PART SIX

Metabolism and Nutrition
Serum Levels of Cancer Antigen 125 and Interleukin-15 in Relation to the Nutrition Status of Peritoneal Dialysis Patients

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Introduction
Cancer antigen 125 (CA125) is produced by human peritoneal mesothelial cells (HPMCs). The concentration of CA125 in dialysis effluent is considered a marker of mesothelial cell mass, their in vivo turnover (1,2), and the biocompatibility of dialysis solutions in stable peritoneal dialysis (PD) patients (3,4). The concentration of CA125 in serum is also a non-specific marker of peritoneal membrane irritation (5).

Interleukin-15 (IL-15) is a 14- to 15-kDa glycoprotein whose mature form consists of 114 amino acids (6). The gene for IL-15 is coded in human chromosome 4q31 (7). Interleukin-15 is a member of the 4α-helix bundle cytokine family, which includes IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and IL-9 (6). As a proinflammatory cytokine, IL-15 plays a significant role in the function of T and B lymphocytes, natural killer (NK) cells, and neutrophils. It influences the production of other cytokines, among them interferon gamma, tumor necrosis factor alpha, IL-10 (increase) and IL-8, and monocyte chemoattractant protein 1 (decrease). Under in vitro conditions, HPMCs produce and secrete IL-15. In continuous ambulatory peritoneal dialysis (CAPD) patients, IL-15 is present in dialysis effluent (8,9).

Inflammation is a factor known to influence nutrition status in dialyzed patients (10,11).
In the present study, we performed a correlation analysis between serum CA125 and IL-15 concentrations and parameters of nutrition in patients treated with PD.

**Patients and methods**

We carried out the study in 42 stable PD patients who showed no clinical signs of infection during the 2 months preceding the study. Mean age of the patients was 52.3 ± 14.4 years, and their time on PD was 24.5 ± 20.1 months (range: 0.4 – 72.8 months). Of the 42 patients, 34 were on CAPD, and 8 were on automated PD. Standard PD solutions were used in all patients.

Causes of end-stage renal disease (ESRD) in the study patients included chronic glomerulonephritis (12 cases), chronic pyelonephritis (8 cases), diabetic nephropathy (8 cases), hypertensive nephropathy (5 cases), polycystic kidney disease (3 cases), and Schönlein–Henoch syndrome (1 case). In 5 cases, the causes of ESRD remained unknown.

Levels of IL-15 were estimated by ELISA (IBL, Hamburg, Germany), and levels of CA125 were estimated by immunoenzymatic assay (Roche Diagnostics, Warsaw, Poland). Other estimated parameters included PD adequacy [Kt/V, protein nitrogen appearance, total creatinine clearance (tCCr), efficacy number], anthropometric markers of nutrition [total body mass (TBM), lean body mass (LBM), total body water (TBW), total fat mass, body surface area (BSA)], peripheral blood morphology (hemoglobin, hematocrit, white blood cell count, platelet count, red blood cell indices), markers of iron metabolism (serum levels of iron and ferritin, transferrin saturation (TSAT), total iron binding capacity), and serum concentration of total protein and albumin. We estimated LBM using anthropometric measurements and also creatinine kinetics. The methods of obtaining the aforementioned data have been previously described (12,13).

After using the Kolmogorov–Smirnov test to analyze the data for normality of distribution, we performed a correlation analysis by the Spearman method. A p value less than 0.05 was defined as statistically significant.

**Results**

Serum IL-15 concentration was 78.9 ± 163.4 pg/mL (median: 25.0 pg/mL; range: 0.0 – 857.1 pg/mL), and CA125 concentration was 20.0 ± 18.6 U/mL (median: 15.1 U/mL; range: 5.6 – 119.1 U/mL). Serum IL-15 concentration was elevated in 29 patients (69%), and CA125 concentration in 5 patients (12%). Serum IL-15 concentration showed a significant correlation with serum albumin concentration (r = −0.442, p = 0.003, Figure 1). Serum CA125 concentration correlated with tCCr (r = −0.322, p = 0.037, Figure 2), with LBM evaluated by the anthropometric method (r = −0.414, p = 0.007, Figure 3) and by creatinine kinetics (r = −0.356, p = 0.020), with TBW (r = −0.360, p = 0.019, Figure 4), with BSA (r = −0.436, p = 0.004, Figure 5), with TSAT (r = −0.436, p = 0.004, Figure 6), with mean cell hemoglobin concentration (MCHC: r = −0.324, p = 0.036, Figure 7), and with serum albumin concentration as a percentage of total protein level (r = −0.547, p = 0.002, Figure 8).

**Discussion**

In PD patients, the existence of a systemic inflammatory state is indicated by systemic activation of monocytes in the absence of peritonitis or other apparent causes of inflammation (14) and by elevated levels of C-reactive protein and proinflammatory cytokines present in serum (15). Our study also confirms chronic inflammation through our observation of elevated serum concentrations of such proinflammatory cytokines as IL-15 (almost 70% of examined stable PD patients) in the absence of clinical signs of infection. The presence of IL-15 may also contribute to
FIGURE 2 Correlation between serum concentration of cancer antigen 125 (CA125) and total creatinine clearance (tCCr).

FIGURE 3 Correlation between serum concentration of cancer antigen 125 (CA125) and lean body mass determined by the anthropometric method (LBM anthr).

FIGURE 4 Correlation between serum concentration of cancer antigen 125 (CA125) and total body mass (TBM).

FIGURE 5 Correlation between serum concentrations of cancer antigen 125 (CA125) and iron.

FIGURE 6 Correlation between serum concentration of cancer antigen 125 (CA125) and transferrin saturation (TSAT).

FIGURE 7 Correlation between serum concentration of cancer antigen 125 (CA125) and mean cell hemoglobin concentration (MCHC).
generation of a local inflammatory state, as suggested by the detection of IL-15 in dialysate by Hausmann et al. (9) and confirmed in our earlier study (8). This persistent systemic and local inflammatory response may be a consequence of unrecognized, clinical, dialysis-related or dialysis-unrelated infections (16). Additionally, the repeated use of bioincompatible dialysis solutions causes a deteriorating effect on the peritoneal membrane (17).

A close relationship exists between inflammation and the nutritional state of dialyzed patients (10,11). The link is the proinflammatory cytokines that, among other things, suppress liver synthesis of albumin (18). In the present study, that situation is reflected in the negative correlation between serum IL-15 and albumin concentration. Proinflammatory cytokines also stimulate protein catabolism, which causes, among other effects, loss of LBM (19).

The present study shows a negative correlation between LBM and serum CA125 concentration, a nonspecific marker of peritoneal membrane irritation (5). Moreover, serum CA125 concentration showed a negative correlation with other parameters connected with nutrition: TBM, serum iron concentration, TSAT, MCHC, and serum albumin concentration as a percentage of total protein level. Some of those parameters correlate with acute-phase proteins. Serum albumin, iron concentration, and TSAT show a significant relationship with serum haptoglobin in PD patients without diabetes (11). Serum albumin and TSAT also correlate with the concentration of C-reactive protein in dialysis patients (20). Those relationships confirm the negative influence of inflammation on nutrition status.

**Conclusion**

We conclude that, in PD patients, elevated serum concentrations of IL-15 and CA125 suggest a persistent inflammatory state that negatively influences nutrition status.

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**References**


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Disturbances in immunity and nutrition status worsen in peritoneal dialysis (PD) patients with advancing age. In the present study, we evaluated variations in total lymphocyte count (TLC) and subset lymphocyte counts (SLCs) with respect to the age of PD patients.

We carried out the study in two groups of PD patients. Group I patients \((n = 12)\) were less than 40 years of age \((35.5 \pm 5.4 \text{ years})\), and their PD duration was \(18.2 \pm 9.4\) months. Group II patients \((n = 14)\) were more than 60 years of age \((67.2 \pm 5.1 \text{ years})\), and their PD duration was \(20.6 \pm 11.0\) months. In group I, 9 patients were taking angiotensin converting enzyme inhibitors (ACEIs); in group II, 10 patients were taking ACEIs. We used flow cytometry to estimate SLCs \((\text{determining CD3, CD4, CD8, CD19, and CD16+56 antigens})\).

In both groups, the mean CD19, CD4, and CD8 counts were lower than the normal ranges. In group II, TLC and CD3 count were also lower than normal. In group I, correlations were seen between age and TLC, CD3, CD19, CD4, and CD8. Correlations were also seen between dialysis duration and TLC, CD3, CD19, and CD4, and between total ACEI dose and CD19 count. In group II, correlations were seen between age and TLC, CD3, and CD8. No correlation was observed between PD duration and TLC or SLCs, but a correlation between total ACEI dose and CD8 count was seen. In patients who were taking enalapril as their only ACEI, a correlation was observed between total enalapril dose and TLC, CD3, and CD8.

Our results confirm data that indicate worse immunity and nutrition status in older PD patients and demonstrate decreasing values of TLC and SLCs with aging in younger and older PD patients alike. Administration of ACEIs negatively influences SLCs independently of age, but decreases in TLC and SLCs are significantly related to PD duration only in younger patients.

**Key words**

Lymphocytes, age, duration of continuous ambulatory peritoneal dialysis, angiotensin converting enzyme inhibitors

**Introduction**

The number of elderly patients with uremia being treated with peritoneal dialysis (PD) is increasing. Continuous ambulatory peritoneal dialysis (CAPD) and automated PD are acceptable forms of renal replacement therapy in elderly uremic patients. The CAPD modality offers many advantages, including hemodynamic stability, steady-state blood chemistries, and better preservation of residual renal function than intermittent hemodialysis does (1).

Several factors appear to be clear predictors of PD outcome—in particular, chronic inflammation, cardiovascular disease, diabetes mellitus, old age, hypalbuminemia, peritonitis, and inadequate dialysis. Age is a predictor of morbidity and mortality in the PD population; and, in general, elderly PD patients with
multiple comorbid diseases have worse outcomes than most younger patients. Disturbances in immunity and nutrition status also worsen in PD patients with advancing age (2).

A combination of socioeconomic and psychological circumstances and biochemical abnormalities is responsible for worsening nutrition status in older people. The decline in immune function with old age is perhaps one of the most dramatic and consequential of those phenomena. Moreover, lymphopenia has an important association with survival in PD patients. Changes in total lymphocyte count (TLC) and subset lymphocyte counts (SLCs) during the course of CAPD can be an indicator of disturbances in immune response and nutrition status and a prognostic index of mortality in CAPD patients (3).

In the present study, we evaluated variations in TLC and SLCs with respect to patient age. The influence of CAPD duration and of administration of angiotensin converting enzyme inhibitors (ACEIs) on TLC and SLCs was concomitantly analyzed.

**Patients and methods**

We carried out the study in two groups of CAPD patients. Group I patients ($n=12$; 7 men, 5 women) were less than 40 years of age ($35.5 \pm 5.4$ years; median: 36.9 years; range: 18.9 – 39.9 years), and their PD duration was $18.2 \pm 9.4$ months. Group II patients ($n=14$; 9 men, 5 women) were more than 60 years of age ($67.2 \pm 5.1$ years; median 66.4 years; range: 60.4 – 76.7 years), and their PD duration was $20.6 \pm 11.0$ months.

In group I, the underlying kidney diseases were chronic glomerulonephritis (3 cases), chronic pyelonephritis (2 cases), diabetic nephropathy (6 cases), and unknown causes (1 case). In group II, the underlying kidney diseases were chronic glomerulonephritis (1 case), chronic pyelonephritis (4 cases), diabetic nephropathy (2 cases), hypertensive nephropathy (3 cases), polycystic kidney disease (3 cases), and unknown causes (1 case).

In group I, 9 patients were taking ACEIs (among the 9, 8 patients received enalapril). In group II, 10 patients were taking ACEIs (among the 10, 9 patients received enalapril). For the patients receiving ACEIs, we calculated the total ACEI dose for the entire individual CAPD course. Recombinant human erythropoietin (rHuEPO), tablets containing iron (210 mg Fe++) and folic acid (0.70 mg), and other drugs and supplements were administered depending on clinical indications.

We determined SLC percentages by flow cytometry with the use of commercially available monoclonal antibodies: CD3, CD4, CD8, CD19, and CD16+56 antigens (Becton–Dickinson, San Jose, CA, U.S.A.). In our laboratory, the normal ranges for the various counts are TLC, $1.5 – 3.5 \times 10^9$/L; CD3, $1.1 – 1.7 \times 10^9$/L; CD4, $0.7 – 1.1 \times 10^9$/L; CD8, $0.5 – 0.9 \times 10^9$/L; CD19, $0.2 – 0.4 \times 10^9$/L; and CD16+56, $0.2 – 0.4 \times 10^9$/L.

Descriptive data are reported as mean ± 1 standard deviation of the mean. Means and standard deviations of TLC and SLCs obtained in both patient groups were compared and related to the respective normal ranges. Correlations between TLC, SLCs, PD duration, ACEI doses, and age were checked using the Spearman test. The results were considered significant if the $p$ value was less than 0.05.

**Results**

Table I presents the TLC and SLC values that were obtained. In both groups, the mean CD19, CD4, and CD8 values were lower than the respective normal ranges. In group II, the TLC and CD3 values were also lower than normal.

In group I, correlations were seen between age and TLC ($r = -0.777$), CD3 ($r = -0.746$), CD19 ($r = -0.781$), CD4 ($r = -0.727$), and CD8 ($r = -0.643$). Correlations were also seen between dialysis duration and TLC ($r = -0.623$), CD3 ($r = -0.637$), CD19 ($r = -0.647$), and CD4 ($r = -0.608$), and between total ACEI dose and CD19 ($r = -0.578$).

In group II, correlations were seen between age and TLC ($r = -0.693$), CD3 ($r = -0.600$), and CD8

<table>
<thead>
<tr>
<th>Table I</th>
<th>Results of estimation of total lymphocyte count (TLC) and subset lymphocyte counts in younger (group I) and older (group II) peritoneal dialysis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I ($n=12$)</td>
</tr>
<tr>
<td>TLC</td>
<td>$1.61 \pm 0.76$</td>
</tr>
<tr>
<td>CD3</td>
<td>$1.22 \pm 0.58$</td>
</tr>
<tr>
<td>CD19</td>
<td>$0.15 \pm 0.12 ^a$</td>
</tr>
<tr>
<td>CD4</td>
<td>$0.61 \pm 0.32 ^a$</td>
</tr>
<tr>
<td>CD8</td>
<td>$0.32 \pm 0.15 ^a$</td>
</tr>
<tr>
<td>CD16+56</td>
<td>$0.21 \pm 0.12$</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>$1.88 \pm 0.49$</td>
</tr>
</tbody>
</table>

| a Mean is below normal range. |
(r = –0.574). No correlation was observed between PD duration and TLC or SLCs. A correlation was seen between total ACEI dose and CD8 (r = –0.535). When patients taking ACEIs other than enalapril were excluded, a correlation was seen between total enalapril dose and TLC (r = –0.586), CD3 (r = –0.554), and CD8 (r = –0.557).

Discussion

Elderly patients exhibit immunosenescence accompanied by high rates of morbidity and mortality associated with infectious diseases. The TLC and SLCs are sensitive to influences of sex, age, hormones, protein and trace element intake, physical effort, stress, and circadian rhythm (4).

Many studies performed in various species have established that age-associated immune decline is characterized by a reduction in both the humoral and the cellular response. Walrand et al. (5) showed that baseline total and cytotoxic T lymphocyte subpopulations were lower in elderly subjects than in adult subjects. McFarlane et al. (6), studying age-associated changes in lymphocyte populations in horses, showed that absolute counts of total lymphocytes, T cells, CD4 cells, and CD8 T cells and B cells were lower in older horses than in young ones. A significant reduction in the percentage of CD8 cells and an increase in the CD4:CD8 cell ratio were observed in the older horse population. In aging dogs, Greeley et al. (7) demonstrated a decline in the absolute numbers of lymphocytes, T cells, CD4 cells, and CD8 cells, independent of sex. Cytokine dysregulation plays a role in the remodeling of the immune system in old age. The percentages of CD3 cells producing tumor necrosis factor alpha and interferon gamma increase with age (8).

In a study by Kawakami et al. (9), aging was associated with a significant reduction in the number of lymphocytes and a decline in mature T cells and helper/inducer T cells, but with increased numbers of activated T cells, suppressor T cells, and natural killer (NK) cells. The authors suggested that cell-mediated immunity in elderly subjects is reduced as a result of malnutrition, because a poor state of nutrition was noted in individuals 60 years of age or older.

An Italian study (10) showed that the absolute numbers of gamma and delta T cells in the peripheral blood of old and very old subjects are reduced. That finding was supported by study from Walrand et al. (5), who showed that, in all subjects, the patient’s nutritional state had a significant effect on total, helper, and cytotoxic T and B lymphocyte counts and that the response of lymphocyte subpopulations to nutritional fluctuation was significantly affected by age.

Changes in TLC and SLCs correspond with various comorbidities observed in the elderly. Age-related changes occur chiefly within the T cell population (number and proliferation), which is consistent with the increased incidence and severity of infection and cancer in elderly subjects (11). Douziech et al. (12) showed that proliferative responses to stimulation with various different mitogens were greater in a young population (20 – 25 years) than in an old one (60 – 87 years).

Our results in the present study confirm data that indicate worse immune status in older PD patients and demonstrate that TLC and SLC values decrease with aging in younger and older PD patients alike. A correlation between age and TLC or SLC values was seen in both study groups. However, no correlation was observed between age and the number of NK cells. Most studies suggest that the NK cell count in peripheral blood in patients with chronic renal failure could be a prognostic marker of susceptibility to infections and malignancy (13). Elderly people with severe medical disorders have low numbers of circulating NK cells and reduced cytotoxicity per NK cell (14).

One of the most important factors influencing the outcome of dialysis treatment is dialysis duration. In the present study, no significant difference in dialysis duration existed between the younger and older group of patients. Studies by Palop et al. (15) and our group (16) have shown a reduction in TLC during the course of CAPD in patients not selected by age. The SLCs can be observed to decrease over the course of CAPD treatment before a fall in TLC is observed (17). In the present study, the reductions in TLC and SLCs were significantly related to CAPD duration only in younger patients.

Administration of ACEIs may reduce the proliferation of T lymphocytes, causing an increase in serum bradykinin concentration (18). Numerous data indicate that rHuEPO has the effect of increasing TLC and SLCs. Huang (19) showed that ACEIs may reduce the serum erythropoietin concentration in patients treated with PD. In this way, ACEIs can indirectly influence TLC and SLCs. Our earlier studies showed a negative correlation between total ACEI dose and...
NK cell count (20). On the other hand, patients treated with ACEIs may require higher rHuEPO doses. It is well known that rHuEPO can improve immune status.

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References

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Anorexia-associated malnutrition is a severe complication that increases mortality in peritoneal dialysis (PD) patients. Ghrelin is a recently-discovered orexigenic hormone with actions in brain and stomach. We analyzed, in 42 PD patients, the possible relationship between ghrelin and appetite regulation with regard to other orexigens [neuropeptide Y (NPY), NO3] and anorexigens [cholecystokinin (CCK), leptin, glucose-dependent insulinotropic peptide (GIP), tumor necrosis factor alpha (TNFα)]. All orexigens and anorexigens were determined in plasma. Eating motivation was evaluated using a visual analog scale (VAS). The patients were divided into three groups: those with anorexia (n = 12), those with obesity associated with high intake (n = 12), and those with no eating behavior disorders (n = 18). A control group of 10 healthy volunteers was also evaluated.

Mean plasma levels of ghrelin were high (3618.6 ± 1533 mg/mL), with 36 patients showing values above the normal range (<2600 mg/mL). Patients with anorexia had lower ghrelin and NPY levels and higher peptide-C, CCK, interleukin-1 (IL-1), TNFα, and GIP levels than did the other patients. Patients with anorexia also had an early satiety score and low desire and pleasure in eating on the VAS and diet survey. We observed significant positive linear correlations between ghrelin and albumin (r = 0.43, p < 0.05), prealbumin (r = 0.51, p < 0.05), transferrin (r = 0.4, p < 0.05), growth hormone (r = 0.66, p < 0.01), NO3 (r = 0.36, p < 0.05), and eating motivation (VAS). At the same time, negative relationships were observed between blood ghrelin and GIP (r = −0.42, p < 0.05), insulin (r = −0.4, p < 0.05), leptin (r = −0.45, p < 0.05), and creatinine clearance [r = −0.33, p = 0.08 (nonsignificant)]. Ghrelin levels were not related to Kt/V or to levels of CCK and cytokines.

Ghrelin plasma levels are elevated in PD patients. Uremic patients with anorexia show relatively lower ghrelin plasma levels than the levels seen in obese patients or in patients with normal appetite. The role of ghrelin in appetite modulation is altered in uremic PD patients, and that alteration is possibly associated with disorders in insulin and growth hormone metabolism.

Key words
Eating behavior disorders, malnutrition, inflammation, ghrelin

Introduction
The deleterious effects of malnutrition in dialysis patients are well recognized. Malnutrition is a common factor in the two major causes of death during dialysis: cardiovascular disease and infection (1). Anorexia is frequently associated with uremia and represents the first step in malnutrition (2). Uremic status complicates appetite regulation because it provokes disorders in adipose tissue, the gastrointestinal system, neuropeptide protection and retention, inflammation, and the central nervous system. We have suggested the possibility that, in uremic patients, a true imbalance exists between anorexigenic and orexigenic mediators (3)—a misbalance that favors the appearance of anorexia.

Ghrelin is a novel 28-amino-acid octanoylated peptide that has been identified in stomach as an endogenous ligand for the growth hormone (GH) secretagogue receptor. Ghrelin is a powerful orexigen in fasting conditions; and, because its postprandial cycle is inverse to the glucose and insulin curves, it has satiating properties. Ghrelin influences eating consumption by increasing the desired meal size, and the peptide has been associated with bulimia—anorexia in non uremic patients (4). Recently, it has been suggested that renal retention may significantly elevate ghrelin plasma levels in patients with renal failure (5). Our hy-
pothesis derives from the contradiction between elevated ghrelin levels and the strong tendency to anorexia in uremic patients. In the present study, we analyzed the relationship between ghrelin plasma levels, other appetite regulators, and markers of nutrition in peritoneal dialysis (PD) patients.

**Patients and methods**

We studied 42 clinically stable PD patients [20 on continuous ambulatory PD (CAPD), 22 on automated PD (APD)]. The 22 male and 26 female patients ranged in age from 22 years to 79 years (mean: 56.9 ± 14 years). Their mean period on PD was 17.5 ± 13.2 months (range: 3 – 63 months). Causes of renal failure were nephrosclerosis (10 cases), glomerulonephritis (8 cases), diabetes (7 cases), chronic pyelonephritis (6 cases), polycystic kidney disease (5 cases), unknown (4 cases), and others (2 cases).

We used a visual analog scale (VAS) adapted from Hill and Blundell [see (6)] to evaluate eating motivation. The VAS includes 5 questions about desire, hunger, sense of fullness, prospective consumption, and palatability to be answered before and after eating. Answers are marked on a horizontal scale (0 – 100 mm).

Anorexia was defined as low eating motivation (personal interview and VAS < 60 mm), low food intake [normalized protein equivalent of nitrogen appearance (nPNA) < 1.1 g/kg/day, daily dietary assessment < 30 kcal/kg], and low values for markers of nutrition [Dialysis Outcomes Quality Initiative clinical practice guideline for nutrition (7)].

Obesity with high food intake was considered when the patient’s body mass index (BMI) measured 25 – 30 kg/m² [grade I, World Health Organization (WHO) criteria (8)], 30 – 40 kg/m² (WHO grade II), or >40 kg/m² (WHO grade III); when eating motivation was high (VAS > 60 mm); and when daily food intake (6) or bulimic criteria [Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (9)] was high.

Normal eating behavior was considered in the absence of anorexia (VAS) and bulimia, with a normal BMI (18.5 – 25 kg/m²) and normal values for markers of nutrition (7).

Using those definitions, we divided the study patients into 3 groups: those with anorexia (n = 12), those with obesity (n = 12) and high food intake, and those without eating behavior disorders (n = 18). We also studied a control group of 10 healthy volunteers (6 men, 4 women).

For all patients and controls, we determined these parameters:

- Dialysis adequacy:
  - Kt/V urea and nPNA
- Markers of nutrition:
  - Long-term: plasma creatinine, albumin, cholesterol (colorimetric method, Hitachi 704: Boehringer Mannheim, Mannheim, Germany), and transferrin (immunonephelometry, Behring Nephelometer: Behringwerte AG, Marburg, Germany)
  - Medium-term: short, half-life plasma proteins such as prealbumin and retinoid-binding protein [RBPI (immunonephelometry, Behring Nephelometer)], serum GH [immunoenzymatic assay, AIA 1200: Tosoh Corporation, Tokyo, Japan; maximum intra-assay and inter-assay coefficients of variation, 5.4% and 3.3% respectively; sensitivity, 0.1 ng/mL (normal, <5 ng/mL)]
  - Short-term: urea nitrogen, serum phosphate, and serum potassium. Mean daily dietary intake was determined from individual 24-hour food records during a 3-day period (including 1 weekend day). Daily calories and intake of carbohydrates, lipids, and protein were calculated for each patient using commercially available computer software [Wander (1990): Sandoz Nutrición, Barcelona, Spain].
- Plasma or serum peptide appetite modulators (radioimmunoassay):
  - Glucose (hexoquinase reaction: Boehringer Mannheim; normal fasting levels, 90 – 120 mg/dL)
  - Insulin (Sorin: Biomedica, Saluggia, Italy; sensitivity, 3 mIU/mL; intra-assay and inter-assay coefficients of variation, 6.6% and 6.2% respectively)
  - Glucose-dependent insulinotropic peptide [GIP (Peninsula Laboratories, Belmont, CA, U.S.A.); normal range, 35 – 52 pg/mL]
  - Cholecystokinin [CCK, 26–33 unsulfated fragment (Peninsula Laboratories); normal value, 12 – 20 pg/mL], which shows anorexigenic action
Leptin (Linco Research, St. Louis, MO, U.S.A.; sensitivity, 0.5 ng/mL; linearity, 100 µg/L; normal range, 3 – 7.8 ng/mL), which has an anorexigenic effect.

Neuropeptide Y [NPY (Peninsula Laboratories); normal range, 220 – 370 pg/mL], a powerful orexigen.

Ghrelin (RIA, 125-Ighrelin: Linco Research; sensitivity, 100 pg/mL; normal range in our 10 healthy volunteers, 900 – 2500 mg/mL), which shows anorexigenic action.

NO [measured as serum NO3, a final metabolite of NO, by capillary electrophoresis; normal range, 90 – 110 µmol/L (10)], a powerful orexigen.

Cytokines with recognized action on the hunger–satiety cycle: tumor necrosis factor alpha [TNFα (ELISA, Medigenix Easia kit): Biosource Europe SA, Nivelles, Belgium); normal, 3 – 20 pg/mL] and interleukin-1 [IL-1 (ELISA, Medigenix Easia kit); normal, <15 pg/mL]

Statistical analysis
Results are given as medians and ranges. Comparisons between study groups were performed using a nonparametric test, the Mann–Whitney rank sum U-test. Spearman regression analysis and the Student t-test were used for paired and unpaired data. A p value less than 0.05 was considered statistically significant.

Results
Table I shows the demographic and hematologic characteristics of the patients at baseline. Patients with anorexia were older and showed lower nPNA, albumin, prealbumin, RBP, and daily food intake. They also showed higher plasma levels of TNFα and IL-1 than were seen in the other groups. Relatively higher plasma levels of anorexigen (CCK, TNFα) and lower levels of orexigenic substances (NPY, ghrelin) were present in the group with anorexia. The contrary was observed in the obese group. Table I also shows a clear difference in ghrelin plasma levels between the dialysis patients and the healthy controls.

Table II shows the data from the VAS. Before and after eating, patients with anorexia scored differently than the other patients, confirming poor appetite in the anorectic group. Patients with obesity presented the opposite eating attitude.

Table III shows significant linear correlations between VAS scores and plasma levels of CCK, NPY, ghrelin, leptin, TNFα, and NO3. Those results effectively confirm the associations between anorexia and CCK, leptin, and TNFα. On the other hand, NPY, ghrelin, and NO3 were associated with higher eating desire.

In PD patients, ghrelin plasma levels were 3618.6 ± 1533 mg/mL as compared with 2084 ± 533.3 mg/mL in healthy controls, p < 0.01. Most patients (n = 36) showed values above the normal range.

We found significant positive linear correlations between ghrelin and some nutritional markers: albumin (r = 0.43, p < 0.05), prealbumin (r = 0.51, p < 0.05), transferrin (r = 0.4, p < 0.05), GH (r = 0.66, p < 0.01), and NO3 (r = 0.36, p < 0.05). Ghrelin also showed negative correlations with GIP (r = –0.42, p < 0.05), insulin (r = –0.4, p < 0.05), leptin (r = –0.45, p < 0.05), and creatinine clearance (r = –0.33, p = 0.08, NS). Ghrelin was not significantly related to Kt/V urea, CCK, or cytokine levels.

Levels of NPY showed a negative linear correlation with IL-1 (r = –0.52, p < 0.05) and TNFα (r = –0.51, p < 0.05), and TNFα and IL-1 showed a positive linear correlation (r = 0.85, p < 0.005). Levels of IL-1 and CCK also showed a positive linear correlation (r = 0.45, p < 0.05), as did levels of IL-1 and GIP (r = 0.46, p < 0.05).

Discussion
Eating behavior is a complex phenomenon with sociocultural and biologic influences, and eating is complicated by the resulting profound metabolic alterations and retention of catabolic end-products (3,4).

Dialysis patients have a strong tendency toward anorexia. They retain substances with anorectic action such as CCK, leptin, corticotropin-releasing hormone, insulin, glucagons, TNFα, IL-1, GIP, lack of NO, C-peptide, α-melanocyte-stimulating hormone (α-MSH), and free tryptophan (11). All of those agents promote the transfer of high concentrations of free tryptophan into cells—most especially in the hypothalamus—resulting in increased serotonin production and loss of appetite [tryptophan–serotonin hypothesis (12)].

Ghrelin, a novel 28-amino-acid polypeptide with GH secretagogue action, increases food intake, fat accumulation, body weight, gastric acid secretion, and stomach motility; it also reduces blood pressure, im-
The high ghrelin plasma levels we found in PD patients correlate negatively with residual renal function (RRF), indicating that RRF is responsible at least in part for ghrelin accumulation. Our findings also confirm findings by Yoshimoto A et al. (5). Importantly, dialysis dose (Kt/V) does not seem to play an important role in uremic ghrelin accumulation, supporting the idea that RRF is more important than dialysis dose in preserving appetite in uremia (11,14). We found a close association between fasting ghrelin plasma levels and eating motivation as measured by VAS (Table III). We also found a positive relationship between fasting ghrelin plasma levels and markers of nutrition. Moreover, patients with anorexia showed relatively lower values of ghrelin than did obese patients or patients with normal appetite (Table I). The anabolic effect of ghrelin is the

### Table I: Demographics and biochemical and nutrition markers in the study groups

<table>
<thead>
<tr>
<th></th>
<th>Anorexic (A)</th>
<th>Patients Obese (O)</th>
<th>Normal appetite (N)</th>
<th>Controls (C)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.4±10</td>
<td>56.3±7.1</td>
<td>49.7±14</td>
<td>31±3.7</td>
<td>A vs. N, &lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O vs. C, &lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N vs. C, &lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A vs. C, &lt;0.001</td>
</tr>
<tr>
<td>PD duration (months)</td>
<td>36.8±32.3</td>
<td>23±11.5</td>
<td>45.5±46.7</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>CCr (mL/min)</td>
<td>0.5±0.45</td>
<td>1.42±1.01</td>
<td>1.38±1.39</td>
<td>101±7</td>
<td>A/O/N vs. C, &lt;0.001</td>
</tr>
<tr>
<td>Daily nPNA (g/kg)</td>
<td>0.87±0.21</td>
<td>1.1±0.25</td>
<td>1.14±0.11</td>
<td>1.4±0.11</td>
<td>A vs. N, &lt;0.05</td>
</tr>
<tr>
<td>Weekly Kt/V urea</td>
<td>2±0.25</td>
<td>1.98±0.33</td>
<td>2.17±0.33</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>174±57.4</td>
<td>211±55.6</td>
<td>188±56</td>
<td>184±50</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7±0.08</td>
<td>4±0.2</td>
<td>3.9±0.4</td>
<td>5±0.4</td>
<td>A vs. O/C, &lt;0.05</td>
</tr>
<tr>
<td>Transferrin (mg/dL)</td>
<td>209±36</td>
<td>262±47</td>
<td>205±50.7</td>
<td>303±57.2</td>
<td>NS</td>
</tr>
<tr>
<td>Prealbumin (mg/dL)</td>
<td>26±7</td>
<td>31±2.9</td>
<td>31±7.5</td>
<td>34±3</td>
<td>A vs. O/C, &lt;0.05</td>
</tr>
<tr>
<td>Retinol protein binding (mg/dL)</td>
<td>8.4±3</td>
<td>11.5±3</td>
<td>13±2</td>
<td>5.3±1.2</td>
<td>A vs. O/N, &lt;0.05</td>
</tr>
<tr>
<td>Lymphocytes/mm³</td>
<td>1298±444</td>
<td>1452±613</td>
<td>1727±480</td>
<td>1877±592</td>
<td>NS</td>
</tr>
<tr>
<td>Growth hormone (ng/mL)</td>
<td>3.4±3.8</td>
<td>4±4.8</td>
<td>2.2±1.4</td>
<td>1.7±1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Diet survey (kcal/day)</td>
<td>1277±467.4</td>
<td>2320±179.4</td>
<td>2006±351</td>
<td>2089±339</td>
<td>A vs. O, &lt;0.01</td>
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<td></td>
<td>A vs. N, &lt;0.05</td>
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<td></td>
<td></td>
<td></td>
<td>O vs. C, &lt;0.05</td>
</tr>
<tr>
<td>Fat (kcal/day)</td>
<td>60.4±28.9</td>
<td>102±23.2</td>
<td>98±22</td>
<td>74.7±15</td>
<td>A vs. O/N, &lt;0.05</td>
</tr>
<tr>
<td>Protein (kcal/day)</td>
<td>63±18</td>
<td>85.7±16.6</td>
<td>83.8±13.7</td>
<td>74.5±21.8</td>
<td>A vs. O, &lt;0.05</td>
</tr>
<tr>
<td>Carbohydrates (kcal/day)</td>
<td>98±41</td>
<td>227±71</td>
<td>155.5±27</td>
<td>248.8±67.2</td>
<td>A vs. O/N/C, &lt;0.01</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>93±33</td>
<td>101±40</td>
<td>105±69</td>
<td>81±5</td>
<td>A vs. O/C, &lt;0.05</td>
</tr>
<tr>
<td>Insulin (mIU/mL)</td>
<td>34±24</td>
<td>41.3±50.7</td>
<td>13.8±4.9</td>
<td>12.4±3.2</td>
<td>A vs. O/N, &lt;0.05</td>
</tr>
<tr>
<td>GIP (pg/mL)</td>
<td>101.2±22</td>
<td>111.8±28</td>
<td>132.6±25</td>
<td>47.1±7.3</td>
<td>A/O/N vs. C, &lt;0.01</td>
</tr>
<tr>
<td>Cholecystokinin (pg/mL)</td>
<td>25.8±3.7</td>
<td>19.9±4.1</td>
<td>21.6±8</td>
<td>10.9±1.8</td>
<td>A vs. O, &lt;0.05</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>44.5±45</td>
<td>110±45</td>
<td>35±28</td>
<td>11.8±8</td>
<td>A vs. O, &lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O vs. N, &lt;0.01</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>N vs. C, &lt;0.01</td>
</tr>
<tr>
<td>Neuropeptide Y (pg/mL)</td>
<td>369±260</td>
<td>463.5±61.6</td>
<td>433.7±61</td>
<td>320.5±48</td>
<td>A vs. O/N/C, &lt;0.05</td>
</tr>
<tr>
<td>Ghrelin (mg/mL)</td>
<td>364±958.6</td>
<td>448±1614.8</td>
<td>4289±1161</td>
<td>2084±533.3</td>
<td>A/O/N vs. C, &lt;0.05</td>
</tr>
<tr>
<td>NO₃ (µmol/L)</td>
<td>175±60</td>
<td>190±35.9</td>
<td>152±26.6</td>
<td>92.7±7.5</td>
<td>A vs. C, &lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N vs. C, &lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O vs. C, &lt;0.05</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>121±43.8</td>
<td>40±11.6</td>
<td>38.2±16</td>
<td>18±4</td>
<td>A vs. O/N, &lt;0.01</td>
</tr>
<tr>
<td>IL-1 (pg/mL)</td>
<td>6.12±0.8</td>
<td>2.1±0.43</td>
<td>2.2±1.34</td>
<td>1±0.8</td>
<td>A vs. O/N, &lt;0.001</td>
</tr>
</tbody>
</table>

PD = peritoneal dialysis; NS = nonsignificant; CCr = creatinine clearance; nPNA = normalized equivalent of protein nitrogen appearance; GIP = glucose-dependent insulinotropic peptide; TNFα = tumor necrosis alpha; IL-1 = interleukin-1.

Proving cardiac function (5,13). The high ghrelin plasma levels we found in PD patients correlate negatively with residual renal function (RRF), indicating that RRF is responsible at least in part for ghrelin accumulation. Our findings also confirm findings by Yoshimoto A et al. (5).

Importantly, dialysis dose (Kt/V) does not seem to play an important role in uremic ghrelin accumulation, supporting the idea that RRF is more important than dialysis dose in preserving appetite in uremia (11,14). We found a close association between fasting ghrelin plasma levels and eating motivation as measured by VAS (Table III). We also found a positive relationship between fasting ghrelin plasma levels and markers of nutrition. Moreover, patients with anorexia showed relatively lower values of ghrelin than did obese patients or patients with normal appetite (Table I). The anabolic effect of ghrelin is the
result of its stimulation of appetite by an unknown mechanism and stimulation of GH–anabolic action (15).

We have successfully used recombinant GH (rGH) to treat malnourished patients on dialysis (16). Notably, treatment with rGH was associated with a dramatic reduction in plasma levels of leptin and in insulin resistance, supporting the possibility of a direct effect on ghrelin (17). Our present results—a positive association between ghrelin and GH, and a negative association between ghrelin and leptin and insulin—support that idea.

Importantly, we found no association between ghrelin and proinflammatory cytokines, which would be the result of independent ghrelin–GH anabolic mechanisms. Moreover, GH release is altered in end-stage renal disease (18), and cytokines may be one of the mechanisms implicated in that alteration (19).

We could consider ghrelin accumulation to be positive. Moreover, RRF may play a crucial role as a

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### TABLE II  Motivation to eat, measured by the visual analog scale

<table>
<thead>
<tr>
<th></th>
<th>Anorexic (A)</th>
<th>Obese (O)</th>
<th>Normal appetite (N)</th>
<th>Controls (C)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desire to eat before lunch</td>
<td>60±6.1</td>
<td>76.6±6</td>
<td>67.8±6.9</td>
<td>72.8±3.9</td>
<td>A vs. O/C, &lt;0.01</td>
</tr>
<tr>
<td>Desire to eat after lunch</td>
<td>8.6±2.2</td>
<td>21.6±4</td>
<td>13.2±5</td>
<td>13.5±8.5</td>
<td>A vs. O, &lt;0.05</td>
</tr>
<tr>
<td>Hunger before lunch</td>
<td>60±6.1</td>
<td>78.3±6</td>
<td>68.6±4.7</td>
<td>74.3±4.5</td>
<td>A vs. O/N, &lt;0.001</td>
</tr>
<tr>
<td>Hunger before lunch</td>
<td>8±4.4</td>
<td>21.6±4</td>
<td>12.8±5.5</td>
<td>17.1±4.8</td>
<td>A vs. O/C, &lt;0.01</td>
</tr>
<tr>
<td>Fullness before lunch</td>
<td>28±8.4</td>
<td>18.8±2.5</td>
<td>12.5±4.2</td>
<td>11.8±4.1</td>
<td>A vs. O/N, &lt;0.01</td>
</tr>
<tr>
<td>Fullness after lunch</td>
<td>81±5.4</td>
<td>59.1±19.6</td>
<td>77±5.6</td>
<td>77±5.6</td>
<td>A vs. O, &lt;0.05</td>
</tr>
<tr>
<td>Prospective consumption before lunch</td>
<td>59±5.5</td>
<td>78.3±4</td>
<td>71.4±3.7</td>
<td>75.7±4.5</td>
<td>A vs. O/N/C, &lt;0.001</td>
</tr>
<tr>
<td>Prospective consumption before lunch</td>
<td>6±2.2</td>
<td>25±5.4</td>
<td>12.3±2.7</td>
<td>13.5±4.7</td>
<td>A vs. O, &lt;0.001</td>
</tr>
<tr>
<td>Palatability</td>
<td>60±7</td>
<td>75±5.4</td>
<td>71.4±4.7</td>
<td>74.3±5.3</td>
<td>A vs. O/N/C, &lt;0.01</td>
</tr>
<tr>
<td>Hunger 2 hours before lunch</td>
<td>34±5.4</td>
<td>58.3±2.6</td>
<td>45±8.6</td>
<td>45.7±5.3</td>
<td>A vs. O/C, &lt;0.01</td>
</tr>
<tr>
<td>Satiety 2 hours after lunch</td>
<td>60±0</td>
<td>39.2±6.6</td>
<td>40.7±17.4</td>
<td>40±0</td>
<td>A vs. O/C, &lt;0.001</td>
</tr>
</tbody>
</table>

* The visual analog scale is measured on the horizontal, with a maximum value of 100 mm.

### TABLE III  Relationship between visual analog scale values and plasma levels of appetite peptide regulators

<table>
<thead>
<tr>
<th></th>
<th>CCK</th>
<th>NPY</th>
<th>Ghrelin</th>
<th>Leptin</th>
<th>TNFα</th>
<th>NO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desire to eat before lunch</td>
<td>0.6ᵇ</td>
<td>0.4ᵃ</td>
<td>-0.4ᵃ</td>
<td>-0.4ᵃ</td>
<td>0.54ᵇ</td>
<td></td>
</tr>
<tr>
<td>Desire to eat after lunch</td>
<td>0.4ᵃ</td>
<td>0.6ᵇ</td>
<td>-0.6ᵇ</td>
<td>-0.6ᵇ</td>
<td>0.4ᵃ</td>
<td>0.5ᵇ</td>
</tr>
<tr>
<td>Hunger before lunch</td>
<td>-0.4ᵃ</td>
<td>0.6ᵇ</td>
<td>-0.6ᵇ</td>
<td>-0.6ᵇ</td>
<td>0.56ᵇ</td>
<td></td>
</tr>
<tr>
<td>Hunger after lunch</td>
<td>0.55ᵇ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fullness after lunch</td>
<td>-0.4ᵃ</td>
<td>-0.4ᵃ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prospective consumption before lunch</td>
<td>0.48ᵃ</td>
<td>0.7ᵇ</td>
<td>-0.34ᵃ</td>
<td>-0.48ᵃ</td>
<td>0.5ᵇ</td>
<td></td>
</tr>
<tr>
<td>Prospective consumption after lunch</td>
<td>0.4ᵃ</td>
<td>0.6ᵇ</td>
<td>-0.4ᵃ</td>
<td>-0.43ᵃ</td>
<td>0.38ᵃ</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ p < 0.05. ᵇ p < 0.01.

CCK = cholecystokinin; NPY = neuropeptide Y; TNFα = tumor necrosis factor alpha.
protective factor against anorexia. Because ghrelin depends on GH release for its anabolic effect, and because uremia alters that cycle, with a resulting retention of many anorexigenic molecules, the final result is anorexia.

Conclusions
Ghrelin plasma levels are elevated in PD patients. Uremic patients with anorexia show relatively lower ghrelin plasma levels than the levels seen in obese patients or in patients with normal appetite. The disordered insulin and GH metabolism in PD patients probably affects the role of ghrelin modulation in appetite control.

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References

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