Atherosclerosis is an important cause of morbidity and mortality in peritoneal dialysis (PD) patients. Oxidative stress plays a role in the pathogenesis of uremic atherosclerosis. Although antioxidant substances (vitamins A and E) are elevated in the plasma of dialysis patients, intracellular and clinical signs of hypovitaminosis are frequently found. Recently, the importance of vitamin/carrier complexes as a marker of vitamin bioavailability has been demonstrated. In the present study, we analyzed vitamin A and E bioavailability, measured as vitamin/carrier complexes, and the relationship of those measurements with clinical atherosclerosis status in PD patients.

We studied 45 patients (15 men, 30 women), who were divided into four groups according to clinical atherosclerotic score (CAS). Five cases were scored as CAS grade 1 (low CAS); 9 as CAS-2; 18 as CAS-3; and 13 as CAS-4. Vitamins A and E and their carriers (prealbumin and retinol binding protein (vitamin A), and cholesterol and triglycerides (vitamin E)) were determined.

Plasma levels of vitamin A were low in 5 patients, normal in 7 patients, and high in 33 patients. By correcting the values for the carrier levels, we created three groups: 24 patients showed low vitamin A/carryer complex (5 from the low plasma vitamin A group, 6 from the normal-value group, and 13 from the high-value group); 11 patients were in the group with normal vitamin A/carryer (1 from the normal plasma vitamin A group, and 10 from the high-value group); and 10 patients were in the group with high vitamin A/carryer. The vitamin A/carryer complex showed a statistically significant, negative linear correlation with CAS and with serum iron.

Low vitamin E plasma levels were found in 1 patient, normal levels in 28 patients, and high levels in 16 patients. When those values were corrected using the carrier values, three groups were also created. The group with low vitamin E/carryer complex contained 24 patients (1 from the low-value group, 22 from the normal-value group, and 1 from the high-value group). The group with normal vitamin E/carryer complex contained 21 patients (15 from the group with high vitamin E values, and 6 from the normal-value group). By univariate logistic regression analysis, significant associations between CAS and vitamin E plasma levels, vitamin E/carryer, age, and serum albumin were found. In the multiple logistic regression analysis, we confirmed that vitamin E/carryer complex, age, and serum albumin showed independent associations with CAS, but not with vitamin-only plasma levels.

Our results in PD patients show a vitamin/carryer complex disorder that results in elevated vitamin mobilization from pool and target cells. Our results and the findings of other researchers about intracellular vitamin A and E deficiencies may change the traditional concept of hypervitaminosis A and E in uremic patients.

Key words
Vitamin A, vitamin E, vitamin/carryer complex, antioxidants, atherosclerosis

Introduction
Reactive oxygen species are factors commonly implicated in the development of tissue injury in renal disorders and in dialysis patients. Antioxidant vitamins decrease tissue damage by trapping organic free radicals and inhibiting lipid peroxidation. Neverthe-
less, supplementation therapy with vitamin A and E is not recommended in such patients, owing to increased serum vitamin levels.

Vitamin A and E deficiencies have been implicated in premature development of atherosclerosis (1). Accelerated atherosclerosis and cardiovascular (CV) complications are recognized as major survival-determining factors for long-term maintenance dialysis patients (2,3). In peritoneal dialysis (PD) patients, the complexity of the situation is increased, because the atherosclerotic process includes specific factors such as hyperglycemia, hyperlipidemia, hyperinsulinemia, obesity, and hypoalbuminemia. In addition, signs of endothelial dysfunction are frequently found in uremic patients (3,4). The adoption of strategies to avoid endothelial injury are therefore relevant.

Increasing plasma levels of antioxidative agents should improve the hyperoxidative status of uremia. Nonetheless, disorders in vitamin A and E intracellular metabolism, bioavailability, membrane receptor, and vitamin/carrier complex have been suggested in uremic patients (1). For instance, plasma levels of vitamin A and its carrier are both increased with respect to skin content of vitamin A. In spite of the increased plasma levels, the vitamin A pool in the skin is not increased, and cutaneous lesions resembling the abnormalities found in hypovitaminosis A (xerosis) are present in uremia. It has therefore been suggested that, in uremia, an alteration occurs in cutaneous vitamin A receptor or in the vitamin A/carrier complex, with reduction of vitamin entry into cells (1,5).

A similar situation may arise with vitamin E status in dialysis patients, induced by an increase in the vitamin E carrier (cholesterol and triglycerides) level. Vitamin E is a scavenger, largely localized within membranes. Because of its high lipid solubility, it plays a special role with regard to the critical membrane lipid targets of free radicals (6). Uremic hyperlipidemia and antioxidant deficiency favoring oxidative stress is an added factor favoring development of atherosclerosis (7). In addition, recent research demonstrates the importance of measuring vitamin/carrier complex as a marker of vitamin bioavailability (8).

Our aim was to study vitamin A and E bioavailability, measured as vitamin/carrier complex, and the relationship of those measurements with clinical atherosclerosis status in PD patients.

**Patients and methods**

We studied 45 clinically stable PD patients. The group included 15 men and 30 women, ranging in age from 27 to 86 years (mean: 51.8 ± 13.9 years); 26 on continuous ambulatory peritoneal dialysis (CAPD), and 19 on automated peritoneal dialysis (APD) [14 on continuous cycling peritoneal dialysis (CCPD), and 5 on nightly peritoneal dialysis (NPD)]. The mean period on PD was 33.5 ± 37 months (range: 1—179 months). No acute or chronic active disorders were present during the 6 months prior to the study. The causes of renal failure were glomerulonephritis (10 cases), diabetes (8 cases), polycystic kidney disease (7 cases), chronic pyelonephritis (5 cases), nephrosclerosis (5 cases), unknown etiology (4 cases), systemic disease (presently inactive, 4 cases), and other causes (2 cases). Patients with cirrhosis, thyroid disease, and familiar dyslipidemias were excluded.

The patients were divided into four groups according to clinical atherosclerosis score (CAS) (9). Five cases were scored CAS grade 1 (lowest atherosclerotic status); 9 were scored CAS-2; 18 were scored CAS-3; and 13 were scored CAS-4.

Prior to starting PD, 32 patients were diagnosed with high blood pressure (HBP); however, only 28 had HBP when the present study was performed. Of the 28, 7 were using angiotensin-converting enzyme inhibitors; 4, calcium-channel blockers; 7, other drugs (α and β adrenergic blockers, direct vasodilators, and angiotensin II—receptor blockers); and 10, combinations of antihypertensive drugs. Lipid-lowering agents were withdrawn 15 days before the study. By echocardiogram, 38 patients showed left ventricular hypertrophy, 6 in severe grade. Low-dose aspirin was used by 8' patients; 36 patients were physically active; and 5’ patients were active smokers.

**Dialysis adequacy**

Dialysis adequacy was assessed by weekly urea Kt/V and normalized protein catabolic rate (nPCR).

**Antioxidants**

Antioxidant levels were determined in fasting conditions. Serum albumin was measured by the colorimetric method (Hitachi 704: Boehringer Mannheim, Mannheim, Germany); transferrin, ceruloplasmin, and vitamin A carriers [prealbumin and retinol binding protein (RBP)] by the immunonephelometric method.
Vitamin’A and E Deficiency and Atherosclerosis in PD Patients

(Behring Nephelometer Terminal S.A.: Behringwerte AG, Marburg, Germany); and bilirubin, glucose, and uric acid by Hitachi 704. Vitamin’A and E plasma levels were analyzed using high-performance liquid chromatography (HPLC). The HPLC instrument used was from Waters Associates, Inc. (Milford, MA, U.S.A.), and consisted of a 600E solvent delivery system, an automatic sample injector model 712 WISP, and a model 481 Lambda Max spectrophotometer programmable multi-wavelength detector injector with a Millennium data station. Vitamins were separated on a 3.9×150 mm resolve C18 column (Waters Associates) packed with 5 µ particles. The solvent was methanol:water (95:5) at a flow rate of 1.2 mL/min. Vitamins were detected at 290 nm. For the analysis, a 100 µL blood serum sample and 100 µL of an internal standard retinol acetate solution in ethanol (1 µg/mL) were mixed on a vortex mixer for 10 seconds. For lipid extraction, 200 µL of spectrograde hexane was added, and the contents were mixed for 60 seconds. Tubes were centrifuged (2000g for 5 minutes) to separate the phases, and then the solvents were transferred to another tube and evaluated in an automatic environment (Speed-Vac: Savant Instruments, Farmingdale, NY, U.S.A.). The lipids were dissolved in 200 µL methanol, and approximately 50 µL of the solution was injected into the chromatograph. Normal values in healthy populations are 8—21 µg/mL α-tocopherol (vitamin’E plasma levels) and 0.4—0.8 µg/mL retinol (vitamin’A plasma levels).

Vitamin’A carrier
Prealbumin and RBP were determined by an immunonephelometric method (BN-100: Behringwerke, Marburg, Germany). Reference values in healthy populations are 10—40 mg/dL for prealbumin and 3—6 mg/dL for RBP.

Vitamin’E carrier
Cholesterol was measured by the colorimetric method, and triglycerides by the Hitachi 704. Normal ranges are 150—250 mg/dL for cholesterol and 50—175 mg/dL for triglycerides.

Vitamin/carrier complexes
Vitamin’A/carry and vitamin’E/carry complexes (µg/mg) were calculated using these equations:

\[
\text{vitamin’A serum levels (µg/mL)} / [ \text{RBP (µg/mL)}] + 7.342
\]

vitamin’E serum levels (µg/mL) / [cholesterol (mg/mL) + triglycerides (mg/mL)]

Normal values for vitamin/carry complexes were obtained from a selected group of healthy individuals (without dyslipidemias): 30 women and 40 men, ranging in age from 25—50 years. The normal range for vitamin’A/carry complex was 0.8—1.2 µg/mg; for vitamin’E/carry complex, it was 6—16 µg/mg.

Statistical analysis
Comparisons between groups of data were performed using the Student t-test and the Mann—Whitney U-test. We also used the linear Spearman regression analysis. A p-value less than 0.05 was considered statistically significant. A multivariate analysis (logistic regression) was performed to analyze the association between the dependent variable (severity of CAS, high or low) and the independent variables, vitamin’E/carry (continuous), age (continuous), and serum albumin (continuous). All variables were analyzed individually against the dependent variable, and multiple regression analysis was then performed. For CAS and multivariate analysis, all patients were grouped as low score (CAS-1 and -2) or high score (CAS-3 and -4). Results are given as median and range.

Results
Table I shows the general analytical data.

Vitamin’A plasma levels were low in 5 patients, normal in 7 patients, and high in 33 patients. By correcting for carrier levels, we created three groups: 24 patients showed low vitamin’A/carry complex (5 from the low plasma vitamin’A group, 6 from the normal-value group, and 13 from the high-value group). The group with normal values of vitamin’A/carry contained 11 patients (1 from the normal-value vitamin’A group, and 10 from the high-value group). The group with normal values of vitamin’A/carry contained 11 patients (1 from the normal-value vitamin’A group, and 10 from the high-value group). Finally, 10 patients were in the group with high vitamin’A/carry levels, all from the high plasma value group.

The vitamin’A/carry complex showed a statistically significant, negative linear correlation with CAS \((r^2 = -0.36p < 0.05)\) and with serum iron \((r^2 = -0.34p < 0.05)\).

With respect to vitamin’E plasma levels, we found low values in 1 patient, normal values in 28 patients, and high values in 16 patients. When those values were corrected for the carrier values, three groups also
emerged. The group with low vitamin E/carrier complex contained 24 patients (1 from the low plasma level group, 22 from the normal-value group, and 1 from the high-value group). The group with normal vitamin E/carrier complex had 21 patients (15 from the group with high vitamin E plasma levels, and 6 from the normal-value group). No patient showed high vitamin E/carrier complex values. Vitamin E/carrier complex showed an inverse correlation with CAS ($r^2 = -0.49$; $p < 0.01$) and with age ($r^2 = -0.34$; $p < 0.05$). Women showed significantly higher vitamin E plasma levels than did men ($6.9 \pm 3.2 \mu g/mL (n = 30)$ vs. $5.5 \pm 1.6 \mu g/mL (n = 6)$, $p < 0.05$).

Vitamin A and E plasma levels showed significant linear correlation with other antioxidant substances. Vitamin A was correlated with transferrin ($r^2 = 0.36$, $p < 0.05$), ceruloplasmin ($r^2 = 0.3$, $p < 0.05$), uric acid ($r^2 = 0.4$, $p < 0.01$), total bilirubin ($r^2 = 0.35$, $p < 0.05$), glucose ($r^2 = 0.38$, $p < 0.05$), vitamin E ($r^2 = 0.36$, $p < 0.05$), vitamin E/carrier ($r^2 = 0.74$, $p < 0.01$), and albumin ($r^2 = 0.28$, $p = 0.054$ (NS)). Vitamin E was correlated with transferrin ($r^2 = 0.4$, $p < 0.05$), ceruloplasmin ($r^2 = 0.38$, $p < 0.05$), uric acid ($r^2 = 0.38$, $p < 0.05$), and albumin ($r^2 = 0.33$, $p < 0.05$).

Using multivariate analysis, no significant associations were found between plasma levels of vitamin A or vitamin A/carrier complex and the remaining variables studied.

By univariate logistic regression analysis, significant associations were found between CAS and vitamin E plasma levels [odds ratio (OR): 0.86; 95% confidence interval (CI): 0.77 to 0.95; $p = 0.04$], vitamin E/carrier (OR: 0.67; 95% CI: 0.5 to 0.9; $p = 0.01$), age (OR: 1.13; 95% CI: 1.04 to 1.23; $p < 0.05$), and serum albumin (OR: 0.086; 95% CI: 0.013 to 0.54; $p = 0.01$).

By multiple logistic regression analysis, we confirmed that vitamin E/carrier complex (OR: —0.63; 95% CI: 0.52 to 0.86; $p = 0.04$), age (OR: 1.38; 95% CI: 1.14 to 1.59; $p = 0.04$), and serum albumin showed an independent association with CAS (OR: —0.4; 95% CI: 0.018 to 0.82; $p = 0.01$).

Discussion

High plasma levels of vitamins A and E have traditionally been found in uremic patients (1). This apparent hypervitaminosis is caused by uremic retention (1,2). Evidence is increasing that, due to their antioxidant effects, those vitamins and vitamin C protect against a number of degenerative disorders, including CV disease, cataracts, and certain types of cancer (11,12).

Uremia is considered a pro-oxidative state (8), manifested in part by accelerated atherosclerosis, which determines survival in long-term maintenance dialysis patients (1,2). Oxidative stress is a complex process implicated in endothelial cell injury and dysfunction (3,4). Several longitudinal and cohort studies have examined the relationship between antioxidant intake and risk of CV events. Patients with angina pectoris showed lower plasma vitamin E concentrations than did normal subjects (13). Hypervitaminosis A in hemodialysis (HD) patients has been associated with anemia and hypercalcemia. Some cases of true vitamin A toxicity have also been reported in such patients (14). Because adequate vitamin A intake is covered by the renal diet, no additional supplementation is recommended; supplementation is, in fact, contraindicated. Nonetheless, clinical signs of vitamin A and E deficiency, with lower tissue levels, have been described in uremic patients (1,5,6). Vitamin/carrier complexes have recently been described as powerful
indicators of intracellular vitamin deficiency in children with cystic fibrosis (8).

Our results showed that 13 of 33 patients with elevated vitamin A plasma levels (39.4%) showed lower levels after correction for transport. In addition, 15 of 16 patients with high vitamin E plasma levels (93.7%) showed normal corrected values, and none showed high corrected values. Those data confirm that dialysis patients with normal or high vitamin plasma levels may have a real deficiency, as demonstrated by measurement of the carrier complex. That situation could increase the oxidative stress that is frequently elevated in uremic patients. Our findings also support that hypothesis, because CAS was negatively associated with vitamin/carrier complex levels, reinforcing the importance of determining the vitamin/carrier complex rather than the vitamin plasma levels.

In uremic patients, the increase in vitamin/carrier levels could induce a higher mobilization of the intracellular vitamin pool, with subsequent poorer vitamin utilization by target cells, as has been demonstrated in children with cystic fibrosis (8). Supporting our hypothesis of lower bioavailability of vitamins A and E in uremic patients owing to high carrier mobilization are the lower concentrations of those vitamins in mucosal and membrane cells (for example, red cells) (1,2,5,6), and the recognized oxidative stress suffered by uremic individuals (3,7). Because abnormalities in cutaneous vitamin A receptors have been hypothesized, but not identified (1), we propose that dialysis patients may suffer high vitamin mobilization, with subsequent pool and target-cell depletion.

By multivariate logistic regression analysis, we found that vitamin E/carrier complex, age, and serum albumin are associated, as independent factors, with atherosclerosis (CAS). Age, hypoalbuminemia, hypertension, and diabetes are recognized risk factors for increased oxidative stress (12).

Finally, to improve the real vitamin deficiency, it may be advisable to use oral supplements to increase vitamin A and E plasma levels and achieve normal vitamin/carrier complex levels. Although many reports exist of hepatotoxicity associated with high doses of vitamin A supplement (15), we believe, on the basis of the Peters and Kelly study (8), that, in maintaining a normal range of vitamin/carrier complex levels, hepatic toxicity is improbable. Moreover, if we intend to improve the nutritional status of dialysis patients with a subsequent increase in prealbumin, RBP, and other proteins (given the strong relationship between vitamins and other antioxidant substances), it would be necessary to give vitamin supplements to maintain adequate levels of vitamin/carrier complex. In addition, malnutrition may modulate the reactive oxygen species that mediate cellular damage through an inability to provide adequate defense if endogenous antioxidants are depleted (16,17). It is also important to control vitamin carrier levels, especially plasma lipids, because hyperlipidemia is present in 70% of dialysis patients (1,2). Moreover, hypercholesterolemia per se increases oxidative stress (12), providing another argument for maintaining an adequately controlled lipid profile in PD patients.

Conclusions
Our findings in PD patients suggest intracellular vitamin A and E deficiencies, in accordance with measurements of vitamin/carrier complex levels. Elevated vitamin mobilization from pool and target cells is indicated, with risk of subsequent inadequate bioavailability. The determination of vitamin/carrier complexes appears to be more important than that of vitamin plasma levels alone. Vitamin supplementation to achieve adequate vitamin/carrier complex levels may be advisable, especially in hyperlipidemic or malnourished patients.

Our results, and the intracellular vitamin A and E deficiencies described by others, may change the traditional concept of hypervitaminosis A and E status in uremic patients.

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4 Nakayama M, Yamada K, Yamamoto Y, et al. Vascu-

Corresponding author:
M. Auxiliadora Bajo, MD, PhD, Servicio de Nefrología, Hospital Universitario La Paz, Pº Castellana nº 261, Madrid 28046 Spain.
Anorexia and protein malnutrition, occasionally associated with obesity, are frequently observed in peritoneal dialysis (PD) patients. Both are recognized risk factors for cardiovascular (CV) morbidity and mortality. Leptin is produced by adipocytes and regulates body-fat mass through a satiety central effect. Leptin accumulates in the uremic state. We analyzed the relationship between plasma leptin levels, nutritional status, obesity, CV risk factors, and atherosclerosis in PD patients.

Leptin was determined using a polyclonal antibody [radioimmunoassay: Linco Research, St. Louis, MO, U.S.A.]. The normal range was 1—7.8 ng/mL. We studied 38 PD patients. Mean leptin levels were 59.1 ± 57.5 ng/mL (elevated in 32 patients). Women (n = 21) showed higher leptin levels than did men (80.4 ± 60.0 ng/mL vs. 32.3 ± 43.3 ng/mL, p < 0.01), in spite of both groups having a similar body mass index (BMI). A statistically significant direct correlation was found between leptin and BMI (r = 0.7, p < 0.01) and triceps skin-fold measurement (r = 0.77, p < 0.01). Leptin levels and renal creatinine clearance (CCr) showed no significant correlation. Independent of BMI, higher leptin levels were associated with parameters considered to be CV risk factors (Framingham study), such as serum triglycerides < 150 mg/dL (n = 29) as compared with > 150 mg/dL (44.2 ± 53.2 ng/mL vs. 80 ± 58.4 ng/mL, p < 0.05), cholesterol < 250 mg/dL (n = 28) as compared with > 250 mg/dL (50 ± 55.6 mg/dL vs. 84.7 ± 57.7 mg/dL, p < 0.05), uric acid < 7 mg/dL (n = 28) as compared with > 7 mg/dL (47 ± 53.7 mg/dL vs. 93.1 ± 36.6 mg/dL, p < 0.05), and the presence or lack of presence of left ventricular hypertrophy [68.8 ± 60 (n = 30) vs. 29.5 ± 23.7 (n = 5), p < 0.05].

The patients were classified into two groups according to a clinical atherosclerosis score (CAS). Nineteen patients had low CAS scores, and they showed higher plasma leptin values than did the other patients (82.4 ± 65.7 ng/mL vs 35.8 ± 36.6 ng/mL, p < 0.05). Twelve patients with anorexia had lower leptin values than did patients with normal appetite (19.2 ± 15.8 ng/mL vs. 91.3 ± 58.8 ng/mL, p < 0.001). In non obese patients (BMI < 25 and CCr > 3 mL/min, n = 14), leptin had a statistically significant direct linear correlation with markers of nutrition, including albumin (r = 0.63, p < 0.05), transferrin (r = 0.4, p < 0.05), cholesterol (r = 0.65, p < 0.05), and triglycerides (r = 0.6, p < 0.05). Finally, plasma leptin levels were notably increased in the PD population, indicating increased production (possibly by chronic hyperinsulinism), or uremic retention, or both. By multivariate analysis, we confirmed the association between leptin levels and sex, leptin and BMI, and leptin levels > 40 ng/mL and sex and LVH.

All of those features suggest that plasma leptin levels could be considered a marker of CV risk and food intake in non obese PD patients without inflammation.

Key words
Leptin, nutrition, cardiovascular risk factors

Introduction
Leptin, a hormone produced by adipocytes (1), regulates body-fat mass through a central satiety effect via neuropeptide Y (NPY) (2). The hormone appears to be cleared by the kidneys, and data on plasma levels are now available for chronic renal failure and for hemodialysis and peritoneal dialysis (PD) patients (3).

Some have speculated that leptin accumulation due to renal failure may be involved in the pathogenesis of uremic anorexia and, in consequence, poor nutritional intake (4,5). Protein malnutrition,
sometimes associated with obesity, is often observed in PD patients (4). Inadequate protein intake and high peritoneal glucose absorption have been involved in the genesis of those nutritional deficits (4,5). Protein malnutrition and obesity (5) are both recognized risk factors for cardiovascular (CV) morbidity and mortality. Obesity is a poorly studied problem in PD patients and is usually associated with insulin resistance, dyslipidemia, CV disease, hypertension, left ventricular hypertrophy (LVH), and diabetes mellitus type 2 (6). Hyperinsulinemia has been implicated as a primary cause of stimulation of obesity, increased sympathetic nerve activity, and hypertension (7). Obesity and hyperinsulinemia are major stimulators of leptin production, which is positively correlated with fat-mass index (8). Visceral fat mass is associated with early CV morbidity and mortality (6).

Recently, leptin action in the regulation of fat distribution has been demonstrated (9). Interestingly, Dunbar et al (10) have demonstrated that leptin injection induces hypertension through renal sympathetic stimulation. We evaluate here the relationship between plasma leptin levels, CV risk factors, atherosclerosis, and nutritional status in PD patients.

Patients and methods
Thirty-eight clinically stable PD patients [32 on continuous ambulatory peritoneal dialysis (CAPD) and 6 on automated peritoneal dialysis (APD)] participated in the study. They included 21 women and 17 men ranging in age from 26 to 85 years. The mean length of time on PD was 34.5 ± 29.2 months (range: 3—126 months). Causes of chronic renal failure were chronic glomerulonephritis (9 cases), pyelonephritis (8 cases), nephrosclerosis (7 cases), polycystic kidney disease (5 cases), systemic disease (3 cases), and unknown etiology (6 cases). Candidates taking thyroid hormones or steroids (including estrogens, contraception, or hormone replacement therapy) were excluded from the study. The presence of anorexia and gastrointestinal symptoms during the preceding months was analyzed. Anorexia was estimated by an interview that guided the patient through a survey of 3-day food intake. Six patients presented anorexia, and 6 patients had anorexia with dyspepsia, nausea, or epigastric pain. Isolated dyspepsia was present in 3 patients, and epigastric pain in 2 patients. Twenty-one patients were asymptomatic.

We used a trans-thoracic 2-D Doppler echocardiogram (HP Sonos’1500, 2.25-MHz transducer: Hewlett-Packard, Andover, MA, U.S.A.) to determine LVH [defined as an indexed left ventricular mass (ILVM) greater than 134 g/m² in men and 110 g/m² in women]. Severe LVH was defined as an ILVM greater than 175 g/m² in men and 150 g/m² in women (Penn convention).

Severe LVH was present in 5 patients; medium-to-mild LVH was present in 25 patients; no LVH was present in 5 patients; and, for 3 patients, no data were available. Hypertension was defined as blood pressure > 135/85 mmHg (2 patients), or a need for antihypertensive drugs (28 patients). Our patients were also classified according to a clinical atherosclerosis score (CAS) (11). For purposes of multivariate analysis, all patients were grouped into two scores: low CAS (CAS-1 and -2) and high CAS (CAS-3 and -4). We also measured certain CV risk parameters (cholesterol, triglycerides, and uric acid). In accord with Framingham and other longitudinal studies, the values considered to be CV risk levels were 250 mg/dL for cholesterol, 150 mg/dL for triglycerides, and 7 mg/dL for uric acid (12,13).

Obesity was considered to be present when body mass index (BMI) [weight (kg)/height (m²)] was greater than 25. Obesity grade I was between 25 and 30; obesity grade II, between 30 and 40; and obesity grade III, greater than 40 (14). Extracellular volume status was evaluated by physical examination, blood pressure, peritoneal fluid balances and weight daily follow-up, and anthropometrical assessment [triceps skin fold (TSF) and mid-arm muscle circumference (MAMC)].

Leptin measurement
We used a polyclonal antibody raised in rabbits against highly purified recombinant human leptin (radioimmunoassay: Linco Research, St. Louis, MO, U.S.A.). At the sample size, the sensitivity limit was 0.5 ng/mL, and the linearity was 100 µg/L. Intra- and inter-assay variation was determined on 5 human serum samples containing varying leptin concentrations, yielding variations of 7.2 ± 0.4 ng/mL (intra-assay) and 25.6 ± 0.9 ng/mL (inter-assay). The intra-assay coefficient was 4.8%, and inter-assay coefficient was 3.5%. Leptin normal range among 115 healthy subjects (61 men and 54 women, aged 15—57 years, BMI 25.4 ± 3.2) was 1—7.8 ng/mL.
Statistical analysis

Linear and Spearman regression analysis were performed. The Student t-test for paired and non-paired data and the nonparametric comparisons test (Mann—Whitney) were also performed. A p' value less than 0.05 was considered statistically significant. Results are expressed as mean±standard deviation throughout the paper.

Univariate and multivariate logistic regression analyses were also performed. The dependent variable (leptin level lower and higher than 40 ng/mL) was analyzed as a dichotomous entry. The independent continuous variables were serum cholesterol, triglycerides, albumin, uric acid, BMI, creatinine clearance (CCr), Kt/V, and age. The other independent variables analyzed were LVH (yes/no), sex (male/female), and CAS (CAS-1 and -2, CAS-3 and -4).

Results

At baseline, our patients showed these results: hemoglobin, 11.7±1.5 g/dL; lymphocyte count, 1764.2±1124 cells/mm³; creatinine, 10.3±2.77 mg/dL; urea, 154.3±42.6 mg/dL; total body protein, 6.8±0.52 g/dL; albumin, 4.04±0.34 mg/dL; transferrin, 253.4±50 mg/dL; cholesterol, 206.6±47.8 mg/dL; triglycerides, 151.2±68.1 mg/dL; ferritin, 352.2±360.6 ng/mL; BMI, 27.2±6.4; TSF, 18.6±10.2 cm; MAMC, 23.47±5.4 cm; leptin, 59.1±57.5 ng/mL; normalized protein catabolic rate (nPCR), 1.02 g/kg daily; and weekly urea Kt/V, 2.1±0.37.

Plasma leptin levels were elevated (>7.8 ng/mL) in 32 patients (84.2%). Values between 7.9 ng/mL and 100 ng/mL were seen in 22 patients; values between 100 ng/mL and 200 ng/mL were seen in 8 patients; and 2 patients showed levels higher than 201 ng/mL. Leptin levels were higher in women than in men [80.4±59.6 ng/mL (n=21) vs. 32.3±43.3 ng/mL (n=17), p<0.01]. The mean BMI did not differ between those groups [28±8 (21 women) vs. 26.3±3.8 (17 men), nonsignificant].

Leptin and renal function

A statistically significant, negative linear correlation was found between CCr and plasma leptin levels when those levels exceeded 13 ng/mL (n=28; r=-0.37, p<0.05). A even stronger correlation was found when plasma leptin levels exceeded 25 ng/mL (n=25; r=-0.5p<0.01). In both cases, we found no relationship between BMI and CCr (r=−0.2 and r=−0.29, both nonsignificant) or BMI and length of time on dialysis (r=−0.19 and r=−0.28, both nonsignificant). However, a significant linear correlation was found between length of time on dialysis and CCr (r=−0.41, p<0.01).

Leptin and BMI

According to the scale in a World Health Organization report (14), 8 patients had a below-normal BMI value (lower than 22.5); 7, a normal BMI (between 22.5 and 25); 14, grade I obesity (BMI between 25 and 30); and 9, grade II obesity (BMI over 30). Overall, a statistically significant direct linear correlation between plasma leptin and BMI appeared (n=38; r=0.7, p<0.01). Obese patients (BMI>25) showed the strongest correlation (r=0.72, p<0.01). In addition, higher plasma leptin levels were found in that group (79.5±57.1 ng/mL vs. 27.9±43.6 ng/mL, p<0.01). No difference in CCr was found between those two groups (1.7±2.09 mL/min vs. 1.06±1.73 mL/min, nonsignificant), nor was the time on PD significantly different (29.2±21 months vs. 42.7±37.9 months, nonsignificant). Furthermore, in all patients, actual dry weight (r=0.53, p<0.01), serum triglycerides (r=−0.36, p<0.05), and weight gained in the last year (n=24: 2.4±3 kg, r=0.44, p<0.05) showed significant linear correlations with leptin levels. Similarly, leptin and TSF and MAMC had significant direct correlations at r=0.77, p<0.01, and r=0.44, p<0.01, respectively.

Leptin and CV risk factors and CAS

Table I shows direct relationships between some CV risk markers and plasma leptin levels. Differences in those results (cholesterol and triglycerides) were similar among patients who received lipid-lowering agents, who had a lower CCr (<3 mL/min), and who were in the group that excluded patients with anorexia. Higher plasma leptin levels were found in patients with LVH, a relationship that could not be explained by differences in BMI (Table I), CCr (1.49±2.12 mL/min vs. 1.45±1.1 mL/min, nonsignificant) or length of time on PD (20.2±13.3 months vs. 39.5±30.5 months, nonsignificant).

Similarly, patients with hypertension showed high leptin values (Table I). Ten patients were scored as CAS-1; 9 as CAS-2; 11 as CAS-3; and 8 as CAS-4. Therefore, 19 were assigned to the low CAS group,
and 19 to the high CAS group. High plasma leptin levels were associated with a high CAS \(82.4 \pm 65.7 \text{ ng/mL vs. } 35.8 \pm 36.6 \text{ ng/mL, } p < 0.05\).

However, the BMI was also different \((29.7 \pm 7.6 \text{ vs. } 24.6 \pm 3.8, p < 0.05)\).

By univariate logistic analysis, significant associations were seen between leptin levels and BMI [odds ratio (OR): 1.36; 95% confidence interval (CI): 1.09 to 1.71; \(p < 0.009\)], sex (OR: 1.58; 95% CI: 1.15 to 2.15; \(p < 0.003\)), serum triglycerides (OR: 1.01; 95% CI: 1.0002 to 1.025; \(p = 0.05\)), and albumin (OR: 9.29; 95% CI: 1.004 to 86.5; \(p = 0.05\)). By multiple logistic regression analysis, we confirmed the association between leptin levels, sex (OR: 80.87; 95% CI: 4.005 to 1633.06; \(p = 0.007\)) and BMI [OR: 1.76; 95% CI: 1.19 to 2.59; \(p = 0.007\) (likelihood ratios model: 29.16; \(p < 0.0001\))], and leptin levels, sex (OR: 19.17; 95% CI: 3.29 to 111.64; \(p = 0.002\)) and LVH [OR: 14.87; 95% CI: 1.17 to 187.72; \(p = 0.04\) (likelihood ratios model: 17.15; \(p < 0.001\))].

**Leptin and nutritional status**

Leptin plasma levels showed significant, positive linear correlations with markers of nutrition in patients with BMI < 25 and CCr < 3 mL/min \(\cdot\) \(h\) = 14): albumin \((r = 0.63, p < 0.05)\), transferrin \((r = 0.4, p < 0.05)\), cholesterol \((r = 0.65, p < 0.05)\), and triglycerides \((r = 0.6, p < 0.05)\). Lymphocyte count, iron, and nPCR did not show linear correlations. Patients with BMI < 25 showed no correlations.

**Discussion**

Leptin is a 16-kDa protein that regulates body weight (1). Several receptors have been isolated from various tissues, including liver, kidney, and brain (2,3). Under experimental conditions, intraventricular leptin injection demonstrated high-affinity binding to receptors in hypothalamic tissue, where it inhibits hunger and subsequent food intake through NPY secretion (3).

Patients on PD show high plasma leptin levels with respect to normal subjects, as has been described for hemodialysis patients. Excess production and accumulation owing to lack of renal excretion have been suggested as possible causes (3).

Among our PD patients, leptin levels higher than 13 ng/mL were associated with lower residual renal function. The high plasma leptin value may repre-

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### Table I: Relationship between plasma leptin levels and cardiovascular risk factors

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Patients (n)</th>
<th>Value</th>
<th>Leptin (ng/mL)</th>
<th>p’ Value</th>
<th>BMI</th>
<th>p’ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid (mg/dL)</td>
<td>28</td>
<td>&lt;7</td>
<td>47±53.7</td>
<td>&lt;0.05</td>
<td>27.4±7.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>&gt;7</td>
<td>93.1±56.6</td>
<td>&lt;0.05</td>
<td>28.5±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>28</td>
<td>&lt;250</td>
<td>50±55.6</td>
<td>&lt;0.05</td>
<td>27.3±6.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>&gt;250</td>
<td>84.7±57.7</td>
<td>&lt;0.05</td>
<td>29.8±4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>29</td>
<td>&lt;150</td>
<td>44±12.3</td>
<td>&lt;0.05</td>
<td>27.4±7.6</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>&gt;150</td>
<td>80±58.4</td>
<td>&lt;0.05</td>
<td>28.5±4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (mmHg)</td>
<td>30</td>
<td>Yes</td>
<td>69.4±59.7</td>
<td>&lt;0.01</td>
<td>27.8±6.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>No</td>
<td>20±23.7</td>
<td>&lt;0.05</td>
<td>26.8±4</td>
<td>NS</td>
</tr>
<tr>
<td>LVH</td>
<td>30</td>
<td>Yes</td>
<td>68±8±60</td>
<td>&lt;0.05</td>
<td>28.3±6.7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>No</td>
<td>29.5±23.7</td>
<td>&lt;0.05</td>
<td>26±3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>15</td>
<td>&lt;4</td>
<td>44±8±51.7</td>
<td>NS</td>
<td>26.8±5.8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>&gt;4</td>
<td>48±4±60.3</td>
<td>NS</td>
<td>27.5±6.9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>25</td>
<td>&lt;25</td>
<td>35.6±38.3</td>
<td>&lt;0.01</td>
<td>28.5±7.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>&gt;25</td>
<td>104.3±62.5</td>
<td>&lt;0.01</td>
<td>28.5±7.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI = body mass index; NS = nonsignificant; LVH = left ventricular hypertrophy.
sent the correction in the uremic state of the normal leptin level (7.8 ng/mL for normal renal function). Similarly, a leptin value lower than 4 ng/mL in normal subjects represents anorexia nervosa (15). That level is not expected among uremic patients, even in intense malnutrition conditions, owing to accumulation of leptin in plasma. In consequence, the accepted normal level must be incremented to interpret correctly the relationship between leptin and nutrition parameters.

Other factors, such as peritoneal glucose absorption could contribute, via insulin release [which increments leptin production (8)], to the higher plasma leptin levels found in PD patients. In fact, ob gene expression has been described to increase after food ingestion in rats, perhaps through direct insulin action on adipocytes (9). Peritoneal dialysis may be considered a model for continuous glucose intake and chronic hyperinsulinism. That could be the mechanism that predisposes to obesity, hyperleptinemia, insulin resistance, and de novo diabetes in PD patients.

Our obese patients showed strongly positive correlations between BMI and plasma leptin levels. Other investigators also reported that relationship in obese non-uremic patients, suggesting a central disorder in the leptin receptor or a post receptor signal transduction defect (16). However, such a relationship was not observed in our non-obese patients.

Other uremic factors may possibly influence leptin production or release (17). The greater insulin resistance usually associated with obesity might also be involved (16). Obesity is associated with increased morbidity and mortality. Longitudinal studies have shown significant incidence of hypertension, high cholesterol levels, hypertriglyceridemia with proportional BMI increment, and hyperuricemia, all related to atherosclerosis progression. Obesity and LVH also represent, per se, a independent coronary risk factor (13). Regardless of BMI, we found higher plasma leptin levels in patients with elevated values of those CV risk factors (Table 1). A significant direct linear correlation between triglycerides and leptin was also seen. The hydrolysis of triglycerides stored in adipose tissue produces free fatty acids, which can inhibit glucose utilization by peripheral tissues. That situation could add to the insulin resistance status found in uremic patients (6). Left ventricular hypertrophy is another known effect of hypertension that increases morbidity and mortality in obese patients (13). In patients with LVH, we found high plasma leptin levels that could not be explained by BMI values.

Sympathetic hyperactivity and hyperinsulinism are commonly associated with obesity (7). Experimentally, intracerebroventricular leptin administration increased sympathetic lumbar and renal nerve activity, elevating mean arterial pressure (10). That finding establishes for the first time a relationship between leptin and CV modulation. Also supporting that hypothesis is the observation that leptin decreases brain NPY levels (2), and that NPY intracerebral injection lowers blood pressure (18). On the other hand, sympathetic stimulation or catecholamines may inhibit leptin production, thus creating a negative feedback loop. In chronic renal failure and dialysis patients, clear evidence exists of sympathetic hyperactivity (19), which may be a product of uremic leptin accumulation. Hyperleptinemia could induce hypertension, CV changes (7,8), and dyslipidemia via adrenal hyperactivity (7), increasing atherosclerosis risk in uremic patients. Furthermore, leptin has been found to stimulate the proliferation of glomerular endothelial cells of the rat (20). The endothelial and metabolic effects of hyperleptinemic states, such as uremia, should be studied.

Leptin is a marker of white fat tissue, and interesting studies have associated the central fat-mass distribution with high coronary risk in non uremic (9) and uremic patients (17). High visceral fat mass has been also associated with coronary disease (9,14). Recent experimental studies have shown that leptin is implicated in the distribution of visceral or intra-abdominal fat (9). We thus wonder if leptin could be a marker of visceral fat. Importantly, in multifactorial analysis, we found a relationship between hyperleptinemia, sex (female), and LVH. In patients with a high CAS score, we found an association between high BMI and worse atherosclerosis status, which supports the hypothesis that leptin could contribute to peripheral insulin resistance, hypertension (through increase in sympathetic activity), dyslipidemia, and CV disease in obese PD patients.

In non-obese patients, plasma leptin levels nonetheless showed significant direct linear correlation with the studied markers of nutrition. In our study group, leptin could therefore be considered
a marker of nutrition and food intake. With respect to patients with anorexia, we found leptin values lower than those exhibited by the rest of the PD population, probably in relation to their low fat mass.

Finally, we suggest that uremic patients with anorexia might have integrity of their hypothalamic leptin receptor. Their increased leptin values might therefore give rise to an upregulation of the hunger—satiety center.

**Conclusion**

Hyperleptinemia in PD patients may be considered a CV risk marker. In non obese patients without inflammation, leptin may be a marker of food intake.

**References**


**Corresponding author:**
M. Auxiliadora Bajo, MD, PHD, Servicio de Nefrolog a, Hospital Universitario La Paz, Paseo de Castellana nº261, Madrid E-28046 Spain.