Serum Leptin Concentrations in Human Immunodeficiency Virus–Infected Men With Low Adiposity

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The product of the obese gene (*ob*) is the protein leptin, which is synthesized in and secreted from adipocytes. Fasting serum leptin concentrations are closely related to body fat content and are higher in obese than in normal-weight individuals. Leptin may contribute to body weight regulation. Overproduction of leptin in certain pathologic conditions such as acquired immunodeficiency syndrome (AIDS) might in principle contribute to the low body fat content associated with body wasting. We measured fasting serum leptin levels by radioimmunoassay in individuals infected with the human immunodeficiency virus (HIV) and in a group of healthy lean men to determine whether HIV infection increases leptin levels. Thirteen HIV-infected men aged 26 to 50 years with a body mass index (BMI) of 15 to 26 kg/m^2 and 4 to 24 kg body fat (7% to 29% body fat) had serum leptin levels (3.4 ± 1.6 ng/mL) that were not elevated compared with the levels in 17 healthy men (4.0 ± 1.4 ng/mL) matched for age (23 to 47 years), BMI (18 to 26 kg/m^2), and body fat (5 to 21 kg; 9% to 28%). In both groups of men, serum leptin concentrations were correlated with percent body fat and body fat content ($P < .001$), and these relationships were not different between the two groups. In both groups, leptin concentrations were not correlated with lean body mass ($P \geq .24$). Energy intake in the HIV-infected men, assessed from 3-day intake records, was within the normal range. These findings extend the hypothesis that circulating leptin concentrations directly reflect adipose tissue mass, even in HIV-infected men with low body fat content. These findings do not support the hypothesis that HIV infection is associated with high circulating leptin concentrations, and suggest that low leptin levels do not stimulate food intake in HIV-infected individuals.

POSITIONAL CLONING of the mouse and human obese (*ob*) gene from adipose tissue indicates that the gene product is a 16-kd protein (designated leptin) that is important in the regulation of body weight.[1] C57BL/6J mice (*ob/ob*) have a mutation in the *ob* gene and undetectable plasma levels of the *ob* protein leptin. These mice are obese and diabetic and have reduced rates of energy expenditure. When the *ob* protein is administered centrally or peripherally to *ob/ob* mice, food intake decreases, energy expenditure increases, and body fat content declines,[2,3] suggesting that circulating leptin plays a role in body weight regulation by adjusting the balance between energy intake and expenditure.

Circulating human leptin is a 146–amino acid polypeptide with 84% homology with the mouse protein. In humans, leptin mRNA is abundant in white adipose tissue (subcutaneous, omental, retroperitoneal, perilymphatic, and mesenteric), but leptin mRNA was not detected in other tissues examined (eg, skeletal muscle, brain, heart, liver, kidney, and pancreas[4,5]). It has recently been reported that human adipocytes are the primary site of leptin gene expression and leptin secretion.[6] Thus, human obesity is associated with high circulating leptin concentrations.[6]

Leptin levels in cachectic individuals with low body fat content have not been reported. It has been proposed that leptin regulates body weight through a feedback system,[7] such that high concentrations of leptin might signal reduced energy intake and increased energy expenditure and result in weight loss, whereas low concentrations might signal increased energy intake and reduced energy expenditure and result in weight gain. A recent study concluded that obese humans might have reduced sensitivity to this leptin signal,[6] but nothing is known about the sensitivity of HIV-infected individuals to circulating leptin. On the basis of studies in rodents, it has been proposed that low circulating leptin levels might signal increased energy intake, reduced physical activity, and subsequent weight gain.[8]

Infection with human immunodeficiency virus type 1 (HIV-1) typically results in involuntary weight loss characterized by a substantial reduction in lean and adipose tissue mass.[9] Weight loss may contribute to reduced immunocompetence in acquired immunodeficiency syndrome (AIDS), and mortality from AIDS-associated cachexia appears to be correlated with the magnitude of lean tissue depletion.[9] The precise reason for HIV-associated wasting is unclear. Because a suppression of food intake and an increase in energy expenditure occur upon exogenous administration of leptin to mice,[2,3] overproduction of endogenous leptin might be postulated to contribute to body wasting associated with HIV infection. Recent evidence suggests that endotoxin or cytokine administration to fasted hamsters increases serum leptin and leptin mRNA in adipose tissue.[10] The increase in serum leptin was correlated with reduced food intake, suggesting that increased leptin may play a role in the anorexia associated with acute infection. Whether this is true in humans chronically infected with HIV is not known. Chronic infection may result in lower leptin levels that might be postulated to increase food intake to restore body weight. To assess these possibilities, we determined fasting serum leptin concentrations in HIV-infected men and in lean healthy men, and correlated leptin levels with body fat content.

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SUBJECTS AND METHODS

Thirteen HIV-infected men aged 26 to 50 years with a body mass index (BMI) not greater than 26 kg/m^2 (range, 15 to 26), less than 25 kg body fat (range, 4 to 24), and less than 29% body fat (range, 7% to 29%) were studied and compared with 17 healthy, lean 23 to 47-year-old men with a BMI not greater than 26 kg/m^2 (range, 18 to 26), no more than 21 kg body fat (range, 5 to 21), and less than 28% body fat (range, 9% to 28%). All HIV-infected men were stable on standard antiretroviral therapy (zidovudine, dideoxyinosine, dideoxycytidine, stavudine, and lamivudine) and other prescription medications for at least 1 month before study. Eight subjects had CD4 lymphocyte counts greater than 200 cells/μL (range, 336 to 634) measured within 30 days of study, no history of opportunistic infection, and less than 10% body weight loss within the previous year. Five subjects had CD4 lymphocyte counts less than 200 cells/μL (range, 0 to 174) and involuntary, unexplained weight loss of at least 10% body weight within the previous year. All five of these men had an active, stable, non–life-threatening, AIDS-defining opportunistic infection (*Mycobacterium avium*-complex or cytomegalovirus retinitis) at the time of study.

Body fat and lean body mass were determined after an overnight fast using a QDR-2000 enhanced-array whole-body dual-energy x-ray absorptiometer (Hologic, Waltham, MA). This instrument uses an x-ray tube to direct photons of two different energy levels through the body and a scanner to detect photons projected through the subject that have been attenuated as a result of tissue (bone, fat, and lean) density differences. Hologic enhanced-array whole-body analysis software (version 5.64A) was used to obtain measures of body mass, lean body mass, and body fat mass and to calculate body fat percentage. Dual-energy x-ray absorptiometry precision is typically 1.5% to 1.8% (coefficient of variation) for body fat percentage, 2.1% for fat mass, and 0.6% for lean tissue.[11,12]

Serum leptin concentration was measured in specimens collected after an overnight fast, using a commercial radioimmunoassay.[13] Briefly, 100-μL aliquots of serum were analyzed in duplicate by mixing with 100 μL ^{125}I-leptin; both tracer and standards were prepared from highly purified recombinant human leptin. Antibody to recombinant human leptin made in rabbits was added, and binding was promoted by overnight incubation at 4°C. Anti-rabbit immunoglobulin G precipitating reagent was added, and after 20 minutes the incubations were centrifuged at 2,000 × *g* for 15 minutes. The supernatants were decanted, bound ^{125}I-leptin in the pellet was determined with a gamma counter, and the concentration of leptin was determined in each specimen by comparison to a standard curve generated from eight standards of 0.5 to 100 ng leptin/mL. Assay precision (coefficient of variation), ranged from 3.4% to 8.3% within runs and 3.6% to 6.2% between runs over a leptin concentration range of 4.9 to 25.6 ng/mL. The assay sensitivity limit was 0.5 ng/mL.[13]

Three-day food intake surveys were obtained from the HIV-infected subjects during an interview with a research dietician. Total energy intake and percent of total calories from dietary protein, carbohydrate, fat, and alcohol were estimated from these surveys using Nutritionist IV software (First DataBank, San Bruno, CA).

The correlation coefficient (r), standard error of the estimate (SEE), and statistical significance of the correlation ($P < .05$) between serum leptin concentration and BMI, body fat content (kilograms and percent of body weight), and lean body mass were calculated using linear regression analysis. The mean ± SD are reported.

The study was approved by the Human Studies Committee at Washington University School of Medicine, and all subjects provided informed consent before participation.

RESULTS

Fasting serum leptin concentrations were 3.4 ± 1.6 ng/mL (range, 1.0 to 7.1) in the HIV-infected men and 4.0 ± 1.4 ng/mL in the control group (range, 1.0 to 6.0; $P = .28$). In HIV-infected subjects, there was a significant correlation between serum leptin concentration and percent body fat ($r = .91$, SEE = 2.56, $P < .001$), body fat content ($r = .91$, SEE = 3.20, $P < .001$), and BMI ($r = .77$, SEE = 2.32, $P = .002$; Fig 1). In control subjects, similar relationships between serum leptin and percent body fat ($r = .85$, SEE = 5.37, $P < .001$), body fat content ($r = .80$, SEE = 3.37, $P < .001$), and BMI ($r = .57$, SEE = 2.26, $P = .018$) were observed, suggesting that HIV infection did not affect the linear relationship between serum leptin and body fat. The slope of the line and the distribution of leptin values versus body fat and BMI were superimposable for the two groups (Fig 1). Serum leptin concentration was not related to lean body mass in either group ($P \geq .24$).

Energy intake was estimated to be 93 to 199 kJ/kg/d (142 ± 40) in the HIV-infected men. Nutrient composition was 0.8 to 1.45 g protein/kg/d (1.17 ± 0.23), with 13% to 24% (19 ± 4%) of calories from protein, 32% to 68% (51 ± 11%) from carbohydrate, 14% to 37% (27 ± 8%) from fat, and 0% to 13% (3 ± 5%) from alcohol. Serum leptin levels were not related to energy, protein, carbohydrate, or fat intake ($P > .14$).

DISCUSSION

These observations suggest that in HIV-infected individuals with low body fat content, fasting serum leptin concentrations are comparable to those measured in age-matched, lean healthy men with a similar body fat content and BMI. Fasting serum leptin levels were not elevated in HIV-infected men, but were directly related to the absolute amount of body fat and the percentage of body weight that is adipose tissue in both the control and HIV-infected subjects. This supports and extends the observation that serum leptin, which is produced predomi-

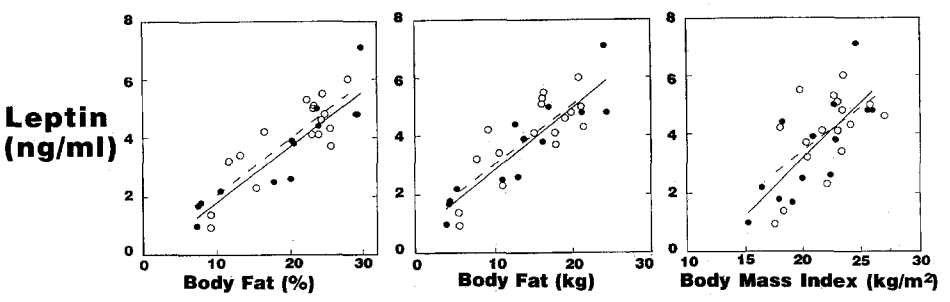

Fig 1. Linear regression analysis of fasting serum leptin concentration and body fat as a % of body weight, body fat content in kg, and BMI in HIV-infected (●) and lean control (○) subjects. In both groups, leptin concentration was significantly correlated with body fat (kg and %, $P < .003$), and the linear relationship was not different between the 2 groups.

nantly in white adipose tissue, is closely associated with the amount of body fat.[4-6] Our findings indicate that the close relationship between body fat content and serum leptin concentration persists even when body adipose stores are very low as a result of HIV infection. In HIV-infected men, a significant relationship was also observed between serum leptin concentration and BMI. This supports the previously reported relationship between BMI and both the amount of *ob* mRNA and the relative amount of *ob* gene expression in white adipose tissue in obese humans both before and after a mild weight loss (10% initial body weight[4,6]). We observed no correlation between serum leptin concentration and lean body mass, consistent with the fact that *ob* mRNA is not detectable, or is at least in low abundance, in nonadipose tissues.[5] Our findings are consistent with the proposition that circulating leptin levels reflect primarily body fat content in both lean healthy men and HIV-infected men.

Weight loss, increased resting energy expenditure, and reduced appetite have been observed when *ob* protein was administered to obese mice.[2,3] HIV infection is also typified by periods of weight loss, elevated metabolic rate, and anorexia, but the pathogenesis of this body wasting is unclear. The low concentrations of serum leptin observed in these HIV-infected men do not support the notion that chronic HIV infection is associated with increased circulating concentrations of leptin. Clearly, the four HIV-infected subjects with the most weight loss, the lowest body fat (despite adequate energy intake), and the lowest CD4 cell count had low circulating leptin concentrations (1.9 ± 0.7 ng/mL). This implies that the cachexia of chronic HIV infection is not associated with increased serum leptin concentrations. These findings do not rule out the possibility that serum leptin levels were transiently increased at some earlier time point when these subjects had an acute infection and fever, were rapidly losing weight, or had severely reduced their food intake (due to increased HIV viral load, acute illness, or medications), and contributed to initiating the weight loss. The postulate that high concentrations of serum leptin signal reduced energy intake and that low concentrations of leptin signal increased energy intake might lead to the prediction that the low serum leptin levels observed in these HIV-infected men would signal increased energy intake to restore body weight. This predicted physiologic effect does not appear to have occurred in the HIV-infected subjects, because the mean daily energy and nutrient consumption was not in excess of standard recommendations (125 to 165 kJ/kg/d and 0.8 to 1.0 g protein/kg/d). Therefore, HIV-infected individuals may be resistant to a potential signal provided by low serum leptin concentrations to increase food intake. It is also possible that, unlike the high levels of leptin reported to reduce food intake in obese and lean mice,[2,3,7] low serum leptin levels do not initiate a signal in the brain that increases energy intake in HIV-infected men.

These findings support the notion that body fat content is the primary regulator of serum leptin concentrations, even at very low body fat contents associated with HIV infection. At least in fasting serum from HIV-infected men, it does not appear that serum leptin is elevated to a level that might contribute to the pathogenesis of weight loss typical of HIV infection. Likewise, it does not appear that the low leptin concentrations were sufficient to trigger increased energy intake in HIV-infected subjects.

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REFERENCES

1. Zhang F, Proenca R, Baffel M, et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372:424-432, 1994

2. Halaas JL, Gajiwala KS, Maffei M, et al: Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546, 1995

3. Pelleymounter MA, Cullen MJ, Baker MB, et al: Effects of the obese gene product on body weight in *ob/ob* mice. Science 269:540-543, 1995

4. Considine RV, Considine EL, Williams CJ, et al: Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. J Clin Invest 95:2986-2988, 1995

5. Masuzaki H, Ogawa Y, Isse N, et al: Human obese gene expression: Adipocyte-specific expression and regional differences in the adipose tissue. Diabetes 44:855-858, 1995

6. Considine RV, Sinha MK, Heiman ML, et al: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292-295, 1996

7. Campfield LA, Smith FJ, Guisez Y, et al: Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-549, 1995

8. Lönnqvist F: The obese gene (*ob*) and human obesity. News Physiol Sci 11:147-148, 1996

9. Kotler DP, Wang J, Pierson RN: Body composition studies in patients with the acquired immunodeficiency syndrome. Am J Clin Nutr 42:1255-1265, 1985

10. Grunfeld C, Zhao C, Fuller J, et al: Endotoxin and cytokines induce expression of leptin, the ob gene product in hamsters. J Clin Invest 97:2152-2157, 1996

11. Pritchard JE, Nowson CA, Strauss BJ, et al: Evaluation of dual energy x-ray absorptiometry as a method of measurement of body fat. Eur J Clin Nutr 47:216-228, 1993

12. Snead DB, Birge SJ, Kohrt WM: Age-related differences in body composition by hydrodensitometry and dual-energy x-ray absorptiometry. J Appl Physiol 74:770-775, 1993

13. Ma ZA, Gingerich RL, Santiago JV, et al: Radioimmunoassay of leptin in human plasma. Clin Chem 42:942-946, 1996