UC Davis

UC Davis Previously Published Works

Title

Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women.

Permalink

<https://escholarship.org/uc/item/9990k8xf>

Journal

Metabolism: clinical and experimental, 47(4)

ISSN

0026-0495

Authors

Dubuc, GR
Phinney, SD
Stern, JS
[et al.](#)

Publication Date

1998-04-01

DOI

10.1016/s0026-0495(98)90055-5

Peer reviewed

Changes of Serum Leptin and Endocrine and Metabolic Parameters After 7 Days of Energy Restriction in Men and Women

Gloria R. Dubuc, Stephen D. Phinney, Judith S. Stern, and Peter J. Havel

Circulating leptin decreases during fasting in rodents and humans; however, the mechanism of the decrease is unknown. The aim of this study was to examine the relationship between decrements of serum leptin concentrations and changes of hormonal (insulin and cortisol) and metabolic (glucose, ketones, and fatty acids) parameters involved in the metabolic adaptation to energy restriction in normal-weight humans. Because there are marked gender differences in circulating leptin, both men and women were studied. The body mass index (BMI), percent body fat (% body fat), and serum leptin, insulin, cortisol, glucose, β -hydroxybutyrate (BOHB), and nonesterified fatty acids (NEFA) were determined in 11 men and 13 women (age, 20 to 41 years; BMI, 21.2 to 26.8 kg/m²) before and during 7 days of energy restriction ($-68\% \pm 1\%$ of daily energy requirements). Weight loss averaged about 4% in both men and women. Leptin in men was 3.7 ± 0.5 and decreased to 2.1 ± 0.4 ng/mL (percent change [% Δ], $-36\% \pm 6.0\%$, $P < .0005$) during restriction. Concurrently, insulin decreased from 7.2 ± 0.6 to 1.8 ± 0.3 μ U/mL (% Δ , $-74\% \pm 4\%$, $P < .0001$). In contrast, leptin was higher in women before (16.2 ± 1.9 ng/mL) and after (6.0 ± 0.8 ng/mL) restriction and decreased more than in men (% Δ , $-61\% \pm 4\%$, $P < .02$ v men), whereas the decrease of insulin in women was less than in men: 10.1 ± 1.9 to 6.1 ± 1.0 μ U/mL (% Δ , $-31\% \pm 9\%$, $P < .0025$; $P < .0005$ v men), perhaps because glucose decreased less in women than in men. Overall, the changes of leptin during fasting were independently correlated with the changes of glucose ($r = .53$, $P < .007$), NEFA ($r = .53$, $P < .01$), and BOHB ($r = .65$, $P < .001$). In addition, the change of leptin correlated with a combined index of the parameters that reflect decreased glucose availability and increased lipolysis $[(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{NEFA})/3]$, $r = .73$, $P < .0001$ or a combined index of parameters that would be expected to limit glucose uptake by adipocytes $[(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{cortisol})/3]$, $r = .48$, $P < .02$. We conclude that there are significant differences between men and women in the responses of leptin and insulin to energy restriction. Furthermore, decreases of circulating leptin during negative energy balance are related to changes of endocrine and metabolic parameters, suggesting that leptin secretion may be regulated by alterations of adipocyte glucose and lipid metabolism, ie, decreased glucose uptake and metabolism and increased lipolysis.

Copyright © 1998 by W.B. Saunders Company

THE DISCOVERY of the *ob* gene and its protein product, leptin,¹ is leading to an increased understanding of the mechanisms regulating energy homeostasis.² Expression of the *ob* gene and circulating leptin concentrations decrease during fasting in humans^{3,4} and animals.^{5,6} The magnitude of the decrease in circulating leptin during fasting cannot be explained by the relatively small decrease in adiposity.^{3,4} Thus, fat mass alone is not the only determinant of circulating leptin concentrations. However, the mechanisms by which leptin secretion is inhibited during periods of negative energy balance are not understood.

Short-term fasting in humans results in concomitant decreases in leptin, glucose, and insulin and increases in free fatty acids, ketones, and cortisol. Kolaczynski et al⁷ found that decreases of circulating leptin during a 60-hour fast were highly correlated with the increase of circulating ketones; however, infusion of β -hydroxybutyrate (BOHB) had no effect on the plasma leptin response to fasting when plasma glucose was maintained with a low rate of glucose infusion. Boden et al³ prevented the decrease of circulating leptin during a 72-hour fast when plasma glucose and insulin concentrations were maintained with a low rate of glucose infusion, suggesting that changes of insulin and glucose may be involved in the leptin response to fasting. In addition, the diurnal pattern of circulating leptin concentrations⁸ is directly related to meal-induced insulin excursions and inversely related to 24-hour cortisol secretion.⁹ We have reported that decrements of plasma leptin are correlated with decrements of plasma insulin during fasting in mice independently of changes in body weight.⁵ Furthermore, we have found that glucose uptake and metabolism regulate leptin secretion from cultured rat adipocytes in vitro.^{9a} Therefore, factors that would be expected to limit glucose

uptake by adipocytes, ie, decreases of insulin and glucose, and increases of glucocorticoids may have a role in the suppression of leptin secretion during fasting. In the present study, we examined the relationship between decrements of circulating leptin and changes in hormonal (insulin and cortisol) and metabolic (glucose, nonesterified fatty acids [NEFA], and BOHB) parameters that either cause or reflect decreased adipocyte glucose uptake or increased lipolysis after 7 days of marked restriction of energy intake in normal-weight men and women.

SUBJECTS AND METHODS

Subjects

Twenty-eight normal-weight subjects were recruited for the project, of whom 13 women (age, 30 ± 2 years; body mass index [BMI], 23.4 ± 0.46 kg/m²) and 11 men (age, 33 ± 2 years; BMI, 24.7 ± 0.5 kg/m²) completed the study. All subjects were recruited through advertisement on the University of California, Davis campus. Initial screening included a standard medical history, physical examination, electrocardiogram, and serum biochemistry panel including serum lipoproteins, a thyroid panel, and fasting serum insulin. None of the subjects participating in the study had a history of obesity. The protocol

From the Department of Internal Medicine, School of Medicine, and Department of Nutrition, University of California, Davis, CA.

Submitted June 3, 1997; accepted August 4, 1997.

Supported by National Institutes of Health Grants No. R29 DK-50129-01A2, DK-07355, DK-28172, and CA-61654, Shaklee Inc, San Francisco, CA and the Juvenile Diabetes Foundation International.

Address reprint requests to Peter J. Havel, DVM, PhD, Department of Nutrition, University of California, Davis, CA 95616.

Copyright © 1998 by W.B. Saunders Company
0026-0495/98/4704-0012\$03.00/0

was approved by the Institutional Human Subjects Review Committee of the University of California, Davis. All subjects provided informed consent. The initial characteristics of the participants are included in Tables 1 and 2.

Caloric Restriction

To investigate the effects of energy restriction on serum leptin and its relationship to endocrine and metabolic parameters, as well as anthropometric measures, the subjects were placed on a 7-day energy-restricted diet. Women received three packets per day of a powdered formula diet (NutraMed, Mount Laurel, NJ) each consisting of 210 kcal, 6 g fat, 11 g carbohydrate, and 27 g protein (total, 630 kcal/d). The men received four packets, or 840 kcal/d. The degree of caloric restriction based on daily energy requirements was estimated according to the method of Cunningham¹⁰ for resting energy expenditure (REE) with an activity factor of 0.35 of REE. With this method of calculation, in which no caloric restriction is zero and absolute fasting is -100%, the degree of energy restriction in women was $-70\% \pm 1\%$, and in men $-66\% \pm 1\%$. One of the male subjects was likely to be significantly more energy-restricted than the other subjects because he was on a strenuous exercise regimen and continued to exercise during the energy restriction period. Therefore, the data were analyzed both with and without this subject included.

To ensure adequate sodium intake, the subjects were instructed to consume at least one and up to three portions of bouillon daily, providing 1 to 3 g/d of sodium. In addition, an intake of 2 to 3 L water per day was recommended to maintain hydration.

Data Collection

Subjects were weighed and body composition was determined by bioelectrical impedance (BodyStat, Isle of Man, England) at baseline and after 7 days of energy restriction. Fasting serum samples were taken at baseline and on day 7 and analyzed for leptin, insulin, glucose, BOHB, NEFA, and cortisol.

Assays and Data Analysis

Serum leptin was determined with a sensitive and specific radioimmunoassay for human leptin with reagents supplied by Linco (St Charles, MO).¹¹ Serum insulin was determined with a radioimmunoassay for human insulin.¹² Serum glucose was measured by the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). BOHB was measured with a kit from Sigma (St Louis, MO), and serum NEFA were measured with a kit from Waco (Richmond, VA). Cortisol was measured using the Clinical Assays Gamma Coat RIA kit by the Radioimmunoassay Core Laboratory of Vanderbilt University School of Medicine.

Within-group mean values were compared with a paired *t* test, and comparisons of mean values in men and women were made using a

Table 2. Anthropometric, Metabolic, and Endocrine Parameters Before and During Caloric Restriction in Women (n = 13)

Parameter	Before	After	Δ	P
Weight (kg)	69.5 $\pm$ 1.4	63.4 $\pm$ 1.3	-2.5 $\pm$ 0.7	.0025
BMI (kg/m ²)	23.4 $\pm$ 0.46	22.5 $\pm$ 0.42	-2.5 $\pm$ 0.2	.0001
%Body fat	27.4 $\pm$ 1.12	26.1 $\pm$ 1.08	-1.4 $\pm$ 0.3	.0005
Total body fat (kg)	18.2 $\pm$ 1.1	16.6 $\pm$ 1.0	-1.6 $\pm$ 0.3	.0005
Leptin (ng/mL)	16.2 $\pm$ 1.9	6.0 $\pm$ 0.8	-10.2 $\pm$ 1.4	.0001
Insulin (μ U/mL)	10.1 $\pm$ 1.9	5.5 $\pm$ 0.7	-4.6 $\pm$ 1.6	.01
Glucose (mg/dL)	78.8 $\pm$ 2.2	73.9 $\pm$ 1.7	-4.9 $\pm$ 2.2	.025
BOHB (mg/dL)	1.33 $\pm$ 0.16	10.43 $\pm$ 2.40	9.11 $\pm$ 2.47	.0025
NEFA (mg/dL)	0.41 $\pm$ 0.05	1.10 $\pm$ 0.08	0.69 $\pm$ 0.08	.0001
Cortisol (μ g/dL)	18.9 $\pm$ 1.8	21.7 $\pm$ 3.0	2.8 $\pm$ 1.9	.15

NOTE. Values are means $\pm$ SE.

two-sample *t* test. Simple and multiple linear regression analyses were used to determine correlation coefficients and the significance of relationships between serum leptin values and changes and absolute values of the BMI, %body fat, absolute body fat, serum insulin, glucose, BOHB, NEFA, cortisol, and the combined indices. All statistics were determined with a statistics software package (StatView for Macintosh; Abacus, Berkeley, CA).

Since there are known to be marked gender differences in circulating leptin concentrations^{13,14} and we found significant differences between men and women in the changes of serum leptin, insulin, and glucose during energy restriction (see the Results), the changes of the different parameters were first normalized to the mean response in men and women separately and then combined for analysis. The decrease in leptin was divided by the mean of the change and then multiplied by 100. Two different combined indices were calculated. The first index combines changes of insulin and two metabolic substrates. These changes reflect decreased glucose availability, specifically the decreases of glucose and insulin, plus increased lipolysis by the increase of serum NEFA: $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{NEFA})/3$. The second index reflects the changes of parameters that would be expected to limit glucose uptake by adipocytes, specifically decreased glucose availability, decreased circulating insulin, and increased circulating cortisol: $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{cortisol})/3$. To calculate these indices, the individual parameters were first normalized to the mean within each sex as already described, added together, and divided by 3. Values from men and women were then combined for analysis.

RESULTS

Correlations Between Baseline Leptin and Baseline Anthropometric and Serum Biochemical Parameters

Correlations between baseline serum leptin concentrations and the baseline BMI, %body fat, total body fat, and serum glucose, insulin, BOHB, NEFA, and cortisol in men and women separately and combined are provided in Table 3. Baseline serum leptin concentrations were significantly correlated with the BMI in men and with BMI %body fat and total body fat in women, but the BMI was not correlated with leptin in men and women combined. Baseline serum leptin concentrations were significantly correlated with baseline insulin in men and women together and with glucose in men only, but were not correlated with baseline BOHB, NEFA, or cortisol concentrations.

Effects of Caloric Restriction on Anthropometric and Serum Biochemical Parameters

After 7 days of caloric restriction, there were significant decreases of the body weight, BMI, %body fat, and total body

Table 1. Anthropometric, Metabolic, and Endocrine Parameters Before and During Energy Restriction in Men (n = 11)

Parameter	Before	After	Δ	P
Weight (kg)	74.7 $\pm$ 1.8	71.6 $\pm$ 1.8	-2.6 $\pm$ 0.6	.005
BMI (kg/m ²)	24.7 $\pm$ 0.5	23.7 $\pm$.04	-1.0 $\pm$ 0.1	.0001
%Body fat	17.2 $\pm$ 1.1	16.7 $\pm$ 1.1	-0.5 $\pm$ 0.2	.02
Total body fat (kg)	12.9 $\pm$ 0.9	12.0 $\pm$ 0.9	-0.9 $\pm$ 0.2	.0025
Leptin (ng/mL)	3.7 $\pm$ 0.5	2.1 $\pm$ 0.4	-1.6 $\pm$ 0.4	.0025
Insulin (mU/mL)	7.2 $\pm$ 0.6	1.8 $\pm$ 0.3	-5.4 $\pm$ 0.6	.0001
Glucose (mg/dL)	87.3 $\pm$ 2.6	65.0 $\pm$ 3.0	-22.3 $\pm$ 4.0	.0002
BOHB (mg/dL)	0.46 $\pm$ 0.27	17.39 $\pm$ 5.01	16.93 $\pm$ 5.07	.005
NEFA (mg/dL)	0.44 $\pm$ 0.04	0.90 $\pm$ 0.12	0.46 $\pm$ 0.13	.0025
Cortisol (mg/dL)	16.65 $\pm$ 1.46	22.91 $\pm$ 2.64	6.26 $\pm$ 2.28	.02

NOTE. Values are means $\pm$ SE.

Table 3. Correlations Between Baseline Serum Leptin Concentrations and Baseline Anthropometric, Metabolic, and Endocrine Parameters

Parameter	Men (n = 11)		Women (n = 13)		Men and Women (N = 24)	
	r	P	r	P	r	P
BMI	.71	.01	.83	.0004	.11	.62
%Body fat	.30	.36	.87	.0001	.89	.0001
Total body fat	.41	.22	.90	.0001	.85	.0001
Glucose	.76	.007	.44	.13	.15	.49
Insulin	.44	.18	.43	.14	.48	.02
BOHB	.04	.70	.44	.14	.28	.19
NEFA	.54	.09	.43	.15	.32	.13
Cortisol	.24	.40	.20	.51	.27	.20

fat in both men and women. Weight loss averaged about 4% of initial body weight in both men and women, and the decreases of total body fat averaged approximately 7% in men and 9% in women. In addition, significant decreases of serum leptin, insulin, and glucose and increases of BOHB and NEFA were observed in both men and women (Tables 1 and 2). Serum leptin decreased more in women than in men (Fig 1A). Even after calculating the percent change (% Δ) of leptin to correct for the higher baseline concentrations of leptin in women, there was a greater proportional decrease of leptin in women than in men (Fig 1B). In addition, after correcting for adiposity by dividing the leptin concentration by the BMI, %body fat, or total body fat, the decrease serum leptin per unit adiposity during the supplemented fast was larger in women than in men (data not shown). Serum insulin was higher in women than in men at baseline. The absolute change of insulin during energy restriction was not different between men and women (Fig 2A), but the percent decrease of insulin in response to energy restriction was significantly greater in men than in women (Fig 2B). Serum glucose decreased more during caloric restriction in men than in women ($P < .001$; Tables 1 and 2). Although, individually, the increase of cortisol was significant only in men, there was a wide range of cortisol responses in women (-4.9 to $+16.8$ $\mu\text{g/dL}$). In men and women together, the change of cortisol was significant ($P < .005$).

Correlation of Changes of Serum Leptin With Changes in Anthropometric, Metabolic, and Endocrine Parameters and Indexes

Relationships between the change of leptin (normalized to the mean change of leptin within men and women) and changes in other parameters are shown in Table 4. The changes of leptin were not significantly related to changes of %body fat or total body fat. There was a relationship between the amount of weight loss as reflected by the change of BMI and the changes of leptin; however, the degree of weight loss ($\sim 4\%$) is not sufficient to independently explain the much larger changes of circulating leptin (-35% to -65%). The changes of insulin and cortisol by themselves were not correlated with the change of leptin during energy restriction. The changes of glucose and BOHB were significantly correlated with the change of leptin in men, women, and men and women combined. The changes of

NEFA correlated with the change of leptin in men and in men and women combined, but not in women alone (Table 4). Furthermore, a combined index of energy restriction ($[\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{NEFA}]/3$; Fig 3) and an index of three factors that would be expected to limit glucose uptake ($[\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{cortisol}]/3$; Fig 4) were both significantly correlated with the changes of serum leptin in men and women alone and with the normalized change of leptin in men and women combined. In addition, by multiple regression analysis, a model combining the parameters in $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{NEFA})/3$ showed a significant relationship with the changes of circulating leptin ($r = .73$, $P < .0001$) that was much stronger than with any one of the parameters alone. Similarly, the change of leptin was related to the $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{cortisol})/3$ index in a multiple regression model ($r = .63$, $P < .02$), and this relationship was stronger than that of the individual components alone.

Fig 1. Comparison of (A) absolute and (B) proportional changes of serum leptin after 7 days of energy restriction in men ($n = 11$) and women ($n = 13$). * $P < .0001$, † $P < .001$; v women.

Fig 2. Comparison of (A) absolute and (B) proportional changes of serum insulin after 7 days of energy restriction in men ($n = 11$) and women ($n = 13$). * $P < .003$ v women.

As discussed in the Methods, one of the male subjects was likely to be significantly more energy-restricted than the others. Indeed, this subject had the largest normalized change of serum leptin, nearly three times the mean change in men (Figs 3 and 4). Therefore, these correlation analyses were also made with this subject excluded. Without this subject, both combined indices, $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{NEFA})/3$ and $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{cortisol})/3$, remained significantly correlated with the normalized change of serum leptin across men and women ($r = .59$, $P < .003$ and $r = .43$, $P < 0.04$, respectively). Finally, the absolute change of leptin was highly correlated with the baseline leptin measurement in men, women, and men and women together (Table 4).

DISCUSSION

In this study, we found that serum leptin decreases in both men and women after 7 days of marked caloric restriction. Leptin decreases by a greater absolute amount and to a

Table 4. Correlations Between the Changes of Serum Leptin Normalized Within Men and Women and Changes of Anthropometric, Metabolic, and Endocrine Parameters and Two Combined Metabolic/Endocrine Indexes

Parameter	Men ($n = 11$)		Women ($n = 13$)		Men and Women ($N = 24$)	
	r	P	r	P	r	P
ΔBMI	.67	.02	.27	.37	.49	.02
$\Delta\%\text{Body fat}$	.13	.70	.19	.53	.13	.53
$\Delta\text{Total body fat}$	.51	.11	.21	.49	.32	.13
$\Delta\text{Glucose}$	.76	.007	.57	.04	.53	.007
$\Delta\text{Insulin}$	.54	.09	.46	.11	.38	.07
ΔBOHB	.68	.02	.73	.005	.65	.0006
ΔNEFA	.70	.02	.30	.31	.53	.01
$\Delta\text{Cortisol}$	.33	.33	.14	.64	.24	.25
$(\Delta\text{glucose} + \Delta\text{IRI} + \Delta\text{NEFA})/3$	.74	.01	.80	.001	.73	.0001
$(\Delta\text{glucose} + \Delta\text{IRI} + \Delta\text{cortisol})/3$	.65	.03	.53	.06	.48	.02
$\Delta\text{Leptin v baseline leptin}$	.97	.0001	.93	.0001	.97	.0001

Abbreviation: IRI, immunoreactive insulin.

proportionally greater degree in women than in men, whereas the proportional decrease of fasting insulin is greater in men. The larger decline of insulin in men versus women may reflect the greater decrement in glycemia observed in men. Serum cortisol was significantly increased in men but not in women, and the cortisol response to energy restriction in women was highly variable. These results indicate that not only are there marked gender differences in circulating leptin concentrations,^{13,14} but there are also gender differences in the responses of leptin, insulin, glucose, and cortisol to energy restriction. Decreases of circulating insulin and increases of cortisol during periods of negative energy balance have been hypothesized to act on the central nervous system to increase the drive for food intake and to decrease energy expenditure.¹⁵⁻¹⁷ Leptin¹ intro-

Fig 3. Correlation between changes of serum leptin after 7 days of energy restriction normalized to the mean changes within men (■) and women (●) separately with a metabolic index combining the changes of serum glucose, insulin, and NEFA. Without the male subject with the greatest change of leptin, $r = .59$ and $P < .003$.

Fig 4. Correlation between changes of serum leptin after 7 days of energy restriction normalized to the mean changes within men (■) and women (●) separately with an index combining changes of factors that would be expected to limit adipocyte glucose uptake, ie, decreases of glucose and insulin and increases of cortisol. Without the male subject with the greatest change of leptin, $r = .43$ and $P < .04$.

duces another peripheral signal of energy status to the central nervous system.² Thus, the relative contribution of these three peripheral signals may differ between men and women, with the contribution of decreased adipose tissue leptin production predominating in women and the decrease of insulin secretion and increase of cortisol secretion predominating in men.

The magnitude of the decreases of serum leptin (35% to 65%) cannot be explained by the relatively small decreases of body weight (~4%) and total body fat (7% to 9%). This is similar to previous observations in studies in fasting humans,^{3,4,7} and suggests that factors other than changes in adiposity are involved in the inhibition of leptin secretion in response to negative energy balance. Although the data are consistent with decreased adipocyte production of leptin during energy restriction, an increase of leptin clearance cannot be ruled out as a contributor to the decline of circulating leptin. At the same time that serum leptin is declining, circulating insulin and glucose decrease and circulating NEFA, BOHB, and cortisol increase. In a previous study, Kolaczynski et al⁷ found a highly significant inverse correlation between the increase of BOHB and the decrease of plasma leptin during a 60-hour fast. When small amounts of glucose estimated to be equivalent to the fasting gluconeogenic rate were infused, both the increase of BOHB and the decrease of leptin were prevented. However, when a BOHB infusion designed to match the increase observed during a 36-hour fast was added to this protocol, there was still no decline in plasma leptin, suggesting that ketones do not directly suppress leptin secretion during fasting.⁷ In another study, plasma leptin did not decrease during a 72-hour fast when glucose was infused at rate sufficient to prevent the decline of glucose and insulin,³ suggesting a role for glucose and/or insulin in the regulation of leptin secretion during fasting.

Although short-term (2 to 4 hours) insulin and glucose infusions have been repeatedly demonstrated not to affect

circulating leptin in humans,¹⁸⁻²⁰ there are now several studies demonstrating increases of plasma leptin after 4 to 6 hours of supraphysiologic insulin administration in which euglycemia is maintained by administering large amounts of glucose.^{21,22} In addition, insulin stimulates *ob* gene expression or leptin secretion in vitro^{18,23} and in animals,^{24,25} and insulin-deficient diabetic animals have a reduced *ob* gene expression and circulating leptin that are reversed by insulin administration.^{25,26} Furthermore, circulating insulin and leptin change in parallel during weight loss on a low-fat diet in humans independently of changes in adiposity.²⁷ Lastly, glucose infusions that simultaneously increase plasma glucose and insulin increase plasma leptin in rhesus monkeys.²⁸ Since insulin increases both glucose uptake and metabolism in adipocytes and inhibits lipolysis, we hypothesized that the reduction of leptin secretion during fasting results from alterations in energy flux through the adipocyte, ie, impaired glucose uptake and increased lipolysis. In support of the hypothesis that leptin secretion is related to adipocyte glucose uptake and metabolism, we have data to demonstrate that blockade of glucose transport or glycolysis inhibits leptin secretion from isolated cultured rat adipocytes (Mueller et al, submitted to Endocrinology).

In the present study in normal-weight humans, we examined the relationship between the changes of circulating leptin after 7 days of marked energy restriction and the changes of several endocrine and metabolic factors that either cause or reflect impaired glucose uptake and increased lipolysis during negative energy balance. Since we found significant gender differences in the changes of serum leptin and other responses to caloric restriction, the responses were normalized to the mean change within each sex and then combined for analysis. Overall, the changes of glucose, BOHB, and NEFA were independently related to the decrease of serum leptin during energy restriction. The decrease of circulating glucose reduces the availability of glucose for transport and metabolism in adipocytes, whereas the increases of NEFA and BOHB are, respectively, direct and indirect indices of lipolysis. When we combined three parameters into an index that would reflect decreased glucose availability and increased lipolysis, $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{NEFA})/3$, or an index that would be expected to decrease glucose uptake by adipocytes, $(\Delta\text{glucose} + \Delta\text{insulin} + \Delta\text{cortisol})/3$, these indices were significantly correlated with the decline of circulating leptin. In this study, there was generally little variability in the degree of energy restriction and the changes of leptin within each sex. Thus, it is likely that the changes of leptin and the measured and endocrine indices would have been more closely related if the degree of energy restriction had been varied. This possibility is suggested by the data from the one male subject who was more energy-restricted by virtue of a high degree of physical activity (see the Methods and Results). Overall, the data from this study suggest that a change of energy flux through the adipocyte or diminished adipocyte glucose uptake and metabolism may be involved in decreasing leptin secretion during negative energy balance.

In summary, circulating leptin decreases in both men and women after 7 days of energy restriction. However, there are significant gender differences in the response of leptin, insulin,

and glucose to energy restriction. The decrease of leptin secretion is related to factors that either cause or reflect decreased adipocyte glucose uptake and increased lipolysis. Thus, the data are consistent with the hypothesis that changes in leptin secretion during fasting and refeeding are regulated by alterations of adipocyte glucose and lipid metabolism.

ACKNOWLEDGMENT

We would like to acknowledge the expert technical assistance of Debbie Porter and Kimber Stanhope; Deborah Metz, RN, for skilled clinical assistance; and Wanda Snead and the Radioimmunoassay Core Laboratory of Vanderbilt University School of Medicine for performing the serum cortisol assays.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425-432, 1994
2. Caro JR, Sinha MK, Kolaczynski JW, et al: Leptin: The tale of an obesity gene. *Diabetes* 45:1455-1461, 1996
3. Boden G, Chen X, Mozzoli M, et al: Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* 81:3419-3423, 1996
4. Weigle DS, Duell PB, Connor WE, et al: Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endocrinol Metab* 82:561-565, 1997
5. Ahren B, Mansson S, Gingerich RL, et al: Regulation of plasma leptin in mice: Influence of age, high-fat diet and fasting. *Am J Physiol* 273:R113-R120, 1997
6. Becker DJ, Ongemba LN, Brichard V, et al: Diet- and diabetes-induced changes of *ob* gene expression in rat adipose tissue. *FEBS Lett* 371:324-328, 1995
7. Kolaczynski JW, Considine RV, Ohannesian J, et al: Response of leptin to short-term fasting and refeeding in humans. *Diabetes* 45:1511-1515, 1997
8. Sinha MK, Ohannesian JP, Heiman ML, et al: Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest* 97:1344-1347, 1996
9. Laughlin GA, Yen SSC: Hypoleptinemia in women athletes: Absence of diurnal rhythm with amenorrhea. *J Clin Endocrinol Metab* 82:318-321, 1997
- 9a. Mueller WM, Gregoire F, Stanhope KL, et al: Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. *Endocrinology* (in press)
10. Cunningham JJ: A reanalysis of the factors influencing basal metabolic rate in normal adults. *Am J Clin Nutr* 33:2372-2374, 1980
11. Ma ZA, Gingerich RL, Santiago JV, et al: Analysis of human plasma leptin by radioimmunoassay. *Clin Chem* 42:942-946, 1996
12. Yalow RS, Berson SA: Immunoassay of endogenous plasma insulin in man. *J Clin Invest* 39:1157, 1960
13. Havel PJ, Kasim-Karakas S, Dubuc GR, et al: Gender differences in plasma leptin concentrations. *Nat Med* 2:949-950, 1996
14. Rosenbaum M, Nicolson M, Hirsch J, et al: Effects of gender, body composition, and menopause on plasma leptin concentrations. *J Clin Endocrinol Metab* 81:3424-3427, 1996
15. Schwartz MW, Figlewicz DP, Baskin DG, et al: Insulin and the central regulation of energy balance: Update 1994, in Negro Vilar A, Underwood LE (eds): *Endocrine Reviews Monographs 2: The Endocrine Pancreas, Insulin Action, and Diabetes*. The Endocrine Society 1994, pp 81-113
16. Strack AM, Sebastian RJ, Schwartz MW, et al: Glucocorticoids and insulin: Reciprocal signals for energy balance. I. *Am J Physiol* 268:R142-R149, 1995
17. Kaiyala KJ, Woods SC, Schwartz MW: New model for the regulation of energy balance and adiposity by the central nervous system. *Am J Clin Nutr* 62:1123S-1134S, 1995 (suppl)
18. Kolaczynski JW, Nyce MR, Considine RV, et al: Acute and chronic effect of insulin on leptin production in humans: Studies in vivo and in vitro. *Diabetes* 45:699-701, 1996
19. Dagogo-Jack S, Fanelli C, Paramore D, et al: Plasma leptin and insulin relationships in obese and nonobese human. *Diabetes* 45:695-698, 1996
20. Pratley RE, Nicolson M, Bogardus C: Effects of acute hyperinsulinemia on plasma leptin concentration in insulin-sensitive and insulin-resistant Pima Indians. *J Clin Endocrinol Metab* 81:4418-4421, 1996
21. Utriainen R, Malmstrom R, Makimattila S, et al: Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. *Diabetes* 45:1364-1366, 1996
22. Havel PJ, Aoki TT, Grecu EO, et al: Leptin/adiposity relationships in intensively treated IDDM and NIDDM and increased plasma leptin after 6 hours of high dose insulin infusion. *Obes Res* 4:15S, 1996 (abstr)
23. Leroy P, Dessolin S, Villageois P, et al: Expression of *ob* gene in adipose cells. *J Biol Chem* 271:2365-2368, 1996
24. Saladin R, De Vos P, Guerre-Millo M, et al: Transient increase in obese gene expression after food intake or insulin administration. *Nature* 377:527-529, 1995
25. Cusin R, Sainsbury A, Doyle P, et al: The *ob* gene and insulin: A relationship leading to clues to the understanding of obesity. *Diabetes* 44:1467-1470, 1995
26. Havel PJ, Uriu-Hare JY, Stanhope KL, et al: Marked and rapid decrease of plasma leptin in insulin deficient diabetes: Reversal by insulin. *Diabetologia* 40:A270, 1997 (suppl 1, abstr)
27. Havel PJ, Kasim-Karakas S, Mueller W, et al: 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 Endocrinol Metab* 81:4406-4413, 1996
28. Havel PJ: Glucose but not fructose infusion increases circulating leptin in proportion to adipose stores in rhesus monkeys. *Exp Clin Endocrinol Diabetes* 105:37-38, 1997