Plasma Leptin Concentrations and Energy Expenditure in Heart Failure Patients

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Leptin, the protein encoded by the *obese* gene, is a newly described hormone implicated in the regulation of energy balance. To examine the possible role of leptin in the energy dysregulation that frequently accompanies chronic heart failure, we examined plasma leptin concentrations and energy expenditure in 18 heart failure patients (aged 71 ± 6 years) and 46 healthy elderly controls (66 ± 6 years). Plasma leptin concentrations were measured by radioimmunoassay, daily energy expenditure by doubly labeled water, and body composition by dual-energy x-ray absorptiometry. Fat mass was lower ($P < .01$) in heart failure patients compared with healthy controls, whereas fat-free mass did not differ between groups. Plasma leptin concentrations were not different between heart failure patients and healthy controls (5.1 ± 4.2 *v* 6.8 ± 4.4 pg/mL) and remained similar after statistical control for fat mass (6.0 ± 3.1 *v* 7.1 ± 3.2 pg/mL). Plasma leptin was related to fat mass in heart failure patients ($r = .92$, $P < .01$) and healthy controls ($r = .69$, $P < .01$). Free-living daily and physical-activity energy expenditures were lower ($P < .01$) in heart failure patients compared with healthy controls. Plasma leptin concentrations were related to both daily ($r = .67$, $P < .01$) and resting ($r = .67$, $P < .01$) energy expenditure in heart failure patients, but not in healthy controls ($r = .09$ and $r = .33$, respectively). In conclusion, we found an association between plasma leptin concentrations and energy expenditure in heart failure patients, but not in healthy controls. Thus, leptin may participate in the regulation of energy expenditure and body energy stores in heart failure patients.

LEPTIN, the protein encoded by the recently discovered *obese* gene, is a newly described hormone implicated in the regulation of energy balance.[1] Leptin is released from adipose tissue[2,3] and is thought to regulate energy balance through its effects on receptors in the central nervous system.[4-6] Leptin has both thermogenic and anorexigenic effects in mice,[4,7,8] but its role in the regulation of energy balance in humans is unclear.

Research on the physiological function of leptin has primarily focused on its role in the pathogenesis of obesity.[4,7-9] However, one role of leptin that has not been explored is its contribution to states of negative energy imbalance (ie, loss of body energy stores) that frequently accompany chronic disease states. Hormonal and metabolic derangements associated with disease may affect the leptin signaling system. Specifically, elevated plasma concentrations of leptin, increased sensitivity to leptin at target tissues, or a combination of both may occur to promote energy imbalance and depletion of body energy stores.

Patients with congestive heart failure represent a model to examine the impact of chronic disease on the leptin signaling system. Heart failure patients frequently experience weight loss[10,11] and exhibit abnormally elevated rates of resting energy expenditure,[12,13] both of which suggest a state of energy dysregulation. To investigate the role of leptin in the regulation of energy expenditure in heart failure, we compared plasma leptin concentrations and their relation to energy expenditure in free-living heart failure patients and healthy elderly controls.

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SUBJECTS AND METHODS

Experimental Subjects

Eighteen patients (aged 71 ± 6 years; 17 men and one woman; 10 African-American and eight white) with heart failure were recruited from the Heart Failure Services of the Baltimore Veterans Affairs Medical Center and University of Maryland Medical System. Patients had a mean resting left ventricular ejection fraction of 22% ± 10% (range, 10% to 37%) measured by radionuclide ventriculography. During the testing period, patients were hemodynamically stable, free of edema, and taking the following medications: diuretics (n = 18), digoxin (n = 17), and vasodilators (angiotensin-converting enzyme inhibitor or hydralazine nitrates, n = 16). There were 10 patients with documented coronary artery disease (defined as a history of myocardial infarction or significant obstruction on cardiac catheterization) and eight patients with dilated cardiomyopathy unrelated to coronary artery disease. Symptoms were categorized as New York Heart Association class II (n = 7), class III (n = 6), or class IV (n = 5). Nine patients were cachectic, as defined by a reported weight loss of greater than 10% of premorbid body weight during the course of the disease. The remaining nine weight-stable patients were designated as noncachectic. All patients were weight-stable at the time of evaluation.

Data from 46 healthy elderly subjects (aged 66 ± 6 years; 44 men and two women; 21 African-American and 25 white) served as a control group. Healthy subjects were recruited by newspaper advertisements and community organizations from Baltimore, MD, and surrounding areas. Healthy subjects met the following criteria: (1) no symptoms or signs of heart disease or diabetes; (2) normal resting electrocardiogram; (3) normal electrocardiogram response to an exercise stress test; (4) absence of medication that could affect cardiovascular or metabolic function; and (5) weight stability (±2 kg) within 6 months before testing. The nature, purpose, and possible risks of the study were explained to each volunteer and heart failure patient before provision of consent. This study was approved by the Institutional Review Board of the University of Maryland. Data regarding the effect of heart failure on energy expenditure has been reported elsewhere (n = 17 heart failure patients and 21 healthy controls).[14]

Leptin Concentrations

After an overnight fast, venous blood was drawn into chilled tubes containing 1.5 mg EDTA/mL blood. Plasma was separated by centrifu-

gation at 4°C and rapidly stored (−70°C) for further analysis. Plasma leptin concentrations were measured in duplicate by radioimmunoassay (Linco, St Louis, MO). The intraassay coefficient of variation was 5.2%.

Dual-Energy X-Ray Absorptiometry

Fat mass and fat-free mass were measured by dual-energy x-ray absorptiometry using a Lunar DPX-L densitometer (Madison, WI). All scans were analyzed using the Lunar Radiation version 1.3z DPX-L program for body composition analysis.

Daily Energy Expenditure

Free-living daily energy expenditure was measured in 17 heart failure patients and 21 healthy controls over a 10-day period using the doubly labeled water technique, as previously described.[15] Each subject consumed a mixed oral dose of ^2H_2O and $H_2{}^{18}O$ (0.075 and 0.15 g/kg body mass, respectively) after providing a baseline urine sample (between 12 noon and 4 PM). Two urine samples were obtained on the morning after dosing to mark the beginning of the measurement period, and 10 days later to mark the end (all between 8 AM and 12 noon). Urine samples were stored in sealed vacutainers at −20°C until analysis in triplicate by isotope ratio mass spectrometry (Optima; Fisons Instruments, Middlewhich, Cheshire, UK). Samples were analyzed for isotopic enrichment of ^2H_2O and $H_2{}^{18}O$ using the off-line zinc reduction procedure of Kendall and Copelan[16] and CO_2 equilibration technique,[17] respectively.

Turnover rates and zero-time enrichment of $H_2{}^{18}O$ and ^2H_2O were determined from the slope and intercept, respectively, of the semilogarithmic plot of urinary enrichment (‰) versus time (days). Isotope dilution spaces were calculated using the equation of Coward.[18] A fixed dilution space ratio of 1.0427 was used following the recommendations of Speakman et al.[19] The rate of carbon dioxide production was calculated using equation 2 from Speakman et al.[19] Carbon dioxide production rates were used to calculate daily energy expenditure from equation 12 of Weir,[20] assuming a respiratory quotient of 0.85.

Physical Activity Energy Expenditure

Physical activity energy expenditure was calculated based on the three-component model of daily energy expenditure: [(0.9 × daily energy expenditure) − resting energy expenditure], assuming the thermic effect of food constitutes 10% of daily energy expenditure in older individuals.[15]

Resting Energy Expenditure

Resting energy expenditure was measured on an outpatient basis in the morning (~8 AM) after a 12-hour overnight fast by indirect calorimetry for 45 minutes using the ventilated-hood method (Deltatrac; Sensormedics, Yorba Linda, CA) as previously described.[21] Energy expenditure was calculated using the equation of Weir.[20]

Statistics

Differences in physical characteristics and energy expenditure variables were assessed by an unpaired Student *t* test. Because plasma leptin concentrations were not normally distributed, differences between heart failure patients and healthy controls were determined by a Mann-Whitney *U* test. Differences in plasma leptin concentrations among cachectic patients, noncachectic patients, and healthy controls were determined by a Kruskal-Wallis *H* statistic. One-way analysis of covariance was used to test for differences in the logarithm (log) of plasma leptin concentrations after statistical control for fat mass. However, for presentation purposes, group-adjusted means for plasma leptin are presented as untransformed values. The relationship between the log of plasma leptin and energy expenditure variables was assessed by linear regression analysis. Partial correlation coefficients between log leptin and energy expenditure variables after removing the effects of fat-free mass were determined by multiple linear regression analysis. All values are expressed as the mean ± SD.

RESULTS

We found no difference in plasma leptin concentrations between African-American and white subjects in either the heart failure or healthy control group. Thus, data were pooled and race was not considered in further analyses. Physical characteristics, plasma leptin concentrations, and energy expenditure of heart failure patients and healthy controls are shown in Table 1. Heart failure patients were older than healthy controls ($P < .01$). No difference in standing height was noted. Heart failure patients weighed less than healthy controls ($P < .01$), primarily due to a lower fat mass ($P < .01$). We found no differences in fat-free mass, measured plasma leptin concentration, or plasma leptin concentration adjusted for fat mass between groups. No difference in plasma leptin concentration was found between heart failure patients and a subgroup (n = 33) of healthy controls (fat mass < 30 kg) either on an absolute basis (5.1 ± 4.2 *v* 5.2 ± 2.2 ng/mL) or after statistical control for fat mass (5.9 ± 2.1 *v* 4.8 ± 2.2 ng/mL). Daily and physical activity energy expenditures were lower in heart failure patients compared with healthy controls (both $P < .01$), whereas no difference in resting energy expenditure was noted.

Plasma leptin concentrations were also examined in a subgroup of heart failure patients (n = 9) who reported significant weight loss (15 ± 6 kg; range, 9 to 25) during the course of the disease (ie, cachectic patients; data not shown in table form). Body weight was lower ($P < .05$) in cachectic patients (64 ± 16 kg) compared with noncachectic patients (80 ± 17 kg) and healthy controls (89 ± 7 kg). Fat mass was lower ($P < .05$) in cachectic heart failure patients (13 ± 10 kg) compared with healthy controls (26 ± 9 kg), and tended to be lower compared with noncachectic patients (22 ± 13 kg). No differences in fat-free mass were noted among the groups (51 ± 9 *v* 58 ± 6 *v* 57 ± 10 kg). Cachectic patients had lower ($P < .05$) plasma leptin concentrations (3.4 ± 1.6 ng/mL) compared with healthy controls (6.8 ± 4.4 ng/mL), but nonsignificantly lower levels

Table 1. Physical Characteristics, Plasma Leptin Concentrations, and Energy Expenditure of Heart Failure Patients and Healthy Controls

Variable	Heart Failure Patients	Healthy Controls
No.	18	46
Age (yr)	71 ± 6	66 ± 6*
Height (cm)	171 ± 8	174 ± 7
Weight (kg)	72 ± 18	89 ± 7*
Fat mass (kg)	18 ± 12	26 ± 9*
Fat-free mass (kg)	55 ± 8	57 ± 10
Leptin (ng/mL)	5.1 ± 4.2	6.8 ± 4.4
Adjusted leptin (ng/mL)†	6.0 ± 3.1	7.1 ± 3.2
Daily energy expenditure (kcal/d)‡	2,113 ± 543	2,679 ± 593*
Physical activity energy expenditure (kcal/d)‡	359 ± 318	817 ± 470*
Resting energy expenditure (kcal/d)‡	1,532 ± 281	1,594 ± 193

NOTE. Values are the mean ± SD.

*$P < .01$.

†Adjusted for fat mass.

‡Heart failure patients, n = 17; healthy controls, n = 21.

compared with noncachectic patients (6.8 ± 5.3 ng/mL). However, after statistical control for fat mass, plasma leptin concentrations were similar among cachectic patients (6.7 ± 3.3 ng/mL), noncachectic patients (7.5 ± 3.1 ng/mL), and healthy controls (6.0 ± 3.1 ng/mL).

The log of plasma leptin concentration was related to fat mass in heart failure patients ($r = .92$, slope = 0.05 ng/mL/kg, y-intercept = 0.53 ng/mL, $P < .01$) and healthy controls ($r = .69$, slope = 0.02 ng/mL/kg, y-intercept = 0.25 ng/mL, $P < .01$; data not shown in figure form).

Figure 1 shows the relationship of the log of plasma leptin concentration to daily energy expenditure ($r = .67$, slope = 0.004 ng · mL^{-1}/kcal · d^{-1}, intercept = −0.12 ng/mL, $P < .01$) and resting energy expenditure ($r = .67$, slope = 0.007 ng · mL^{-1}/kcal · d^{-1}, intercept = −0.42 ng/mL, $P < .01$) in heart failure patients. The log of plasma leptin concentration was not related to daily or resting energy expenditure in healthy controls ($r = .09$ and $r = .33$, respectively; data not shown in figure form). The relationship of the log of plasma leptin concentration with both daily energy expenditure and resting energy expenditure persisted in heart failure patients after statistical control for fat-free mass (partial correlation $r = .54$ and $r = .48$, respectively, both $P < .05$). The log of plasma leptin concentration showed a tendency for a relationship to physical activity energy expenditure in heart failure patients ($r = .44$, $P = .08$), but not in healthy controls ($r = -.05$, NS).

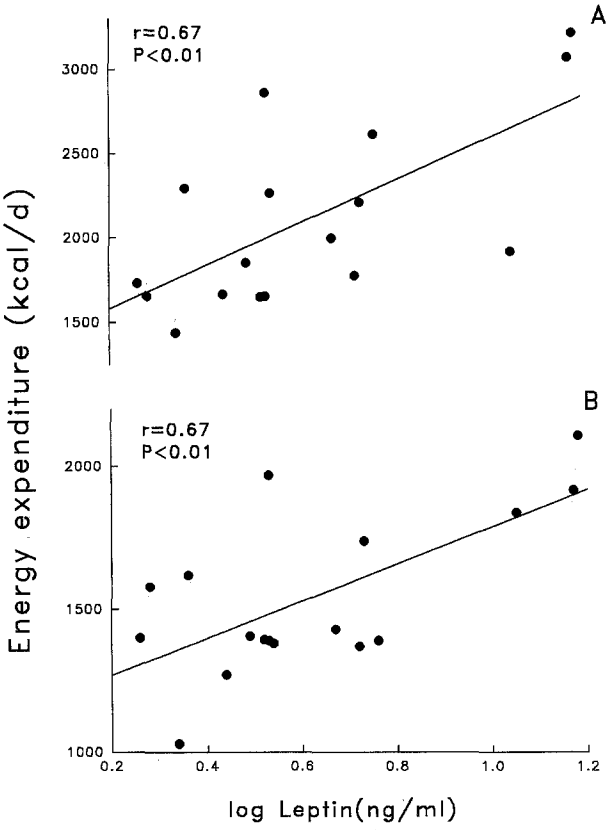

Fig 1. Relationship between the log of plasma leptin concentrations and daily energy expenditure (A) and resting energy expenditure (B) in heart failure patients.

DISCUSSION

The objective of this study was to investigate the possible role of leptin in the regulation of energy expenditure in chronic heart failure. The major finding is the relationship between plasma leptin concentrations and energy expenditure variables in heart failure patients. Plasma leptin concentrations were positively related to free-living daily energy expenditure and resting energy expenditure. These findings suggest that plasma leptin concentrations may contribute to the regulation of energy expenditure in heart failure patients.

Interestingly, no relationship was found between plasma leptin concentrations and energy expenditure in healthy elderly controls. This finding suggests that plasma leptin concentrations do not contribute significantly to variation in daily energy expenditure in the healthy elderly. Relative to this point, we have shown that physical activity energy expenditure is the primary determinant of variation in daily energy expenditure in the healthy elderly[21] and also contributes to variation in resting energy expenditure.[22] Thus, factors such as the habitual physical activity level may be more important than plasma leptin concentrations in determining both daily and resting energy expenditure in healthy elderly individuals.

The stronger relationship of leptin to energy expenditure in heart failure patients may be explained by their reduced level of physical activity energy expenditure (56% less than controls). That is, the role of leptin as a determinant of energy expenditure in heart failure patients is unmasked because a large portion of the confounding influence of physical activity energy expenditure has been removed. The reduction in physical activity associated with heart failure may therefore allow for hormonal regulatory systems such as leptin to play a significant role in the regulation of daily and resting energy expenditure. Collectively, our findings suggest a tighter coupling of plasma leptin concentrations and energy expenditure in heart failure patients compared with healthy controls.

Because leptin is related to energy expenditure in heart failure patients, elevated plasma leptin concentrations could contribute to elevated levels of energy expenditure observed in this population.[12,13] However, no difference in plasma leptin concentration was found between heart failure patients and healthy controls after statistical control for differences in fat mass. Similar results were obtained when plasma leptin concentrations were examined in a subgroup of cachectic heart failure patients or when compared with a subgroup of non-obese healthy controls (<30 kg fat mass). Thus, our findings do not suggest that heart failure patients are characterized by abnormally elevated plasma leptin concentrations.

The absence of differences in plasma leptin concentrations between groups may be surprising, given the lower fat mass in heart failure patients and the positive linear relationship between the log of plasma leptin concentrations and fat mass. However, log-transformed data do not accurately reflect the true relationship between plasma leptin concentrations and fat mass. If this relationship is examined before log transformation, a curvilinear relationship is noted (Fig 2). In our data set of healthy subjects (N = 67), the slope between plasma leptin concentration and fat mass is less steep (0.15 ng/mL/kg) in individuals with less than 30 kg fat mass, but increases sharply thereafter (0.53 ng/mL/kg). Thus, plasma leptin concentrations

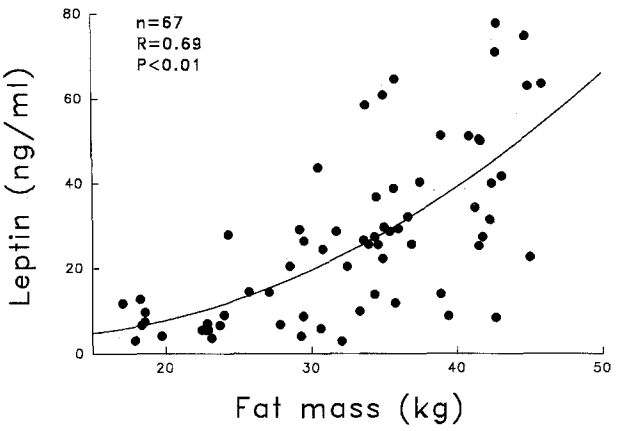

Fig 2. Relationship between plasma leptin concentrations and fat mass in 67 healthy adults ($r = .69$, $P < .01$; 22 men and 45 women aged 56 to 73 years).

change little per unit of fat mass in individuals below a threshold level of adiposity (~30 kg fat mass). A similar relationship was reported between plasma leptin concentration and percent body fat by Considine and et al.[9] Thus, the magnitude of group differences in fat mass noted in the present study is not likely to yield large differences in plasma leptin concentrations.

Several caveats associated with our results should be considered. First, studies that examine changes in plasma leptin concentrations and energy expenditure during the dynamic phase of weight loss are needed to further clarify leptin's role in the regulation of energy balance in heart failure patients. Second, leptin may contribute to weight loss in heart failure patients by decreasing energy intake. Indeed, a large portion of the weight-reducing effect of leptin in laboratory animals can be attributed to its anorexigenic effects.[4,7,8] Further studies are needed to examine the role of leptin in the regulation of food intake in heart failure patients.

In conclusion, we found that plasma leptin concentrations were related to energy expenditure variables in heart failure patients, but not in healthy controls. Thus, leptin may participate in the regulation of energy expenditure and body energy stores in heart failure patients.

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