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Fasting Insulin Levels Influence Plasma Leptin Levels Independently from the Contribution of Adiposity: Evidence from Both a Cross-Sectional and an Intervention Study*

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ABSTRACT

The aim of the present investigation was to determine whether leptinemia is only a reflection of the status of fat stores or if insulinemia has a significant influence over leptin levels.

Study 1 focused on the association between fasting plasma insulin and leptin in subjects of the Québec Family Study who were first classified as either high- or low-insulin individuals and were then individually matched on the basis of fat mass (FM). In Study 2, 19 men and 23 women took part in a 15-week weight loss program that consisted of drug therapy (fenfluramine, 60 mg/day) or placebo coupled to an energy-restricted diet (−2930 kJ/day). Body weight, FM, and fat-free mass (assessed by underwater weighing) as well as visceral and sc abdominal and mid-thigh adipose tissue measured by computed tomography were assessed before and after weight loss. Blood samples were drawn and analyzed for fasting plasma insulin and leptin before and after weight loss.

In Study 1, significant positive associations were noted between $\log_{10}$ transformed fasting insulin and leptin in both men ($r = 0.55$, $P < 0.0001$) and women ($r = 0.48$, $P < 0.0001$). Moreover, after having carefully matched high-insulin to low-insulin individuals on the basis of FM, significantly lower leptin levels were observed in the low-insulin groups, in men (5.5 *vs.* 8.1 ng/mL, $P < 0.05$) as well as in women (18.7 *vs.* 24 ng/mL, $P < 0.05$). Results from Study 2 showed significant reductions of body weight, FM, fat-free mass, visceral abdominal tissue, sc abdominal tissue, and mid-thigh adipose tissue levels in men and women in response to the weight loss protocol. Moreover, the decrease in fasting plasma insulin was the only significant correlate of changes in fasting plasma leptin levels during weight loss, even after corrections for changes in FM in both men ($r = 0.50$, $P < 0.05$) and women ($r = 0.46$, $P < 0.05$).

These results suggest that in a population characterized by a wide range of adiposity hyperinsulinemia has the potential to modulate leptin levels beyond what can be explained by total adiposity. Moreover, this relation also seems to exist in a dynamic setting (*i.e.* during weight loss) because changes in insulin were independent predictors of the changes in leptinemia in both men and women after correction for changes in FM. (*J Clin Endocrinol Metab* **85:** 4231–4237, 2000)

ONE OF THE important scientific breakthroughs of the 20th century was the discovery of insulin in the 1920s (1). First thought to be entirely devoted to glucose disposal, insulin has since then been shown to be, on countless occasions, related to increased body weight. In fact, insulin is now considered as an important component of the plurimetabolic syndrome, more commonly referred to as Syndrome X (2). Fortunately, modest weight loss can considerably reduce insulinemia (3, 4), thereby contributing to decrease the risk for morbidity and, presumably, mortality.

Beyond the fact that increased body fat stores is generally accompanied by an increase in insulin levels, which ultimately translates into a risk for health, hyperinsulinemia might also be implicated in the neurobiological regulation of energy metabolism in obese individuals when they are faced with a fattening lifestyle. In this sense, Vollenweider *et al.* (5) have shown that during a euglycemic-hyperinsulinemic clamp the increase in insulin was associated with an increase in sympathetic nervous system (SNS) activity, which is also in accordance with the results of Berne *et al.* (6). Apart from its influence on SNS activity, insulin has also been shown to inhibit food intake in animals by modulating the expression of neuropeptide Y (7). These experimental data fit well with population studies that demonstrated that individuals who display greater insulin levels are generally also those who experience the smallest weight gain over time (8–10).

The question remains as to whether insulin is directly involved in the regulation of energy-metabolism-related variables or whether these observations are rather the result of an intricate hormone interplay. A fresh look has been brought on this issue with the recent cloning of the *ob* gene (11). As for insulin, leptin fluctuates considerably with body weight changes and more particularly with adiposity (12, 13). Moreover, leptin administration has also been shown to inhibit food intake (14) possibly by decreasing the expression

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of neuropeptide Y (15) and to increase energy expenditure (16) and fat oxidation (17) in animals. Increased leptin levels have been shown to be associated with an increased SNS activity (18). The decrease in fat oxidation that normally occurs in response to weight loss in humans (19, 20) also seems to be partly explained by the decrease in leptinemia (21).

The common effects of insulin and leptin on energy metabolism and the fact that these two hormones both fluctuate in the same direction in response to body weight variations raise the question as to whether they could regulate each other, at least partly. This issue has been a matter of debate over the last years. In this sense, it has been shown that sustained hyperinsulinemia stimulates leptin messenger RNA (mRNA) expression (22, 23) although acute changes in insulin levels do not seem to affect the expression of this protein (24). Some studies have also been conducted to assess the potential of insulin to influence the *ob* gene product. In fact, increases in insulin have been shown to increase leptin levels in obese males (25) even in physiological amounts (26), a finding that is also supported by that of Couillard *et al.* (27), who demonstrated a positive association between insulin and leptin. Moreover, other results showed that changes in insulin levels during weight loss were associated with changes in leptin even after corrections for changes in adiposity and other confounding factors in women (28, 29).

The aim of the present study was, hence, 2-fold. First, we wanted to assess whether leptinemia would be different between groups of identical levels of fat mass (FM) but with either high- or low-insulin levels (Study 1). Second, we also wanted to verify whether changes in leptin levels that occur during weight loss are, in part, attributable to changes in insulin levels in obese men and women (Study 2). A secondary aim of these studies was to determine which anthropometric component best predicts fasting leptin levels.

Subjects and Methods

Study 1

Subjects (161 men and 196 women) from this study were selected from the Phase II cohort of the Québec Family Study. Caucasian nuclear families from the greater Québec City area were recruited to participate in this study, which received approval from the Laval University Medical Ethics Committee.

Study 2

Forty-two subjects (19 men and 23 premenopausal women) took part in a 15-week drug therapy-energy restriction intervention. Subjects were given either fenfluramine (60 mg) daily or a placebo for the duration of the program (men = 5 and 14 and women = 4 and 19, placebos and drug-tested individuals, respectively). This treatment was coupled to a non macronutrient-specific energy intake restriction of 2930 kJ/day (700 kcal/day). To achieve this energy restriction, the Good Health Eating Guide (food exchange system) (30) was explained to each subject by a nutritionist. During this program, subjects visited the laboratory every 2 weeks for a control session during in which they were weighed and asked about compliance. If subjects had lost less than 1 kg during this time period and if it was ascertained that is was not due to noncompliance, the energy restriction was adjusted to ensure a progressive weight loss throughout the duration of the program. This visit also served as a way to verify drug compliance and to renew subjects' prescription. Subjects gave their written consent to participate in this study, which received approval of the Laval University Medical Ethics Committee.

It is important to note that following the suspension of fenfluramine and dexfenfluramine further to a potential association with disturbances in cardiac valvular function (31, 32), all subjects (including placebos) were subjected to an echocardiogram. After this assessment, a detailed evaluation of cardiac valvular function was performed by cardiologists who detected no abnormalities in response to the use of fenfluramine according to the Food and Drug Administration criteria (33).

Anthropometric measurements

Body weight was taken with a standard beam scale. Body density was determined by hydrodensitometry (34). The closed circuit helium dilution method (35) was used to assess the residual lung volume. The Siri formula (36) was used to estimate the percentage of body fat from body density. Fat mass (FM) was calculated from the derived percentage of body fat and total body weight. Fat-free mass (FFM) was then simply calculated as a subtraction of FM from total body weight.

Computed tomography (CT) measurements

CT was performed on a Siemens Somaton DRH scanner (Siemens, Erlangen, Germany) according to the methodology described by Sjöström *et al.* (37). Briefly, the subjects were examined in the supine position with both arms stretched above their head. CT scans were performed at the abdominal level (between L4 and L5 vertebrae) in Study 1 and at both the abdominal and femoral (mid-thigh) levels in Study 2, using a radiograph of the skeleton as a reference to establish the position of scans to the nearest millimeter (38). Total adipose tissue areas were calculated by delineating these areas with a graph pen and then computing the total adipose tissue surfaces with an attenuation range of −190 to −30 Hounsfield units (37), as described previously (38). The abdominal visceral adipose tissue (VAT) area was determined by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal sc adipose tissue (SAT) area was calculated by subtracting the VAT area from the total adipose tissue area. The mid-thigh adipose tissue (TAT) accumulation level was measured for both the right and the left leg (at mid-distance between the iliac crest and the knee joint). For practical purposes, the mean of the two legs was calculated and this value was used in all subsequent analyses.

Plasma insulin and leptin concentrations

Blood samples were collected in tubes containing EDTA and Trasylol (Miles Pharmaceutics, Rexdale, Ontario, Canada) through a venous catheter from an antecubital vein in the fasting state. Plasma insulin concentration was determined by RIA with polyethylene glycol separation (39). Fasting plasma leptin concentrations were determined with a highly sensitive commercial double-antibody RIA (Human Leptin Specific RIA Kit; Linco Research, Inc., St. Louis, MO), which detects relatively low leptin levels of (0.5 ng/mL) and which does not cross-react with human insulin, proinsulin, glucagon, pancreatic polypeptide, or somatostatin.

Statistical analysis

Study 1. Jump Software 3.1.6.2. (SAS Institute, Inc., Cary, NC) was used for all analyses. Stepwise multiple regression analyses were first performed on the whole samples of men and women to assess which adipose tissue compartment best predicted leptin levels. Simple correlation analyses were performed between fasting plasma insulin and leptin in both men and women. Scores were normalized by $\log_{10}$ transformation because the distributions of both variables were skewed. To determine whether fasting insulin levels exert an influence over fasting leptin levels, men and women were first categorized as either low-insulin (≤50 pmol/L) or high-insulin (≥60 pmol/L) individuals. Then, subjects from the low-insulin group were individually matched within each gender, one by one, on the basis of their FM with subjects from the high-insulin group. The matching criterion was a difference 0.3 kg or less. When more than one possible match could be obtained for FM, the subject with the lowest insulin level in the low-insulin group or the highest insulin level in the high-insulin group was retained. This was done to obtain the widest possible gap in insulin level between both groups. Characteristics of subjects in the high-insulin (24 men and 46

women) and low-insulin (24 men and 46 women) groups were compared by unpaired *t* tests. Differences were considered significant at $P < 0.05$. All data are presented as mean ± SEM.

Study 2. Jump Software 3.1.6.2. (SAS Institute, Inc.) was used for all analyses. Multivariate ANOVA for repeated measures was first performed on all variables to assess the effects of gender, treatment (drug or placebo), time, and their interactions. These analyses did not reveal any significant differences between subjects who received placebo and those who were subjected to drug therapy. Hence, subjects were pooled for all remaining analyses. However, because gender effects as well as gender·time interactions were observed, the analyses were performed separately in men and women. Paired *t* tests were used to verify the effects of this weight loss on the subjects' characteristics. Stepwise multiple regression analyses were then performed in men and women to assess which adipose tissue compartment best predicted leptin levels before and after weight loss. Simple correlation analyses were finally performed between the changes in insulin levels and those in leptin levels corrected for the changes of FM that occurred during weight loss. All data are expressed as mean ± SEM.

Results

Study 1

Figure 1 shows simple correlations between normalized insulin and leptin in a large sample of men and women. Significant positive associations were observed between these two variables in both men (r = 0.55, $P < 0.001$) and women (r = 0.48, $P < 0.001$). Figure 2 shows results of fasting plasma insulin and leptin in both men and women matched for FM. Comparisons between groups revealed that FM was identical in high- and low-insulin individuals. As expected, insulin levels were significantly greater in men (83.5 *vs.* 32.3 pmol/L, $P < 0.001$) and women (90.4 *vs.* 29.1, $P < 0.001$) of the high fasting plasma insulin level groups. Leptin levels were also significantly higher in men (8.1 *vs.* 5.5 ng/ml, $P < 0.05$) and women (24.0 *vs.* 18.7 ng/ml, $P < 0.05$) of the high fasting insulin groups compared with the low-insulin subjects.

Table 1 shows results from a stepwise multiple regression analysis to identify the best anthropometric predictor of fasting plasma leptin levels in large cohorts of men and women. This analysis revealed that in men as in women, the best predictor of fasting plasma leptin levels was FM explaining 52% and 54% of the variance, respectively ($P < 0.001$). Adding L4L5 SAT to the model increased the prediction level in men (54%, $P < 0.04$). In women, adding FFM to the model increased the prediction from 54% to 55% of the variance ($P < 0.01$).

Because high-insulin men tended to display higher VAT and lesser FFM levels, analyses were performed to verify whether the VAT/FFM ratio would be more closely associated with fasting plasma insulin and insulin area under the curve in response to an oral glucose challenge. These analyses revealed that the strongest correlates of fasting insulin levels or of insulin area under the curve was total FM for both men and women (results not shown).

To investigate whether other characteristics differed between groups, unpaired comparisons were performed on variables that might also influence fasting plasma leptin levels. The results of these analyses are shown in Table 2. Except for age in women, none of the other variables were statistically different between groups.

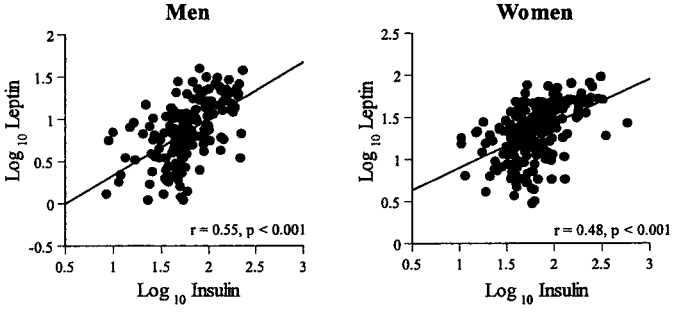

FIG. 1. Correlations between fasting plasma leptin levels and insulin levels normalized by $\log_{10}$ transformation, in men and women.

Study 2

The before and after weight loss characteristics of subjects are shown in Table 3. This program significantly reduced body weight (−11 and −9%, $P < 0.001$) and FM (−26 and −14%, $P < 0.001$) in men and women, respectively, and FFM in women only (−5%, $P < 0.01$). Moreover, L4L5 SAT (−21 and −10%, $P < 0.001$) and VAT (−38 and −18%, $P < 0.001$), as well as TAT (−19 and −12%, $P < 0.001$), were all significantly decreased in both men and women, respectively.

Fasting plasma insulin was significantly reduced by weight loss from 122.7 ± 11.6 to 96.1 ± 15.6 pmol/L in men (−22%, $P < 0.05$), whereas in women a nonsignificant (n.s.) decrease from 108.3 ± 13.9 to 99.0 ± 14.5 was observed (−9%, n.s.). Fasting leptinemia also decreased in response to weight loss in both men (12.7 ± 1.0 to 7.9 ± 1.0, −38%, $P < 0.001$) and women (37.2 ± 3.0 to 27.6 ± 2.8, −26%, $P < 0.001$).

Stepwise multiple regression analyses were performed on leptin values before and after weight loss (Table 4). Before weight loss, the best predictor of leptin levels in men was L4L5 SAT (36%, $P = 0.007$) and in women, FM (40%, $P = 0.001$). After weight loss, L4L5 SAT remained the best predictor of fasting leptin levels in men (49%, $P < 0.001$). Similarly, FM was still the best predictor of leptin levels in women after weight loss, explaining 63% of the variance ($P < 0.001$).

The strongest correlate of changes in plasma leptin levels during weight loss was the change in fasting plasma insulin for both men (r = 0.65, $P < 0.01$) and women (r = 0.48, $P < 0.01$). However, there were strong trends for the correlation between the changes in FM and those in plasma leptin levels in men (r = 0.40, n.s.) and women (r = 0.41, $P = 0.053$).

The changes in leptin levels were adjusted for changes in FM to determine whether the changes in insulin levels contributed independently to changes in leptin levels during weight loss. Figure 3 shows the results of these analyses. After correction of the changes in leptin levels for those in FM, significant associations were still observed between changes in leptin levels and those in fasting plasma insulin in both men (r = 0.50, $P < 0.05$) and women (r = 0.46, $P < 0.05$).

Discussion

These studies were conducted to clarify the nature of the relation between fasting insulin and leptin levels after having controlled for the contribution of adipose tissue to this relation in both men and women. Here, the most significant

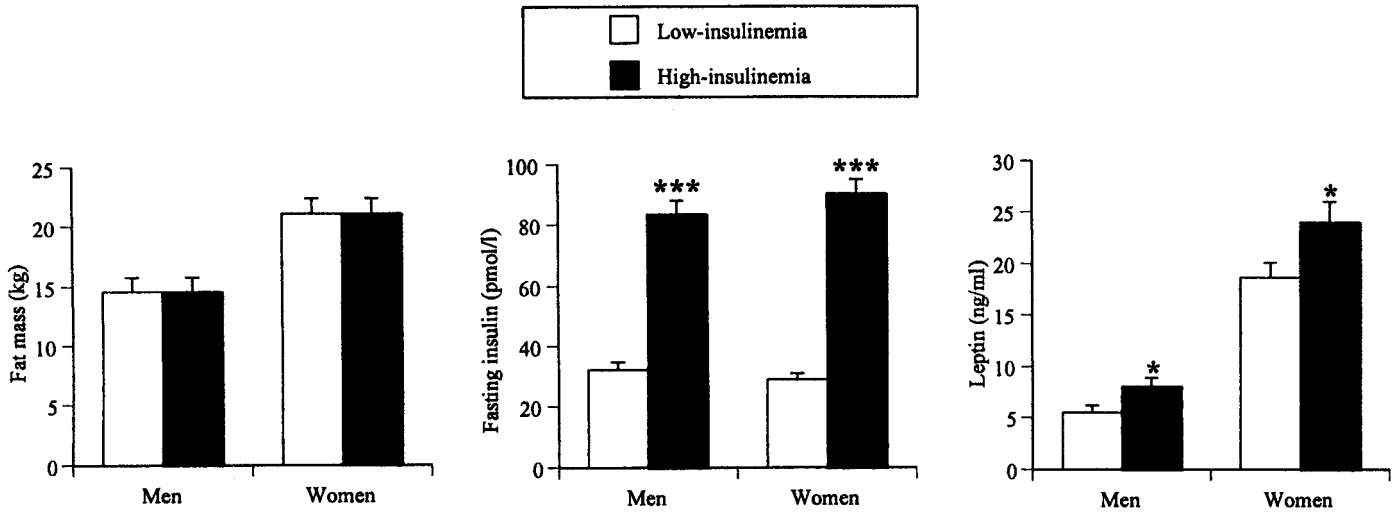

FIG. 2. Comparison of FM, fasting plasma insulin, and fasting plasma leptin levels between low- and high-insulin subjects individually matched for FM. *, $P < 0.05$; ***, $P < 0.001$.

TABLE 1. Stepwise multiple regression analysis examining predictors of fasting leptin levels in the whole sample of men and women who participated in Study 1

Dependent variable	Step no.	Predicting variable	r^2	P
Men				
Log_{10} leptin	1	FM	0.52	<0.001
	2	L4L5 SAT	0.53	<0.05
Women				
Log_{10} leptin	1	FM	0.54	<0.001
	2	FFM	0.55	<0.01

Variables entered in the model: 1) dependent variable, log_{10} leptin; 2) independent variables (FFM, FM, L4L5 SAT, L4L5 VAT).

findings are: 1) in both men and women who displayed identical amounts of total fat and similar fat distribution, individuals characterized by higher insulin levels also display higher leptin levels (Study 1); and 2) in response to weight loss, positive associations between changes in fasting plasma leptin and insulin levels could still be observed after adjustment of the changes in fasting plasma leptin levels for those in FM (Study 2).

The fact that leptin is primarily produced and secreted by the adipose tissue (11, 40) is commonly recognized. However, whether specific adipose tissue stores are more closely related to leptin levels than others is less clear. It was initially thought that leptin levels were the reflection of total body adiposity because obese and reduced-obese individuals generally display a leptinemia that is proportional to their adiposity (12, 13). It has also been shown that the strongest correlate of leptin levels in men and women was total FM in large samples of men and women, varying widely with respect to their degree of obesity (27) and in normal weight as well as in reduced-obese women (41). Furthermore, the most important determinant of changes in leptin levels still seems to be changes in total FM during weight loss in obese women (42). In contrast, other studies have shown that specific depots of adipose tissue are more closely related to fasting plasma leptin levels than total adiposity (43, 44). Moreover, *in vitro* studies have also demonstrated that SAT was more closely associated with leptin secretion than VAT (45, 46). Our results support the concept that total adipose tissue mass is a better reflection of circulating levels of leptin. For instance, Study 1 demonstrates that in large samples of men and women FM is the best predictor of fasting leptin levels. However, in the intervention study with a smaller sample size, total FM was the best predictor of fasting leptin levels before and after weight loss in women, whereas in men stronger relations were noted between leptin levels and L4L5 SAT. Thus, although total adiposity seems to remain the best predictor of leptin levels as demonstrated by the data on large samples of men and women, there might still be a significant contribution of specific adipose tissue stores to leptinemia, particularly in men. We are well aware that there are some limitations related to isolating the best correlate of fasting plasma leptin among different adipose tissue stores when these different compartments are also quite closely intercorrelated. Because VAT is not as closely associated with leptin levels as SAT (45, 46), it would be expected that a stronger relation be observed between SAT and leptin levels than between total FM and leptin in viscerally obese men, since these individuals present a larger proportion of their total FM as VAT. However, it is possible that in leaner men or in women who generally present a much larger proportion of their total FM as SAT, it might be harder to discriminate between total fat and SAT because these two variables might be more closely associated. Indeed, correlation analyses revealed that SAT was more closely associated with total FM in women in Studies 1 and 2, as well as in men in Study 1 (results not shown). For these reasons, our results lend support to previous findings that have shown that both total FM and SAT are important determinants of fasting plasma leptin. However, they also raise the possibility that isolating the best correlate of fasting plasma leptin levels among different compartments of adipose tissue might be confounded by close intercorrelations between these compartments.

Some investigators have reported that in response to energy restriction the most striking decline in leptin levels is seen before any major changes in adipose tissue stores occur

TABLE 2. Comparison of the characteristics of subjects in either the high- and low-insulin groups individually matched for FM (Study 1)

Variables	Men		Women	
	Low-insulin	High-insulin	Low-insulin	High-insulin
Age (yr)	39.3 ± 3.2	35.7 ± 3.8	42.3 ± 2.1	31.5 ± 2.0[a]
Body weight (kg)	75.7 ± 1.7	71.0 ± 2.9	65.7 ± 1.8	66.8 ± 1.9
FM (kg)	14.6 ± 1.2	14.6 ± 1.2	21.1 ± 1.3	21.1 ± 1.3
FFM (kg)	61.1 ± 1.2	56.4 ± 2.2	44.6 ± 0.8	45.7 ± 0.7
L4L5 VAT (cm^2)	86.8 ± 9.7	106.8 ± 12.5	78.1 ± 7.1	80.4 ± 6.9
L4L5 SAT (cm^2)	160.6 ± 16.4	181.6 ± 15.4	273.4 ± 22.0	313.2 ± 20.1

Means ± SEM; n = 24 and 46 in groups of low- and high-insulin groups in men and women, respectively.
[a] $P < 0.001$.

TABLE 3. Characteristics of men and women who participated in Study 2 before and after weight loss

Variables	Men		Women	
	Before	After	Before	After
Weight (kg)	102.6 ± 2.1	91.9 ± 1.9[a]	92.6 ± 2.9	84.7 ± 2.9[a]
FM (kg)	38.3 ± 1.4	28.7 ± 1.2[a]	46.0 ± 9.6	40.4 ± 2.1[a]
FFM (kg)	64.4 ± 1.6	63.2 ± 1.5	46.6 ± 1.2	44.3 ± 1.0[a]
L4L5 VAT (cm^2)	208.5 ± 13.9	129.5 ± 13.4[a]	152.5 ± 9.1	124.7 ± 8.0[a]
L4L5 SAT (cm^2)	394.1 ± 14.6	310.3 ± 14.2[a]	557.3 ± 29.5	501.3 ± 32.5[a]
TAT (cm^2)	80.1 ± 2.7	64.7 ± 2.6[a]	186.2 ± 7.9	164.0 ± 7.9[a]

Means ± SEM.
[a] $P < 0.001$, respectively.

TABLE 4. Stepwise multiple regression analysis examining predictors of leptin levels in obese men and women before and after weight loss

Dependent variable	Step no.	Predicting variable	r^2	*P*
Men				
Before weight loss				
Leptin	1	L4L5 SAT	0.36	<0.01
After weight loss				
Leptin	1	L4L5 SAT	0.49	<0.001
Women				
Before weight loss				
Leptin	1	FM	0.4	0.001
After weight loss				
Leptin	1	FM	0.63	<0.001

Variables entered in the model: 1) dependent variable, leptin; 2) independent variables (FM, FFM, L4L5 SAT, L4L5 VAT, TAT).

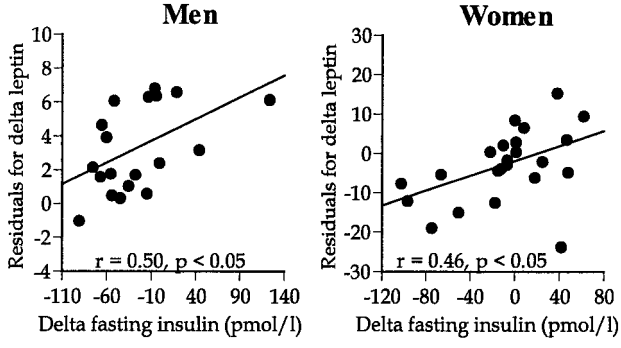

FIG. 3. Correlations between changes in fasting plasma leptin levels corrected for the contribution of changes in FM and changes in fasting plasma insulin levels in response to a 15-week weight loss program in men and women.

(47–49). This observation suggests that factors other than adiposity *per se* influence leptin levels. Fasting plasma insulin could be one of these factors as positive associations between fasting insulin levels and leptinemia before (27, 41, 43, 50) and after correction for adiposity (43, 50) have been reported. We were able to further investigate this issue by using a matching strategy that allowed us to have either high-insulin or low-insulin individuals with identical amounts of total FM. This strategy permitted us to assess to what extent increased insulin can affect leptinemia. In this case, the high-insulin group displayed fasting leptin levels that were 32% and 22% higher than what was observed in the low-insulin group in men and women, respectively. These results are in accordance with those of Segal *et al.* (51), who reported that insulin resistance is associated with elevated plasma leptin levels in lean men matched for body fat. In addition, our results also show this to be true for both men and women of varying degrees of adiposity.

The findings from Study 1 are reinforced by the fact that other variables known to influence plasma leptin levels were not different between the two groups, in both men and women. The fact that women with low-insulin levels were older than high-insulin women is of concern. However, it should be noted that some correlation analyses were performed in men and women both on the whole samples and on the subsets that resulted from this matching strategy to determine whether or not an association could be observed between age and leptinemia, and this was not the case (results not shown). Moreover, levels of fasting plasma leptin are not different between pre- and postmenopausal women (43, 52, 53).

Although observational data can be useful, intervention studies have the potential to significantly add to the understanding of the interplay that takes place between hormones that fluctuate during weight loss. In this sense, results have shown that during weight loss leptin level changes are related to changes in insulin levels in men and women (54), even after correction for adiposity in women (28, 29, 42). Our results are in accordance with currently available literature. However, because women have been shown to have higher *ob*

gene expression (55) as well as higher plasma (21) and cerebrospinal fluid levels of leptin (56), and because it has also been reported that there are significant gender differences in the response of insulin and leptin to weight loss (57), we cannot easily extrapolate these results to men. To our knowledge, our observations are the first to show that after having controlled for changes in adiposity that occur during weight loss, changes in leptin are also associated with changes in insulin in men. Furthermore, our observations fit well with studies that have experimentally produced hyperinsulinemia and then measured its impact on leptin mRNA expression and circulating leptin levels. Although acute increases in insulinemia (2–5 h) did not affect leptin mRNA expression (24) or levels (22), it has been demonstrated that experimentally induced prolonged hyperinsulinemia (from 24–72 h) increases serum leptin levels (22) dose dependently (25), even in physiological amounts (26).

In summary, these studies lend support to the notion that variability in insulin levels contributes to variations in leptin levels independently from the influence of adiposity in both men and women. They confirm that in individuals of identical adiposity level, high fasting insulin is associated with elevated fasting leptin levels. In addition, they emphasize that variations in leptinemia are correlated with the changes in insulin levels during weight loss independently of changes in adiposity. Thus, fasting leptin levels are influenced by insulin both before and during weight loss in men and women.

References

1. **Banting FG, Best CH.** 1987 Nutrition classics. *The Journal of Laboratory and Clinical Medicine*, vol. VII, 1922: The internal secretion of the pancreas. Nutr Rev. 45:55–57.
2. **Reaven GM.** 1988 Role of insulin resistance in human disease. Diabetes. 37:1495–1507.
3. **Wing RR, Jeffery RW.** 1995 Effect of modest weight loss on changes in cardiovascular risk factors: are there differences between men and women or between weight loss and maintenance. Int J Obes. 19:67–73.
4. **Goldstein DJ.** 1992 Beneficial health effects of modest weight loss. Int J Obes. 16:397–415.
5. **Vollenweider P, Tappy L, Randin D, et al.** 1993 Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest. 92:147–154.
6. **Berne C, Fagius J, Pollare T, Hemjdahl P.** 1992 The sympathetic response to euglycemic hyperinsulinemia. Diabetologia. 35:873–879.
7. **Schwartz MW, Sipols AJ, Marks JL, et al.** 1992 Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology. 130:3608–3616.
8. **Folsom AR, Vitelli LL, Lewis CE, Schreiner PJ, Watson RL, Wagenknecht LE.** 1998 Is fasting insulin concentration inversely associated with rate of weight change? Contrasting findings from CARDIA and ARIC study cohorts. Int J Obes. 22:48–54.
9. **Valdez R, Mitchell BD, Haffner SM, et al.** 1994 Predictors of weight change in a bi-ethnic population. The San Antonio Heart Study. Int J Obes. 18:85–91.
10. **Schwartz MW, Boyko EJ, Kahn SE, Ravussin E, Bogardus C.** 1995 Reduced insulin secretion: an independent predictor of body weight gain. J Clin Endocrinol Metab. 80:1571–1576.
11. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425–432.
12. **Maffei M, Halaas J, Ravussin E, et al.** 1995 Leptin levels in human and rodent; measurement of plasma leptin and OB RNA in obese and weight reduced subjects. Nat Med. 1:1155–1161.
13. **Considine RV, Shina MK, Heiman ML, et al.** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese subjects. N Engl J Med. 334:292–295.
14. **Halaas JL, Gajiwala KS, Maffei M, et al.** 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 269:543–546.
15. **Stephens TW, Basinski M, Bristow PK, et al.** 1995 The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature. 377:530–532.
16. **Pelleymounter MA, Cullen MJ, Baker MB, et al.** 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 269:540–543.
17. **Hwa JJ, Fawzi AB, Graziano MP, et al.** 1997 Leptin increases energy expenditure and selectively promotes fat metabolism in ob/ob mice. Am J Physiol. 272: R1204–R1209.
18. **Snitker S, Pratley RE, Nicolson M, Tataranni PA, Ravussin E.** 1997 Relationship between muscle sympathetic nervous activity and plasma leptin concentration. Obes Res. 5:338–340.
19. **Larson ED, Ferraro RT, Robertson DS, Ravussin E.** 1995 Energy metabolism in weight-stable postobese individuals. Am J Clin Nutr. 62:735–739.
20. **Buemann B, Astrup A, Madsen J, Christensen NJ.** 1992 A 24-hr energy expenditure study on reduced-obese and non-obese women: effect of β-blockade. Am J Clin Nutr. 56:662–670.
21. **Doucet E, Imbeault P, St-Pierre S, et al.** 2000 Changes in energy expenditure and substrate oxidation resulting from weight loss in obese men and women: is there a significant contribution of leptin? J Clin Endocrinol Metab. 85:1550–1556.
22. **Kolaczynski JW, Considine RV, Ohannesian J, et al.** 1996 Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. Diabetes. 45:1511–1515.
23. **Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF.** 1996 Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab. 81:4162–4165.
24. **Vidal H, Auboeuf D, De Vos P, et al.** 1996 The expression of ob gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. J Clin Invest. 98:251–255.
25. **Boden G, Chen X, Kolaczynski JW, Polansky M.** 1997 Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest. 100:1107–1113.
26. **Saad MF, Khan A, Sharma A, et al.** 1998 Physiological insulinemia acutely modulates plasma leptin. Diabetes. 47:544–549.
27. **Couillard C, Mauriege P, Prud'homme D, et al.** 1997 Plasma leptin concentrations: gender differences and associations with metabolic risk factors for cardiovascular disease. Diabetologia. 40:1178–1184.
28. **Carantoni M, Abbasi F, Azhar S, Schaaf P, Reaven GM.** 1999 Can changes in plasma insulin concentration explain the variability in leptin response to weight loss in obese women with normal glucose tolerance? J Clin Endocrinol Metab. 84:869–872.
29. **Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL, Stern JS.** 1996 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.
30. **Canadian Diabetes Association**. 1994 Diabetic food exchange system. Ottawa, Canada.
31. **Khan MA, Herzog CA, St Peter JV, et al.** 1998 The prevalence of cardiac valvular insufficiency assessed by transthoracic echocardiography in obese patients treated with appetite-suppressant drugs. N Engl J Med. 10:713–718.
32. **Weissman NJ, Tighe JFJ, Gottdiener JS, Gwynne JT.** 1998 An assessment of heart-valve abnormalities in obese patients taking dexfenfluramine, sustained-release dexfenfluramine, or placebo. Sustained-Release Dexfenfluramine Study Group. N Engl J Med. 10:725–732.
33. **Prud'homme D, Langlais M, Samson MP, et al.** 1999 Lack of major cardiac valvular abnormalities in asymptomathic obese men and women following a 3-month fenfluramine or dexfenfluramine treatment. Int J Obes. 23:S175.
34. **Behnke AR, Wilmore JH.** 1974 Evaluation and regulation of body build and composition. Englewood Cliffs, NJ: Prentice-Hall; 20–37.
35. **Meneely EA, Kaltreider NL.** 1949 Volume of the lung determined by helium dilution. J Clin Invest. 28:129–139.
36. **Siri WE.** 1956 The gross composition of the body. Adv Biol Med Physiol. 4:239–280.
37. **Sjostrom L, Kvist H, Cederblad A, Tylen U.** 1986 Determination of total adipose tissue and body fat in women by computed tomography, ^{40}K, and tritium. Am J Physiol. 250:E736–E745.
38. **Ferland M, Despres JP, Tremblay A, et al.** 1989 Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr. 61:139–148.
39. **Desbuquois B, Aurbach GD.** 1971 Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab. 37:732–738.
40. **Masuzaki H, Ogawa Y, Isse N, et al.** 1995 Human obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. Diabetes. 44:855–858.
41. **Guven S, El-Bershawi A, Sonnenberg GE, et al.** 1999 Plasma leptin and insulin levels in weight-reduced obese women with normal body mass index; relationships with body composition and insulin. Diabetes. 48:347–352.
42. **Rissanen P, Makimattila S, Vehmas T, Taavitsainen M, Rissanen A.** 1999 Effect of weight loss and regional fat distribution on plasma leptin concentration in obese women. Int J Obes. 23:645–649.
43. **Baumgartner RN, Ross RR, Waters DL, et al.** 1999 Serum leptin in elderly people: associations with sex hormones, insulin, and adipose tissue volumes. Obes Res. 7:141–149.
44. **Bennett FI, McFarlane-Anderson N, Wilks R, Luke A, Cooper RS, Forrester TE.** 1997 Leptin concentration in women is influenced by regional distribution of adipose tissue. Am J Clin Nutr. 66:1340–1344.

45. **Van Harmelen V, Reynisdottir S, Eriksson P, et al.** 1998 Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes. 47:913–917.
46. **Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S.** 1997 Depot- and sex-specific differences in human leptin mRNA expression; implications for the control of regional fat distribution. Diabetes. 46:342–347.
47. **Wisse BE, Campfield LA, Marliss EB, Morais JA, Tenenbaum R, Gougeon R.** 1999 Effect of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentrations in obese women. Am J Clin Nutr. 70:321–330.
48. **Geldszus R, Mayr B, Horn R, Geisthovel F, von zur Muhlen A, Brabant G.** 1996 Serum leptin and weight reduction in female obesity. Eur J Endocrinol. 135:659–662.
49. **Wadden TA, Considine RV, Foster GD, Anderson DA, Sarwer DB, Caro JS.** 1998 Short- and long-term changes in serum leptin dieting obese women: effects of caloric restriction and weight loss. J Clin Endocrinol Metab. 83:214–218.
50. **Haffner SM, Mykkanen L, Rainwater DL, Karhapaa P, Laakso M.** 1999 Is leptin concentration associated with the insulin resistance syndrome in nondiabetic men? Obes Res. 7:164–169.
51. **Segal KR, Landt M, Klein S.** 1996 Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. Diabetes. 45:988–991.
52. **Haffner SM, Mykkanen L, Stern MP.** 1997 Leptin concentrations in women in the San Antonio Heart Study: effect of menopausal status and postmenopausal hormone replacement therapy. Am J Epidemiol. 146:581–585.
53. **Kohrt WM, Landt M, Birge Jr SJ.** 1996 Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. J Clin Endocrinol Metab. 81:3980–3985.
54. **Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL.** 1999 Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. Diabetes. 48:839–847.
55. **Lonnqvist F, Arner P, Nordfors L, Schalling M.** 1995 Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med. 1:950–953.
56. **Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte Jr D.** 1996 Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nat Med. 2:589–593.
57. **Dubuc GR, Phinney SD, Stern JS, Havel PJ.** 1998 Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. Metabolism. 47:429–434.