification of a method for determination of residual lung volume. *Medicine and Science in Sports and Exercise* 12: 216-218, 1980.

128. Wisse, B. E., L. A. Campfield, E. B. Marliss, J. A. Morais, R. Tenenbaum, and R. Gougeon. Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentrations in obese women. *American Journal of Clinical Nutrition* 70: 321-330, 1999.

129. Zeng, J., B. Patterson, S. Klein, D. Martin, S. Dagogo-Jack, W. Kohrt, S. Miller, and M. Landt. Whole body leptin kinetics and renal metabolism in vivo. *American Journal of Physiology* 273: E1102-6, 1997.

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