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The Relationship between Plasma Leptin and Nutritional Status in Chronic Hemodialysis Patients

Leptin serves an important role in suppressing appetite in mice and is known to be elevated in chronic renal failure (CRF) patients. But clinical significance of leptin as an appetite-reducing uremic toxin, remains to be determined. So we studied the relationship between plasma leptin and nutritional status in 46 chronic hemodialysis (HD) patients. Pre HD leptin was measured and divided by body mass index (BMI) to give adjusted leptin levels. KT/V_{urea} (K, dialyzer urea clearance; T, duration of HD; V, volume of distribution of urea), C-reactive protein (CRP), plasma insulin and nutritional parameters such as serum albumin, normalized protein catabolic rate (nPCR), subjective global assessment (SGA), BMI and mid-arm muscle circumference (MAMC) were also measured. Mean plasma leptin levels were 8.13±2.91 ng/mL (male 3.15±0.70; female 14.07±6.14, $p<0.05$). Adjusted leptin levels were positively correlated with nPCR (male r=0.47, $p<0.05$; female r=0.46, $p<0.05$), SGA (male r=0.43, $p<0.05$; female r=0.51, $p<0.05$) and MAMC (male r=0.60, $p<0.005$; female r=0.61, $p<0.05$). They did not correlate with KT/V_{urea}, serum albumin, hematocrit, bicarbonate, insulin and CRP. Presence of DM and erythropoietin therapy had no effect on leptin levels. These results suggest that leptin is a marker of good nutritional status rather than a cause of protein energy malnutrition in chronic HD patients.

Key Words: *Nutrition disorder; Hemodialysis*

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INTRODUCTION

Leptin, a 16-kDa protein synthesized by the obese gene and secreted by adipocytes (1, 2), is known to play an important role in suppressing appetite in mice (2). Administration of recombinant leptin to ob/ob mice, which have a genetic defect in leptin production, reduces food intake and increases energy expenditure (3-5). It has also been demonstrated that leptin levels in humans have a positive correlation with body fat indices and are elevated in obese patients (2, 6). It is postulated that as adipocytes increase in size with increased food intake, there is increased leptin binding to receptors in the hypothalamus that inhibits further food intake (7).

It is known that plasma leptin is partly cleared by the kidney and is elevated in chronic hemodialysis (HD) patients (8). Elevated leptin levels may then have an important influence on decreased appetite which is commonly observed in patients with end stage renal disease. As leptin receptor antagonists are likely to be developed for clinical use, it would be of obvious clinical importance if hyperleptinemia could be proven to contribute to uremic anorexia. To address this question, we studied the relationship between plasma leptin levels and nutritional status in chronic HD patients.

MATERIALS AND METHODS

Patients

In this cross-sectional study, 46 patients (25 males and 21 females; age, 46.0±1.7 years) with chronic HD (duration of HD, 38.9±5.3 months) were investigated. All patients were free of acute illness within the past three months, and none were receiving corticosteroids. Their body mass index (BMI), defined as dry weight in kilograms divided by the square of the height in meters, was 21.3±0.3 kg/m^2. The cause of chronic renal failure was diabetic nephropathy in 16 patients, chronic glomerulonephritis in 15 patients, hypertension in four patients, unknown in nine patients and miscellaneous in the remainder.

The patients received two or three 4-hr sessions of HD

per week using bicarbonate-buffered dialysate at the outpatient HD unit of Chunchon Sacred Heart Hospital (Chunchon, Korea). The dialysis membrane used in all these patients was composed of modified cellulose (Hemophane) with a surface area of 1.1 m^2. Twenty-three patients received recombinant human erythropoietin and most patients were on antihypertensive medication (ACE inhibitor, calcium-channel blockers, alpha and beta-blockers, vasodilator) as well as other drugs commonly used in chronic HD such as phosphate- and potassium-binders, and vitamin B, C, and D supplements.

Dialysis adequacy (KT/V_{urea}: K, dialyzer urea clearance, mL/min; T, duration of HD, min; V, volume of distribution of urea, mL) and normalized protein catabolic rate (nPCR) as a marker of protein intake were calculated by variable volume, single pool urea kinetic calculator obtained from a web site (HDCN, Hypertension Dialysis Clinical Nephrology, http://www.hdcn.com/) (9). The contribution of residual renal function was included in the calculation.

Biochemical analyses

Samples were nonfasting and collected in the morning (from 7 a.m. to 8 a.m.) immediately after initiation of HD. Circulating leptin is unaffected by food ingestion (10) and is not removed by modified cellulose membranes (8). Plasma was separated by centrifugation at 4℃ and kept frozen at -70℃ until leptin concentration was assayed.

Plasma leptin levels were determined by radioimmunoassay (Linco Research, St. Louis, Mo, U.S.A.; normal range, 1-7.8 ng/mL), using polyclonal antibody raised in rabbits against highly purified recombinant human leptin. Due to the effect that body fat has on plasma leptin levels, the data were reanalyzed after expressing leptin values per unit of BMI (adjusted leptin level). The assay limits of detection and linearity were 0.5 and 100 ng/mL in plasma. Leptin level >100 ng/mL was reassayed in dilution. The intra-assay coefficient of variation ranged from 3.4 to 8.3%, and interassay coefficients of variation ranged from 3.6 to 6.2%.

Plasma insulin and intact parathyroid hormone (PTH) levels were measured with specific radioimmunoassays. Urea, albumin, hematocrit, bicarbonate, C-reactive protein (CRP) were measured by standard techniques.

Subjective global assesment

Modified subjective global assesment (SGA) used in the Canada-USA Peritoneal Dialysis Study (11) were used to evaluate the overall protein-energy nutritional status. The SGA included four items (weight loss over past six months, anorexia, subcutaneous fat, and muscle mass) scored on a seven-point Likert scale. Scores of 1-2 represented severe malnutrition, 3-5 moderate to mild malnutrition, and 6-7 normal nutrition. SGA scores were determined by a clinician who was not part of the investigating team.

Anthropometric measurements

Skinfold thickness was measured with a Harpenden caliper at the triceps and subscapular sites in the fistula-free arm. Each measurement was repeated three times and the median value was recorded. The mid-arm muscle circumference (MAMC) was derived from the triceps skinfold thickness (TSF) and mid-arm circumference (MAC) as follows: $MAMC=MAC-(\pi \times TSF)$ (12).

Statistical analyses

Data analysis was performed using a statistical software program (SPSS for windows 7.5). All results were given as mean±SEM. A two-tailed p value less than 0.05 was considered statistically significant. The nonparametric Mann-Whitney U-test or unpaired t test was used to test differences between two groups as appropriate. Because of the extreme values in the distribution of plasma leptin, Spearman's correlations were used to assess the relationship between plasma leptin levels and other variables.

RESULTS

Mean values and gender differences for leptin levels and other variables are shown in Table 1. Female patients had significantly higher leptin levels compared with male patients.

Correlations of leptin levels with other variables are shown in Table 2 and Fig. 1. Marked influence of gender on leptin levels lead to the correlates of leptin levels being evaluated separately for male and female patients. Plasma leptin and adjusted leptin levels were positively correlated with nPCR, SGA score, TSF, subscapular skin fold thickness (SSF) and MAMC in both gender. They were not correlated with plasma KT/V_{urea}, insulin, serum albumin, hematocrit, bicarbonate, CRP and PTH. Presence of DM and erythropoietin therapy also had no influence on leptin levels (Data are not shown).

DISCUSSION

Protein-energy malnutrition is present in a large pro-

Table 1. Mean values and gender differences for leptin levels and other variables

Variables	Mean	Gender difference		Significance
		Male	Female	
Plasma leptin (ng/ml)	8.13±2.91	3.15±0.70	14.07±6.14	p=0.038*
Adjusted leptin	0.37±0.13	0.14±0.03	0.65±0.27	p=0.016*
Plasma insulin (mU/L)	21.7±1.9	23.2±3.0	19.9±2.3	NS
KT/V_{urea}	1.20±0.04	1.10±0.05	1.32±0.05	p=0.002
nPCR (g/kg/day)	1.16±0.04	1.18±0.06	1.13±0.05	NS
SGA score (1-7)	4.49±0.26	4.44±0.39	4.55±0.35	NS
TSF (mm)	10.0±0.6	9.8±0.8	10.3±0.9	NS
SSF (mm)	13.7±0.6	13.5±0.9	14.0±0.9	NS
MAMC (cm)	21.6±0.3	22.9±1.9	20.7±1.9	p=0.022
Albumin (g/L)	3.83±0.07	3.92±0.10	3.72±0.09	NS
Hematocrit (%)	25.6±0.6	27.0±0.9	23.9±0.7	p=0.007
Bicarbonate (mEq/L)	18.0±0.5	18.1±0.6	17.9±0.9	NS
CRP (mg/dl)	0.57±0.13	0.62±0.17	0.52±0.18	NS
PTH (pg/ml)	104.8±14.9	93.7±18.9	118.3±23.7	NS

*Mann-Whitney U-test. all others, Student's t test. Results are shown as mean±SEM

Table 2. Spearman's correlations (r) of leptin levels with other variables

Variables	Plasma leptin		Adjusted leptin	
	Male	Female	Male	Female
KT/V_{urea}/session	0.28	0.25	0.34	0.29
KT/V_{urea}/week	0.18	0.28	0.23	0.23
nPCR	0.46*	0.47*	0.47*	0.46*
SGA score	0.44*	0.53*	0.43*	0.51*
BMI	0.41*	0.62†	-	-
TSF	0.60†	0.71†	0.60†	0.72†
SSF	0.56†	0.65†	0.57†	0.73†
MAMC	0.64†	0.63†	0.60†	0.61*
Insulin	0.11	0.18	0.09	0.14
Albumin	0.18	0.11	0.14	0.09
Hematocrit	0.18	0.20	0.14	0.25
Bicarbonate	0.19	0.13	0.21	0.17
CRP	-0.18	-0.03	-0.15	-0.05
PTH	0.30	-0.22	0.31	-0.20

*p<0.05, †p<0.005

portion of patients with chronic HD (13, 14) and is associated with increased morbidity and mortality (15, 16). This may be a consequence of multiple factors, but reduced food intake due to anorexia is probably the most important. Since leptin is thought to be an appetite inhibitor, it has been suggested that elevated leptin in end stage renal disease may contribute to anorexia and malnutrition in patients with chronic HD (8, 17, 18).

But in the present study, we found no evidence that leptin is a mediator of malnutrition in chronic HD patients. On the contrary, both nPCR, a marker of protein intake and SGA score, a well-proven and reliable parameter for good nutritional status, (19) positively correlated with plasma leptin and adjusted leptin levels in both gender. Among anthropometric data, the parameters for fat mass (TSF, SSF) positively correlated with leptin levels as in healthy subjects, and the parameter for musculature (MAMC) also positively correlated with leptin levels in both gender. These results suggest that leptin is a marker of good nutritional status rather than an appetite-reducing uremic toxin in chronic HD patients. In fact, in the previous studies, the results have been conflicting as some indirect evidence from cross-sectional studies (17, 18) suggests that leptin may mediate anorexia in uremic patients, whereas others (20, 21) have not been able to find any proof of leptin being a mediator of anorexia in chronic renal failure. The reasons for the discrepant result are not clear. However in studies which suggest leptin as a anorectic uremic toxin, the number of patients on HD is relatively small (19 and 22 patients) (17, 18) as compared with the present study and the effect of uremic inflammatory state on leptin level and protein metabolism is not considered, which make it difficult to judge the biologic importance of leptin as a appetite-reducing uremic toxin.

In the present study, leptin levels were also not so high as compared with leptin levels of other studies (8, 22) which demonstrated 2- to 4-fold elevation in chronic HD patients compared with healthy controls. However, elevated leptin level is not a universal finding in advanced CRF (17, 23), and these results might be due to relatively lower fat mass (BMI, 21.3±0.3 kg/m^2) of our patients compared to Caucasian patients in other studies (BMI, 24.3-24.7 kg/m^2) (8, 22).

There are data (24, 25) suggesting that hyperinsulinemia may affect plasma leptin levels in CRF. But in the present study, leptin levels did not correlate with insulin

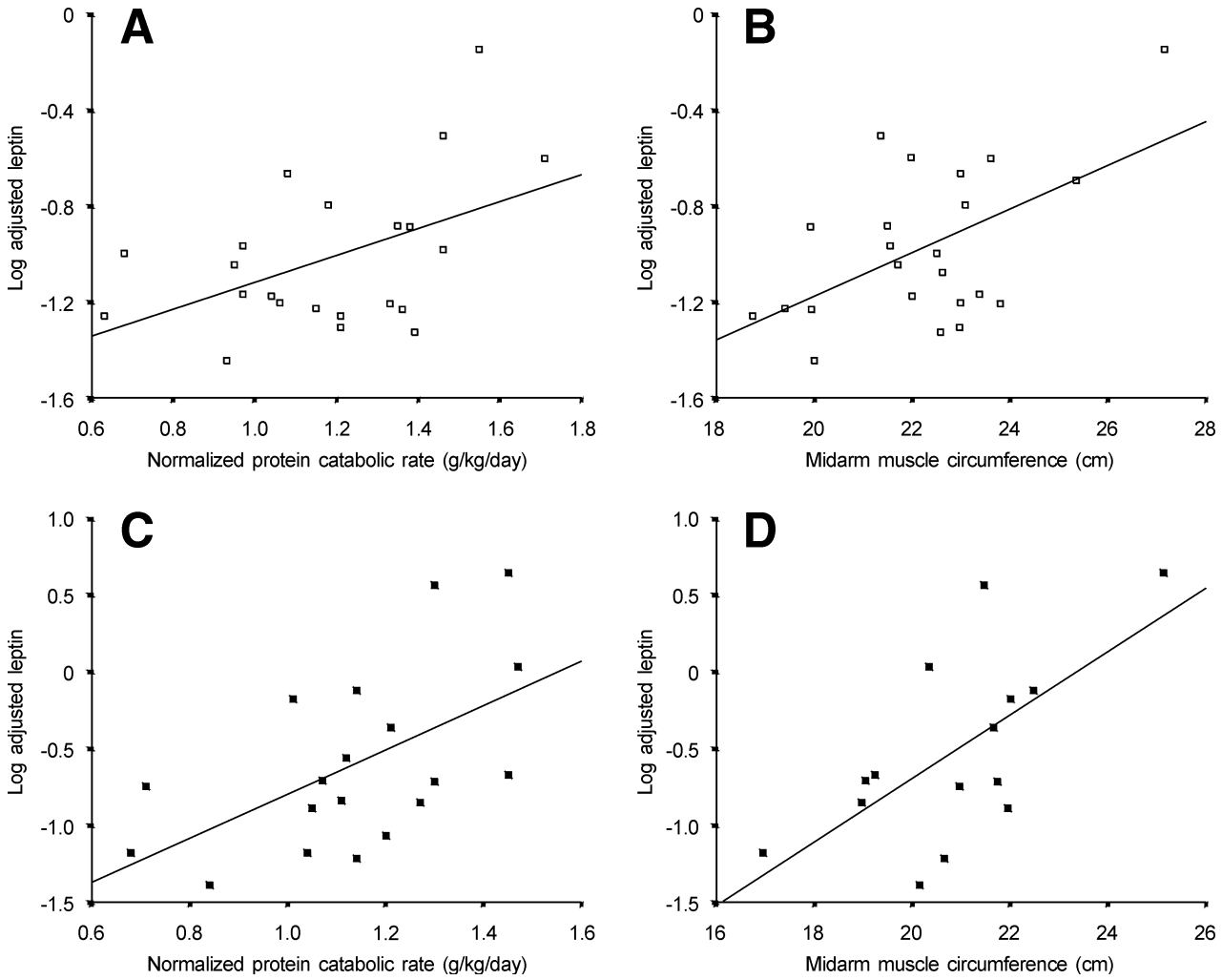

Fig. 1. Correlations of adjusted leptin (plasma leptin/body mass index) levels with normalized protein catabolic rate (nPCR) and mid-arm muscle circumference (MAMC) in male patients (upper panel, open squares) and female patients (lower panel, closed squares). Adjusted leptin levels are log-transformed because the relationship of adjusted leptin levels with nPCR and MAMC are not linear. Pearson's correlation coefficients were: A, r=0.48 ($p<0.05$); B, r=0.54 ($p<0.01$); C, r=0.56 ($p<0.05$); D, r=0.65 ($p<0.01$). Data were missing for some patients.

levels. We think that any fasting relationship of insulin with leptin might be lost because insulin values are not fasting in our study. Chronic inflammation is another possible reason for elevated leptin level in CRF (22). It has been demonstrated that cytokines induce both an increase in leptin mRNA concentrations and anorexia in animals (26). Because cytokines are known to induce catabolism of endogenous protein (27), nPCR may not reflect the real dietary protein intake of the patients, and positive correlations between leptin levels and nPCR could be due to a chronic inflammatory state in CRF. But in the present study, leptin levels did not correlate with CRP level, a marker of ongoing inflammatory process, and all patients were clinically stable without acute illness, with none receiving corticosteroids.

For leptin to suppress appetite in chronic HD patients, we have to assume that leptin is present mostly in its free bioactive form and that there is a normal transport of leptin across the uremic blood-brain barrier. Within the hypothalamus, leptin binds to its receptor and decreases production of neuropeptide Y. A reduction in neuropeptide Y leads to a decrease in appetite and increase in energy expenditure (28). So there are three possible explanations for the apparent resistance to anorectic effect of leptin in chronic HD patients. First, it may be possible that elevated leptin levels are due to an excess of bound form and degradation products of leptin rather than free bioactive form. But recent studies (8, 21) have shown that circulating leptin in chronic HD patients primarily existed in the free bioactive form and was of the same molecular weight as intact leptin. Second, a decreased efficiency in transportation of leptin across the

blood-brain barrier as in nonuremic obese subjects (29) may result in an apparent leptin resistance in CRF patients. Finally, CRF patients may have defects in leptin receptor or post receptor signal transduction pathways, even though no deleterious mutations in the human leptin receptor have yet been identified (30).

In summary, the present study has demonstrated positive correlations of leptin levels with nPCR, SGA score and parameter for musculature as well as parameters for fat mass in chronic HD patients. These results suggest that leptin is a marker of good nutritional status rather than a cause of protein energy malnutrition in chronic HD patients. Further, confirmatory studies are needed to clarify the exact pathophysiological significance of leptin in uremic patients.

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