Leptin, Body Composition, and Indices of Malnutrition in Patients on Dialysis

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Abstract. A cross-sectional study was performed in a group of dialysis patients and control subjects to identify the determinants of serum levels of leptin, the protein product of the obese (ob) gene. Twenty-eight patients on dialysis (19 patients on hemodialysis [HD] and nine patients on peritoneal dialysis [PD]) and 41 healthy control subjects were studied. For each subject, blood was drawn for measurement of serum leptin levels and body composition was analyzed by dual-energy x-ray absorptiometry. Regression analyses were performed to determine the predictors of leptin levels, the independent contribution of HD and PD, and the relationship between leptin levels and markers of malnutrition and protein intake in the patients on dialysis. As expected, percentage of body fat was strongly correlated with leptin levels in the group as a whole and in each subgroup when analyzed separately. However, the slope of the relationship was significantly greater for dialysis patients than for control subjects ($P < 0.05$). Multivariate regression analysis showed that patients on HD and PD had higher leptin levels than control subjects even after adjustment for age, gender, and percentage of body fat. Univariate analyses were performed to assess the relationship between leptin levels and markers of nutritional status such as albumin, blood urea nitrogen, protein catabolic rate (PCR), transferrin, cholesterol, and lean body mass per height. There was a significant negative correlation between leptin levels and serum albumin ($r = -0.598$, $P < 0.001$) and between leptin and PCR ($r = -0.433$, $P < 0.05$) in the patients on dialysis. It is concluded that leptin levels adjusted for percentage of body fat are increased in dialysis patients compared with control subjects, particularly in those on PD. In addition, increased leptin levels are associated with low serum albumin levels and PCR in dialysis patients. (J Am Soc Nephrol 9: 1080–1084, 1998)

Anorexia is a common complication of uremia that usually worsens as renal failure progresses and improves slightly with the institution of renal replacement therapy (1). Despite this improvement, many maintenance dialysis patients do not consume enough protein or calories to maintain neutral nitrogen balance (2). Consequently, malnutrition and reduced lean body mass occur frequently in dialysis patients (3–5). Markers of malnutrition such as low serum albumin, protein catabolic rate (PCR), blood urea nitrogen (BUN), and creatinine are associated with higher morbidity and mortality in this population (6–8). The causes of the anorexia that accompanies renal failure are not completely understood, but many investigators suspect that accumulation of uremic toxins is an important factor. Candidate uremic toxins include phosphorus, parathyroid hormone, metabolic acids, and additional unknown dialyzable substances (4,8).

The recent discovery of leptin, the product of the obesity gene in mice (9), suggests another possible mediator of the anorexia of renal failure. Leptin is a 16-kD protein that is produced by adipocytes (10–14). In humans, serum leptin levels are highly correlated with measures of body fat content, and leptin is thought to be important in the control of appetite and metabolic rate (10,13–15). It has been proposed that leptin acts as a marker of fat stores under steady-state conditions but also increases or decreases with perturbations in energy balance. Thus, individuals have a set-point that is determined by their fat stores, but changes from that set-point may still occur and contribute to the regulation of metabolism and appetite (14,16). Leptin is primarily cleared from the circulation by the kidneys in rats (17). In mice, administration of leptin leads to anorexia and increased energy expenditure (13,18,19), both of which occur in dialysis patients (20). Leptin levels are significantly increased in dialysis patients (21–23) compared with control subjects, a difference that persists even after correction for body mass index (BMI) (22,23). However, no study has examined the relationship between leptin and directly measured body fat content in dialysis patients, nor have any of the reports thus far included patients on peritoneal dialysis or examined the relationship between leptin and markers of nutritional status in dialysis patients.

We hypothesized that increased leptin levels in dialysis patients might be associated with the malnutrition that is common in this population. The aims of the current investigation were to determine whether serum leptin levels are higher in dialysis patients when corrected for body fat as measured by dual-energy x-ray absorptiometry (DEXA), to investigate

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whether leptin levels are different in patients on hemodialysis (HD) or peritoneal dialysis (PD), and to evaluate whether leptin levels correlate with indices of malnutrition in dialysis patients.

Materials and Methods

Patients

Twenty-eight maintenance dialysis patients and 41 healthy control subjects were recruited for the study. The dialysis group included 19 patients on HD and nine patients on PD from the University of California Renal Center at San Francisco General Hospital. Patients were stable on dialysis for at least 6 mo before study enrollment. Patients on HD were dialyzed three times per week using high-flux polysulfone membranes (F-80, Fresenius, Bad Homburg, Germany) with a minimum Kt/V of 1.0. Patients on PD had a minimum daily Kt/V of 0.29. Patients were excluded if they were taking corticosteroids or if they had illnesses other than end-stage renal disease that could potentially cause anorexia or malnutrition, including infection with HIV, malignancy, or bacterial infections requiring intravenous antibiotics within 3 mo before enrollment.

Healthy subjects were recruited from the community. The protocol was approved by the Committee on Human Research at the University of California, San Francisco. All study procedures were performed in the General Clinical Research Center or the dialysis center at San Francisco General Hospital.

Study Protocol

Blood was drawn from patients on HD immediately before the first dialysis session of the week after at least 4 h of fasting. After clotting, serum was separated and stored frozen at −20°C until assayed for leptin levels. All samples were analyzed in a single batch. Immediately after dialysis on the same day, height and weight were measured while the patients were wearing only a hospital gown. Intraperitoneal fluid was drained from patients on PD before measurement of height, weight, and body composition.

Body composition was measured using a Lunar model DPX dual-energy x-ray absorptiometer (Madison, WI). This instrument scans with x-ray sources that produce dual-energy photon beams. The ratio of the mass attenuation coefficients of the two photon energies varies in a linear manner with the proportions of lean and fat in soft tissue, and the weights of fat and lean tissue are calculated from algebraic equations using the known attenuation ratios for fat and lean.

Patients on dialysis had additional blood tests to measure serum albumin, BUN, creatinine, transferrin, and cholesterol, and to calculate PCR. PCR is an indirect measure of protein intake in dialysis patients under steady-state conditions and is calculated from dialysis urea removal and serum urea levels.

Control subjects and patients on PD had blood drawn to measure serum leptin levels after an overnight fast. Body composition measurements were performed immediately afterward in the same manner as for the subjects on HD.

Assays

Serum leptin was measured using a human leptin RIA kit (Linco Research, St. Charles, MO) that has been characterized previously (24). The lower limit of detection of the assay is 0.5 ng/ml. Duplicate analyses were performed on all samples, and the average value was used for data analysis.

Statistical Analyses

Characteristics of the groups were compared by ANOVA with *post hoc* Scheffé tests. A *t* test was used to compare the slopes of the correlations between percentage of fat and leptin in patients on dialysis and control subjects. Multiple regression analysis was performed using the natural logarithm of leptin as the dependent variable because the relationship between percentage of body fat and leptin is not linear. Independent variables included age, gender, percentage of body fat by DEXA, and HD and PD status as indicator variables. Univariate correlations were performed using Spearman rank correlations, because leptin levels were not normally distributed. Computations were performed using STATISTICA software (StatSoft, Inc., Tulsa, OK).

Results

The characteristics of the study population are summarized in Table 1. Patients on HD were older and were more likely to be men than control subjects and those on PD. The PD group had significantly higher BMI than the control and HD groups, and patients on HD had less body fat than control subjects. Mean serum leptin concentration was significantly higher in the PD group than in either of the other two groups.

Figure 1 shows the relationship between the natural logarithm of leptin and percentage of body fat for patients on dialysis and control subjects and illustrates that the differences in body composition between groups do not explain the dif-

Table 1. Characteristics of study population[a]

Variable	Control	HD	PD
Age (yr)	36 ± 10	48 ± 12[b]	43 ± 15
Gender (M/F)	21/20	17/2[c]	5/4
Percentage of body fat[d]	26.4 ± 8.8	20.0 ± 9.7[b]	28.1 ± 9.9
BMI (kg/m^2)	23.5 ± 3.3	23.4 ± 3.1	30.9 ± 9.3[c]
LBM/height (g/m)	27.8 ± 5.6	29.2 ± 4.2	32.5 ± 5.0
Leptin (ng/ml)[e]	5.4; 1.1 to 19.3	3.5; 0.8 to 156	10.5; 3.3 to 266[c]

[a] HD, hemodialysis; PD, peritoneal dialysis; BMI, body mass index; LBM, lean body mass.
[b] $P < 0.05$, significant compared with control group.
[c] $P < 0.05$, significant compared with both other groups.
[d] Body fat and LBM were measured by dual-energy x-ray absorptiometry.
[e] Leptin levels are reported as median and range because the data are not normally distributed; other continuous variables are reported as mean ± SD.

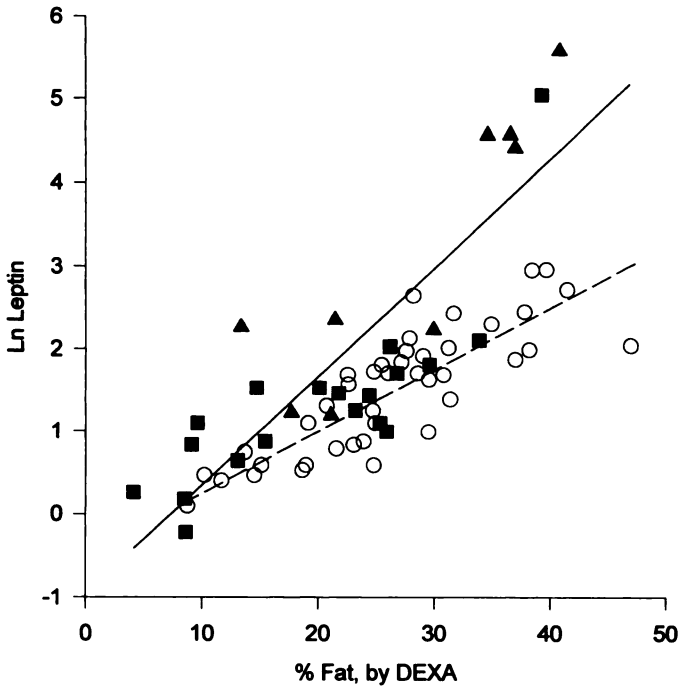

Figure 1. Relationship between percentage of body fat and leptin levels. Leptin is depicted as the natural logarithm of serum leptin, because the relationship between percentage of body fat and leptin level is not linear. Open circles, control subjects; filled squares, hemodialysis (HD) patients; filled triangles, peritoneal dialysis (PD) patients. The dashed line shows the regression for the control subjects, and the solid line is the regression for the dialysis patients. The slope of the regression for dialysis subjects is significantly greater than for control subjects (0.124 *versus* 0.072; $P < 0.05$).

ferences in leptin levels. Body fat was a strong predictor of leptin levels in both groups, but the slope of the relationship was significantly greater for dialysis patients than for control subjects ($P < 0.05$).

Multiple regression analysis was performed to assess the independent contribution of HD and PD to leptin levels while adjusting simultaneously for clinically significant variables. The results are shown in Table 2. The natural logarithm of the serum leptin level was used as the outcome variable in the model because the relationship between percentage of body fat and serum leptin level was not linear. Age, HD, PD, and percentage of body fat were significant predictors of leptin levels in this model. As shown in Table 2, each 1% increase in body fat was associated with a 9.3% increase in leptin level. After adjustment for the other variables in the model, HD status was associated with a 92% increase in leptin level, and PD was associated with a 400% increase. Two data points from different groups were identified as outliers, but when the analysis was repeated excluding these subjects, the results were unchanged with the exception that the relationship between age and leptin level was no longer statistically significant. Figure 2 is a plot of the leptin levels predicted by the model against the actual leptin levels observed. Overall, the model had an r^2 of 0.771, and the residuals were normally distributed. Thus, taken together, age, gender, percentage of body fat, and type of dialysis explained 77.1% of the variability in leptin levels in these subjects.

To determine whether leptin levels were associated with nutritional status in patients on dialysis, univariate analyses were performed between leptin levels and laboratory markers of nutritional status in these patients. Separate analyses were performed for patients on HD and for all patients on dialysis because of the possibility that dialysis modality could be a confounding factor. Results are summarized in Table 3. As in subjects with normal renal function, leptin levels were highly correlated with percentage of body fat in patients on dialysis. In addition, leptin levels were negatively correlated with serum albumin, the strongest nutritional predictor of morbidity and mortality in dialysis patients. There was a negative correlation between leptin levels and PCR that was statistically significant in the group of dialysis patients as a whole, although not in the patients on HD when considered alone. In addition, BUN, creatinine, and transferrin had negative correlations with leptin levels, although not all reached statistical significance. There was no association between cholesterol or lean body mass per height and leptin (not shown).

Discussion

The results of the present study confirm recent reports that leptin levels are significantly increased in dialysis patients compared to control subjects with normal renal function when corrected for body composition and extend these findings in

Table 2. Predictors of leptin levels

Variable	Regression Coefficient[a]	SEM	*P* Value	% Change in Leptin Level per Unit Change in Predictor Variable
Age	−0.014	0.007	0.043	−1%
Gender	0.172	0.187	0.363	19%
Percentage of body fat[b]	0.089	0.010	<0.001	9.3%
HD	0.652	0.190	0.001	92%
PD	1.618	0.214	<0.001	400%

[a] Dependent variable is natural logarithm of serum leptin concentration.
[b] Percentage of body fat was measured by dual-energy x-ray absorptiometry.

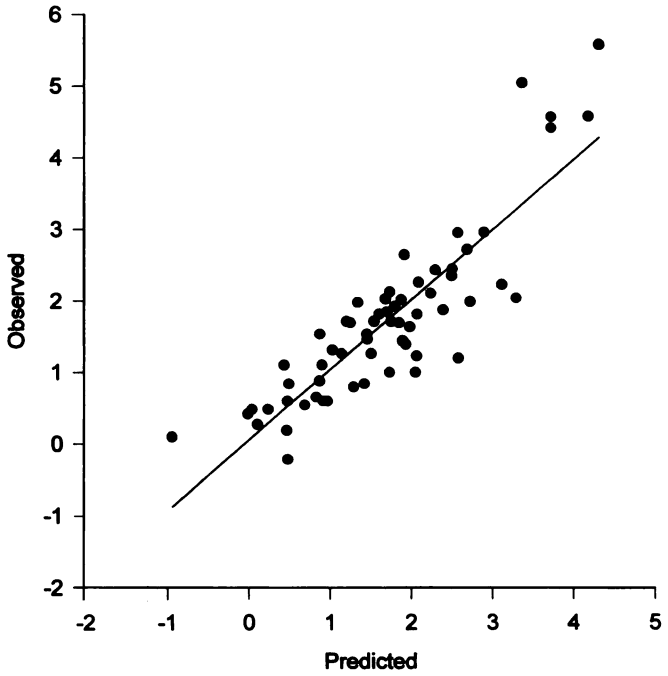

Figure 2. Predicted *versus* observed leptin levels. Leptin levels are expressed as the natural logarithm of serum leptin concentration. The predictor variables included in the model were age, gender, percentage of body fat by dual-energy x-ray absorptiometry, being on HD, and being on PD. Overall, the model had an r^2 of 0.771 and thus explained 77.1% of the variability of the natural logarithm of the serum leptin concentration.

several ways. In our analysis, leptin levels were adjusted for percentage of body fat as measured by DEXA rather than BMI. In previous analyses, BMI was used when comparing dialysis patients with control subjects (22,23). However, BMI does not measure body fat directly and may not be comparable in dialysis patients and healthy individuals because there is evidence that dialysis patients have decreased lean body mass compared with control subjects (5). Therefore, correcting for BMI alone does not rule out the possibility that increased leptin levels in dialysis patients are due to differences in body composition. DEXA has been shown to be highly accurate in measuring body fat content in healthy subjects and in dialysis patients (5,25–27), and the current results indicate that leptin levels are increased in dialysis patients compared with control subjects even when adjusted for percentage of body fat by DEXA.

Leptin levels are considerably higher in patients on PD than in patients on HD or subjects with normal renal function after correcting for other predictors of leptin levels, including percentage of body fat, age, and gender. There was no difference in residual renal function or time on dialysis between the patients on PD and HD in the study, so it seems unlikely that differing renal excretion rates account for the higher leptin levels in patients on PD. It is possible that high-flux HD more efficiently removes leptin than PD, but leptin levels do not decline after dialysis with modified cellulosic membranes (23). Another possibility is that patients on PD produce more leptin than those on HD. Patients on PD have higher glucose and insulin levels than patients on HD (28), and there are many reports that link hyperinsulinemia and insulin resistance with high leptin levels in humans (29–31). In a recent study, Segal *et al.* (29) showed that the increased leptin levels seen in insulin-resistant subjects are independent of body fat content. Perhaps the continual glucose absorption and increased insulin levels lead to increased leptin production in patients on PD.

To assess whether increased leptin levels are associated with anorexia or malnutrition in patients on HD, the correlation between leptin levels and laboratory markers of malnutrition, including serum albumin, PCR, BUN, creatinine, lean body mass per height, transferrin, and cholesterol, was examined. Low serum albumin is the strongest nutritional predictor of morbidity and mortality in dialysis patients, and serum albumin was negatively correlated with leptin levels in this study. PCR is an indirect measure of protein intake in dialysis patients in steady-state conditions that is based on urea generation. Low levels are associated with increased morbidity and mortality (6). PCR was negatively correlated with leptin levels when all dialysis patients were considered together, as was transferrin. These data suggest that high leptin levels in these patients are associated with poor nutritional status. However, it cannot be determined from this study whether increased leptin levels lead to decreased nutritional intake and poor nutritional status or whether decreased nutritional intake causes both the increased

Table 3. Univariate correlations between serum leptin levels and indices of nutritional status in HD patients[a]

Variable	HD Patients		All Dialysis Patients	
	r	*P* Value	*r*	*P* Value
Albumin	−0.557	0.013	−0.598	0.0008
PCR	−0.450	0.061	−0.433	0.039
BUN	−0.411	0.081	NA[b]	
Creatinine	−0.404	0.086	NA[b]	
Transferrin	−0.396	0.104	−0.433	0.0122
Percentage of body fat	0.848	<0.0001	0.816	<0.0001

[a] PCR, protein catabolic rate; BUN, blood urea nitrogen.
[b] Not analyzed because BUN and creatinine are not comparable between HD and PD patients.

leptin levels and the markers of poor nutritional status. Additional studies are needed to directly assess the relationship between leptin levels and nutritional intake in dialysis patients.

In summary, this study demonstrates that serum leptin levels are increased in dialysis patients compared with subjects with normal renal function and are significantly greater in patients on PD than patients on HD after adjustment for age, gender, and percentage of body fat. Leptin levels in dialysis patients are negatively correlated with albumin and PCR, suggesting a possible role of leptin in nutrition. Additional studies are needed to describe the relationship between insulin levels and leptin in dialysis patients, and to clarify the relationship between nutritional intake and leptin levels.

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