PART FOUR Metabolism and Nutrition
The Relationship Between Residual Renal Function, Protein Catabolic Rate, and Phosphate and Magnesium Levels in Peritoneal Dialysis Patients

Residual renal function (RRF) is an important factor in the well-being of peritoneal dialysis (PD) patients. Serum phosphate has been correlated with long-term morbidity and mortality. We wished to determine if RRF contributes to a lower level of serum phosphate and magnesium. We also investigated the relationship between protein catabolic rate (PCR) and phosphate and magnesium.

We collected data related to serum phosphate, serum magnesium, PCR, and RRF in 100 PD patients. The Pearson correlation coefficient was used to study the correlation between RRF and magnesium, RRF and phosphate, PCR and magnesium, and PCR and phosphate.

No relationships were seen between PCR and serum phosphate, and RRF did not influence the serum magnesium level. But a very striking direct

Disappointing and unsuccessful. Cardiovascular and valvular heart diseases have been shown to be related to serum phosphorus and the calcium↔phosphorus product in dialysis patients (1,2). Morbidity and mortality have been related in dialysis patients to high levels of phosphate and calcium↔phosphorus product (3). On the other hand, magnesium, another serum electrolyte of similar size, accumulates only slightly in dialysis patients, mainly if the patients avoid taking medications with a high magnesium content.

The purpose of the present study was to investigate whether protein catabolic rate (PCR) and residual renal function (RRF) play an active role in control of the two electrolytes serum phosphate and serum magnesium. As RRF decreases in dialysis patients, does that decrease contribute to higher levels of serum phosphate and serum magnesium and, consequently, to higher mortality and morbidity?

Patients and methods
Data related to serum phosphate, serum magnesium, PCR, and RRF were collected in 100 peritoneal dialysis (PD) patients. All 100 patients were well dialyzed, with a weekly Kt/V of more than 2.1. The Pearson correlation coefficient was used to study the correlation between RRF and magnesium, RRF and phosphate, PCR and magnesium, and PCR and phosphate.

Results
Table I presents the results of the study. Surprisingly, PCR seemed to have no relationship with serum phosphate, and RRF did not influence the serum magnesium level. But a very striking direct
relationship \((p \leq 0.0001)\) was seen between PCR and magnesium. Most importantly, an inverse correlation was seen between RRF and serum phosphate. As RRF deteriorates, dialysis patients have more problems keeping their abnormal phosphate levels within an acceptable range.

**Discussion**

We could not find a good relationship between RRF and magnesium. That finding is probably related to the fact that magnesium, like potassium, is easily dialyzed. As we increased the total daily PD dialysis fluid to compensate for the loss of RRF, we were able to remove enough magnesium, thus replacing the role that RRF plays in eliminating that solute. The average magnesium in our 100 PD patients was 0.93 mmol/L. We are in the process of investigating whether a relationship exists between the daily quantity of PD fluid and the level of serum magnesium.

A significant correlation existed between magnesium level and PCR \((p = 0.0001)\), showing that protein intake influences the serum magnesium level significantly.

It seems that PD does not adequately replace RRF, as pointed out by Bargman et al (4). In our PD patients, we kept the \(Kt/V\) at a level of 2.1 and above, gradually increasing the daily amount of dialysis fluid to replace the loss of RRF over the years. Unfortunately, even if we try to compensate for the loss of RRF by increasing the PD dose, that increase does not help much in keeping the serum phosphate level within an acceptable range. As RRF is lost, serum phosphate increases an inverse relationship between RRF and serum phosphate level. That was not the case with magnesium. The difference between magnesium and phosphate is related to the fact that phosphate is poorly removed by dialysis, whereas magnesium is easily removed. Although the molecular size of the two electrolytes is not greatly different, the hydrophilic property of phosphate causes it to act like a large molecule, and it is not easily dialyzable.

We were intrigued by the fact that we could not find a relationship between PCR and phosphate level. That result is probably related to the fact that phosphate serum level depends on many factors such as diet, RRF, hyperparathyroidism, and intake of vitamin D. Vitamin D given to our patients mobilizes phosphate from bones and increases phosphate absorption in the gut.

The control of phosphate in dialysis patients is of great importance. Patients on PD seem to have a slight advantage over hemodialysis patients: serum phosphate seems to be slightly lower in PD patients, as pointed out by Hoar et al (5). That finding may be related to the fact that PD preserves RRF longer (6,7).

Abnormally high serum phosphate and high calcium ↔ phosphate product contribute to calcified valvular disease. They also contribute to the accelerated arteriosclerosis with vessel calcifications that are frequently seen in our dialysis population (2). Residual renal function seems to play a significant role in the control of serum phosphate. Patients on PD have the advantage of preserving their RRF longer than hemodialysis patients (6). That advantage helps them to maintain a lower serum phosphate level for a longer time.

**Conclusion**

Because of the significant relationship between serum phosphate (and phosphate ↔ calcium product) and morbidity and mortality in dialysis patients, it is of primary importance to better control serum phosphate by trying to preserve RRF as long as possible. To preserve RRF, drugs that decrease renal function, or drugs that are toxic to the kidneys, should be avoided as much as possible. Preserving RRF helps to control serum phosphate.

**References**


3 Block GA, Hulbert—Shearon TE, Levin NW, Port FK.


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Correlation Between Oxidized Low-Density Lipoprotein and Other Factors in Patients on Peritoneal Dialysis

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Oxidized low-density lipoprotein (Ox-LDL) has been related with progression of atherosclerosis. Some studies reported increased Ox-LDL levels in hemodialysis (HD) patients. The levels of Ox-LDL in peritoneal dialysis (PD) patients have not yet been clarified. We measured Ox-LDL in PD patients and investigated the related factors.

We measured plasma Ox-LDL, total cholesterol (TC), triglycerides (TG), and LDL-cholesterol (LDL-c) levels in 18 PD patients (mean age: 55.5 ± 9.8 years; mean duration of dialysis: 2.7 ± 1.4 years) and in 24 HD patients as controls. We compared Ox-LDL levels in patients with diabetes mellitus (DM) and ischemic heart disease (IHD). And we looked at the correlation between Ox-LDL levels and adequacy of PD.

Levels of Ox-LDL were significantly higher in the PD patients (2.24 ± 1.14 ng/µg LDL protein) than in the HD patients (1.43 ± 0.90 ng/µg LDL protein), and other lipids were higher in the PD patients. The Ox-LDL did not correlate with other lipids. The Ox-LDL levels of the PD patients with DM or IHD were higher than those of non DM or non IHD patients. The adequacy of PD did not correlate with Ox-LDL.

Patients on PD, especially those with DM or IHD, showed elevated Ox-LDL levels. Special attention should be paid to the level of Ox-LDL and atherosclerosis in PD patients.

Key words
Oxidized low-density lipoprotein, atherosclerosis

Introduction
Oxidized low-density lipoprotein (Ox-LDL) in plasma has been related to progression of atherosclerosis. The atherosclerosis leads to many complications for example, ischemic heart disease (IHD) and cerebral stroke. Many dialysis patients have complications from those diseases.

Some studies report that Ox-LDL levels are increased in hemodialysis (HD) patients (1). The cause of the increase in Ox-LDL is being debated. But Ox-LDL levels in peritoneal dialysis (PD) patients have not been clarified. We therefore measured plasma Ox-LDL levels in PD patients and investigated the related factors.

Patients and methods

Patients and controls
We studied 18 PD patients ranging in age from 40 to 75 years (mean: 55.3 ± 9.8 years; all men). The PD duration ranged from 0.3 to 5.8 years (mean: 2.7 ± 1.4 years). Among the 18 PD patients, 4 had diabetes mellitus (DM) and 3 had IHD.

A group of 24 HD patients was recruited as a control. The HD patients ranged in age from 41 to 75 years (mean: 59.8 ± 9.6 years; all men). The HD duration ranged from 0.3 to 4.9 years (mean: 2.26 ± 1.4 years). Among the 24 patients, 8 had DM, and 1 had IHD (Table 1).

The PD and HD patients were all outpatients of St. Luke’s International Hospital.

Blood sampling
In the PD patients, blood was drawn at the time of routine laboratory investigations on the outpatient treatment day. In the HD patients, blood was drawn at the time of routine laboratory investigations on the first treatment day of the week, just before the HD treatment. We measured plasma levels of Ox-LDL, total cholesterol (TC), triglycerides (TG),
and LDL-cholesterol (LDL-c). We correlated Ox-LDL level and adequacy of PD [weekly creatinine clearance (CCr), Kt/V, peritoneal function, and residual renal function]. We defined residual renal function as a urine volume greater than 300 mL daily.

**Ox-LDL measurement**
We measured Ox-LDL using the sandwich ELISA method of Itabe (2).

**Statistical analysis**
Data are expressed as mean ± standard deviation. The Mann—Whitney U-test was used to examine differences between groups for statistical significance. The Spearman correlation coefficient (r) was used for analysis of the relationship between two variables. A value of \( p < 0.05 \) was considered significant.

**Results**

**Ox-LDL and lipids**
The level of Ox-LDL was significantly higher in the PD group (2.24 ± 1.14 ng/µg LDL protein) than in the HD group (1.43 ± 0.90 ng/µg LDL protein). The TC level was 185.7 ± 38.5 mg/dL in the PD patients and 146.1 ± 23.9 mg/dL in the HD patients (\( p < 0.01 \)). The TG level was 200.1 ± 1.4 mg/dL in the PD patients and 124.8 ± 74.3 mg/dL in the HD patients (\( p < 0.05 \)). The level of LDL-c was 101.7 ± 44.1 mg/dL in the PD patients and 77.3 ± 17.9 mg/dL in the HD patients (\( p < 0.05 \)).

Levels of TC, TG, and LDL-c were higher in the PD patients than in the HD patients. The level of high-density lipoprotein cholesterol (HDL-c) was 44.0 ± 13.8 mg/dL in the PD patients and 43.9 ± 13.8 mg/dL in the HD patients. The Ox-LDL level did not correlate with TC, TG, or LDL-c in the two groups (Table II).

**Ox-LDL and characteristics of patients**
The level of Ox-LDL did not correlate with age and dialysis duration in the two groups.

**Ox-LDL and complications**
The Ox-LDL level of the PD patients with DM was 3.22 ± 0.88 ng/µg LDL protein; it was 1.39 ± 0.72 ng/µg LDL protein in the HD patients. Ox-LDL level of the PD patients without DM was 2.06 ± 1.06 ng/µg LDL protein; it was 1.45 ± 1.0 ng/µg LDL protein in the HD group. The Ox-LDL level of the PD patients with DM was higher than that of the non DM PD patients. Also, the Ox-LDL level of the PD group was higher than that of the HD group [Figure 1(A)]. The Ox-LDL level of the PD patients with IHD was 3.53 ± 1.13 ng/µg LDL protein; it was 1.25 ± 0.95 ng/µg LDL protein in HD patients. The Ox-LDL level of the PD patients without IHD was 1.99 ± 0.98 ng/µg LDL protein; it was 1.44 ± 0.92 ng/µg LDL protein in HD patients. The Ox-LDL level of the PD patients with IHD was higher than that of non IHD PD patients. Also, the Ox-LDL level in the PD group was higher than that in the HD group [Figure 1(B)].

**Ox-LDL and adequacy of PD**
The Ox-LDL level did not correlate with Kt/V, weekly CCr, peritoneal function, or the glucose concentration of the dialysate. The adequacy of PD did not correlate with Ox-LDL level.

**Discussion and conclusions**
Some reports have indicated that lipid levels are increased in PD patients (3); other reports have indicated that lipid levels are normal in such patients (4). We showed that lipids were normal without TG and that lipids were higher in PD patients than in HD patients. The Ox-LDL level is high in chronic renal failure (1), indicating that the uremic state causes oxidative stress. Others have reported that HD is associated with the development of oxidative stress and disturbances involving oxygen free radicals (5,6). The dialyzer and endotoxins in dialysate are thought to be sources. But, as we showed in the present report, the Ox-LDL level was higher in PD patients than in HD patients. That finding suggests that other factors are at work in PD patients.

We think that PD dialysate is one of the factors increasing oxidative stress. Owing to the dialysate

| Table I Characteristics of the peritoneal dialysis (PD) and hemodialysis (HD) patients |
|---------------------------------|-----|-----|
| Patients (n) | PD | HD |
| Age (years) | 55.3 ± 9.8 | 59.8 ± 9.6 |
| Dialysis duration (years) | 2.7 ± 1.4 | 2.26 ± 1.4 |
| Diabetes mellitus (Y/N) | 4/14 | 8/16 |
| Ischemic heart disease (Y/N) | 3/15 | 1/23 |

Y/N = yes/no.
glucose concentration and pH difference, peritoneal sclerosis and changes in peritoneal function occur. Those effects suggest that PD dialysate may not be biocompatible for PD patients. We think that dialysate may be a cause of the increasing oxidative stress, and that protection from that stress can be achieved by changing the dialysate component.

In our study, PD patients with DM or IHD (or both) showed elevated Ox-LDL levels. We suspect that PD itself may increase the risk of atherosclerosis. We think that special attention should be paid to the level of Ox-LDL and atherosclerosis in PD patients.

References

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| TABLE II | Oxidized low-density lipoprotein (Ox-LDL) and lipid levels in the peritoneal dialysis (PD) and hemodialysis (HD) patients |
|-----------|--------------------------------------------------|--------------------------------------------------|
|           | PD                                               | HD                                               |
|           | Ox-LDL (ng/µg LDL protein)                       | Ox-LDL (ng/µg LDL protein)                       |
|           | 2.24±1.14                                        | 1.43±0.90                                        |
|           | T-cho (mg/dL)                                    | T-cho (mg/dL)                                    |
|           | 185.7±38.5                                       | 146.1±23.9                                       |
|           | TG (mg/dL)                                       | TG (mg/dL)                                       |
|           | 200.1±1.4                                        | 124.8±74.3                                       |
|           | LDL-c (mg/dL)                                    | LDL-c (mg/dL)                                    |
|           | 101.7±44.1                                       | 77.3±17.9                                        |
|           | HDL-c (mg/dL)                                    | HDL-c (mg/dL)                                    |
|           | 44.0±13.8                                        | 43.9±13.8                                        |

**T-cho** = total cholesterol; **TG** = triglycerides; **LDL-c** = low-density lipoprotein cholesterol; **HDL-c** = high-density lipoprotein cholesterol; **NS** = nonsignificant.
Usefulness of Bioelectrical Impedance Analysis in Monitoring Nutrition Status and Survival of Peritoneal Dialysis Patients

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Malnutrition is highly prevalent in peritoneal dialysis (PD) patients and is associated with higher mortality. Lower serum levels of markers of nutrition such as albumin, creatinine, prealbumin, and total cholesterol are important risk factors in PD patients. Usefulness of bioimpedance analysis (BIA) in hemodialysis (HD) patients has been reported. In the present study, we prospectively examined the relationship of bioimpedance indexes to the nutrition status and survival of 45 PD patients who were followed for more than 1 year.

On patient enrollment, a BIA was performed (Bioelectrical Impedance Analyzer, Model BIA-101: RJL Systems, Clinton Township, MI, U.S.A.). Monthly blood was analyzed for biochemical markers. The mean age of the study group was 50 ± 15 years. Of the 45 patients, 56% were female and 24% were diabetic. Mean body mass index was 25.7 ± 5.1. Mean resistance, reactance, capacitance, and phase angle were 524 ± 106 Ω, 57 ± 20 Ω, 678 ± 223 pF, and 6.2 ± 1.7 degrees respectively.

Patients with diabetes had lower capacitance (555 pF vs. 713 pF; p = 0.007) and phase angle (5.35 degrees vs. 6.4 degrees, p = 0.05) than patients without diabetes. During the study period, 4 patients died. Patients who survived had higher capacitance (486 ± 163 pF vs. 697 ± 218 pF; p = 0.07) and phase angle (4.65 ± 0.73 degrees, vs. 6.34 ± 1.67 degrees, p = 0.008) than those who did not survive. The Kaplan—Meier method was used to compute observed survival. The cumulative observed survival of PD patients with an enrollment phase angle > 6 degrees was significantly (p = 0.01) higher than that of patients with an enrollment phase angle < 6 degrees.

Reactance was directly correlated with albumin (r = 0.52, p < 0.0001) and total protein (r = 0.44, p < 0.05). Capacitance was directly correlated with body mass index (r = 0.35, p < 0.05), albumin (r = 0.32, p < 0.05), and blood urea nitrogen (BUN) (r = 0.44, p < 0.01), and inversely correlated with body weight (r = —0.51, p < 0.0001). Phase angle was directly correlated with all of the biochemical markers of nutrition, such as albumin (r = 0.54, p < 0.01), total protein (r = 0.38, p < 0.05), creatinine (r = 0.28, p < 0.01), and BUN (r = 0.39, p < 0.05). By stepwise multivariate regression analysis, body weight (β = —0.60 p < 0.0001) and total protein (β = 0.32, p = 0.012) were significant determinants of resistance. Body weight (β = —0.31 p = 0.02) and albumin (β = 0.59, p < 0.0001) were significant predictors of reactance. Serum albumin (β = 0.53, p < 0.0001) was the only best predictor of phase angle in PD patients.

The BIA indices reflect nutrition status in PD patients, and may be useful in monitoring nutrition interventions.

Key words
Nutrition, bioelectrical impedance analysis (BIA), phase angle

Introduction
Bioelectrical impedance analysis (BIA) is a widely used and proven method for evaluating a patient’s body composition (1). It is relatively inexpensive, easy to use, safe, noninvasive, and portable. It also requires little operator training. Owing to relationships between various chronic diseases and body composition, the use of BIA measurement in body composition assessment has received much attention.

Undernutrition leads to a change in body composition and tissue function that is associated with
clinical complications. The early detection of alterations in body composition may well provide for early indication of developing malnutrition. Some reports have proposed BIA as an accurate and reproducible measure of body composition in various patient populations (2—4). Use of BIA in end-stage renal disease patients undergoing maintenance hemodialysis has been validated by several workers (5—7).

Malnutrition is highly prevalent in peritoneal dialysis (PD) patients. It is now well established that lower levels of serum markers of nutrition such as albumin, creatinine, and prealbumin are associated with increased mortality in PD patients (8—11). An assessment of malnutrition in PD patients should rely not only on serum biochemical measures, but also on several other indices of nutrition status, simultaneously including an analysis of body composition. In this study, we evaluated the usefulness of BIA in assessing nutrition and predicting survival of PD patients.

Patients and methods
Into this study, we enrolled 45 PD patients attending Long Island College Hospital’s outpatient facility from November 2000 to April 2001. The patients were followed until January 2002. Demographic and clinical data such as age, race, sex, body weight, height, cause and duration of end-stage renal disease (ESRD), diabetes, hypertension, and human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), were collected on enrollment.

Laboratory analysis
Non fasting blood samples were collected from the PD patients at the routine monthly visit and a multiphasic biochemistry screen including albumin, creatinine, blood urea nitrogen (BUN), total cholesterol, and prealbumin was performed.

Bioelectrical impedance analysis
On the day of the monthly blood collection, patients underwent BIA post dialysis (Bioelectrical Impedance Analyzer, Model BIA-101: RJL Systems, Clinton Township, MI, U.S.A.). The BIA measurements were taken by the same operator using an impedance plethysmograph (800’mA and 50’kHz). The BIA test was performed by placing four small electrodes on a hand and a foot of the patient, who was lying flat. The BIA analyzer was connected to the electrodes and the patient’s electrical impedance values, called resistance and reactance, were measured. Body composition, including body mass index (BMI), was determined using Cyprus’1.0 (RJL Systems).

Statistical analysis
Continuous variables are reported as mean± standard deviation. For selected comparisons between two group means, parametric (t-test) or nonparametric (Mann—Whitney test) were used wherever applicable. Correlations are reported as either the Pearson correlation coefficient or the Spearman rank correlation coefficient. Patient survival was analyzed with death as the final event. Transfer to another center or switch to another modality was regarded as censored information. Observed survival of PD patients was computed by the Kaplan—Meier method (12). The log rank test was used to compare survival curves. Calculations were performed using SPSS for Windows’9.0.1 (SPSS Inc., Chicago, IL, U.S.A.).

Results
The mean age of the PD patients was 50±15’years. Of the 45 patients, 24% had diabetes, and 56% were female. The causes of ESRD were diabetes (15%), hypertension (38%), glomerulonephritis (13%), polycystic kidney disease (7%), obstruction (2%), HIV (7%), and other/unknown (18%). The ethnic composition of the population was 70% African American, 7% white, and 23% Hispanic. The mean time on PD at enrollment was 55± 49’months. At enrollment, the mean serum albumin, creatinine, total protein, and BUN were 3.79± 0.6’g/dL, 11.9± 4.0’mg/dL, 7.38± 0.76’g/dL, and 48.5± 16’mg/dL respectively. Mean weight was 73.35± 16.36’kg. Mean body mass index was 25.7± 5.1. Mean resistance, reactance, capacitance, and phase angle were 524± 106’Ω, 57± 20’Ω, 678± 223’pF, and 6.2± 1.7’degrees respectively. Patients with diabetes had lower capacitance (555’pF vs. 713’pF, p’ = 0.007) and phase angle (5.35’degrees vs. 6.4’degrees, p’ = 0.05) than non diabetic patients.

The minimum, maximum, and mean follow-up were 2.76’months, 12.24’months, and 6.93’months respectively. During the study period, 4’patients died. Patients who survived had higher capacitance (486’pF vs. 697’pF, p’ = 0.07) and phase angle (4.65’degrees vs. 6.34’degrees, p’ = 0.008) than those who did not survive.
We stratified the patients by phase angle. The cumulative observed survival of PD patients with an enrollment phase angle $\oplus 6$ degrees was significantly ($p'= 0.01$) higher than that of patients with an enrollment phase angle $< 6$ (Figure 1).

Table I shows the correlations of BIA parameters with age and indices of nutrition in PD patients. Reactance ($r'= -0.36, p < 0.01$), phase angle ($r'= -0.47, p < 0.01$), and capacitance ($r'= -0.35, p < 0.05$) decrease with increasing age. Reactance was directly correlated with albumin ($r'= 0.52, p < 0.0001$) and total protein ($r'= 0.44, p < 0.05$). Capacitance was directly correlated with BMI ($r'= 0.35, p < 0.05$), albumin ($r'= 0.32, p < 0.05$), and BUN ($r'= 0.44, p < 0.01$), and inversely correlated with body weight ($r'= -0.51, p < 0.0001$). Phase angle was directly correlated with all the biochemical markers of nutrition such as albumin ($r'= 0.54, p < 0.01$), total protein ($r'= 0.38, p < 0.05$), creatinine ($r'= 0.28, p < 0.01$), and BUN ($r'= 0.39, p < 0.05$).

![Figure 1](Observed survival by phase angle (degrees) in peritoneal dialysis.)

**Discussion**

In the present study, we showed that, in PD patients, BIA parameters are correlated with levels of various serum markers of nutrition, such as albumin, creatinine, total protein, and BUN. Among all the BIA parameters, phase angle was strongly and consistently correlated with all the serum markers of nutrition (Table I). Phase angle appears to more comprehensively represent the patient’s nutrition status than resistance, reactance, and capacitance. That finding suggests the importance of phase angle in monitoring the nutrition status of PD patients. The importance of phase angle in monitoring the nutrition status of HD patients has been reported (13). Significantly lower levels of phase angle and capacitance in PD patients with diabetes as compared with patients who are non diabetic may be due to the fact that diabetic patients were more malnourished than non diabetic patients. We have previously reported lower levels of serum markers of nutrition in diabetic patients as compared with non diabetic patients (14).

Recently, several reports in the literature used BIA to analyze body composition in evaluating the nutrition status of dialysis patients (15—17). Passadakis et al (18) reported that BIA phase angle seems to be a

<table>
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<th>Variable</th>
<th>Age</th>
<th>Body weight</th>
<th>BMI</th>
<th>Total protein</th>
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<td>Reactance</td>
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<td>$-0.24$</td>
<td>$-0.14$</td>
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<td>$0.52^a$</td>
<td>$0.04$</td>
<td>$0.27$</td>
</tr>
<tr>
<td>Phase angle</td>
<td>$-0.47$</td>
<td>$0.13$</td>
<td>$0.1$</td>
<td>$0.38^b$</td>
<td>$0.54^b$</td>
<td>$0.28^a$</td>
<td>$0.39^b$</td>
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<tr>
<td>Capacitance</td>
<td>$-0.35$</td>
<td>$-0.51$</td>
<td>$0.35^b$</td>
<td>$0.04$</td>
<td>$0.32^b$</td>
<td>$0.38$</td>
<td>$0.44^a$</td>
</tr>
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*a* $p < 0.01$.

*b* $p < 0.05$.

*c* $p < 0.0001$.

BMI = body mass index; BUN = blood urea nitrogen.
simple method for the routine assessment of nutrition status in CAPD patients. Ikizler et al (6) showed that reactance values by BIA are a reliable indicator of hospitalization in HD patients. Chertow et al (5,19) reported that body cell mass and total body water derived from BIA were in excellent agreement with those determined by dual energy X-ray absorptiometry (DEXA) in a group of HD patients. They concluded that BIA is a valid and reliable method for assessing nutrition in maintenance HD patients. Recently Edefonti et al (20) reported that BIA is more sensitive than anthropometry in detecting alterations in body composition in children on PD. The BIA technique has also been reported to be a promising predictive tool for assessing nutrition in PD patients (7,21). However, phase angle did not correlate with body weight and BMI of PD patients, which agrees with the previous report on HD patients (13).

Over the past decade, parameters of nutrition have emerged as powerful predictors of mortality in dialysis patients (8,22). We have shown that demographic and biochemical indices reflecting nutrition status can predict long-term survival in PD (9,10,14,23—25). One of the most important observations in the present study was that phase angle was significantly associated with survival of PD patients. Phase-angle values were significantly higher among survivors than among non survivors ($p=0.008$). We observed that patients with phase-angle values below 6˚degrees at enrollment had significantly ($p=0.01$) lower 1-year survival than those with higher phase-angle values (Figure 1). Chertow et al (26) reported that, in HD patients, phase angles less than 4˚degrees were associated with an increased relative risk of death, even after adjustment for case mix and several indicators of nutrition. Prognostic importance for BIA-derived phase angle in HIV patients has also been reported (27). Maggiore et al (13) reported that, compared with the usual nutrition parameters, phase angle appeared to be a better prognostic index of HD patient mortality.

Conclusions
During the study period, only 4 patients died. At enrollment, those patients had much lower values for phase angle, capacitance, and reactance than did survivors. Diabetic patients had significantly lower phase-angle values (6.4˚degrees vs. 5.3˚degrees, $p=0.05$) and capacitance (713˚pF vs. 555˚pF; $p=0.0007$) than did non diabetic patients. Observed survival of patients with phase angles $\geq 6$˚degrees was significantly higher than that for patients with phase angles $<6$˚degrees. The BIA indexes were significantly correlated with age and markers of nutrition. Among the BIA parameters, phase angle was strongly and consistently correlated with all the nutrition markers, such as albumin, total protein, creatinine, and BUN. By stepwise multivariate regression analysis, determinants of resistance were body weight and total protein, determinants of reactance were body weight and albumin, and the determinant of phase angle was albumin.

Measurements of BIA during longitudinal clinical follow-up of dialysis patients and correlations of BIA analysis with serum markers of nutrition and clinical outcomes have not been evaluated. Further studies with longer follow-up of patients are required to determine the predictive power of phase angle or other BIA parameters, or any association between phase angle and other BIA parameters with morbidity and cause-specific mortality.

Acknowledgments
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References
Usefulness of BIA in PD Patients


17 Dumler F. Use of bioelectric impedance analysis and dual-energy X-ray absorptiometry for monitoring the nutritional status of dialysis patients. ASAIO J 1997; 43:256—60.


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Effects of Human Recombinant Erythropoietin on Inflammatory Status in Peritoneal Dialysis Patients

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Treatment with recombinant human erythropoietin (rHuEPO) in dialysis patients has been associated with improvement of nutritional and immune status through an increase of cytokine production [such as tumor necrosis factor α (TNFα)]. The high cytokine production can be a double-edged sword owing to the relationship of cytokines with the systemic inflammatory process, which has been associated with many complications of uremic status. Our aim was to analyze the medium-long term effects of rHuEPO treatment on uremic inflammatory markers.

We studied 45 peritoneal dialysis (PD) patients divided in two groups: a rHuEPO group (40—70 subcutaneous units/kg weekly) and a control group (no rHuEPO). The treated group was analyzed in four periods. Period 1 (rHuEPO-1) included 24 patients who had been using rHuEPO at long-term. Period 2 (rHuEPO-2; n = 21) looked at the patients 2 months after rHuEPO withdrawal. Period 3 (rHuEPO-3; n = 19) looked at the patients after 2 months under rHuEPO therapy. Period 4 (rHuEPO-4; n = 17) looked at the patients after 4 months on rHuEPO treatment.

With the reintroduction of rHuEPO, we observed a progressive, statistically significant (p < 0.05), and temporary increase in TNFα plasma levels, from 44 ± 24 pg/mL (rHuEPO-2) to 76.8 ± 25 pg/mL (rHuEPO-3), and then to 83 ± 27 pg/mL (rHuEPO-4). But in the long term, TNFα decreased [33.5 ± 10 pg/mL (rHuEPO-1)]. Similarly, albumin increased in the short term (3.73 ± 0.5 g/dL to 4 ± 0.5 g/dL, and then to 4 ± 0.43 g/dL), and then decreased (3.8 ± 0.44 g/dL). The normalized protein catabolic rate (nPCR) increased from 1 ± 0.2 g/kg daily to 1.12 ± 0.3 g/kg daily (rHuEPO-4). Long term, nPCR decreased to 1.06 ± 0.3 g/kg daily. Leptin initially increased (60.1 ± 48 ng/mL to 42.8 ± 22 ng/mL, and then to 38 ± 18.2 ng/mL); it also increased in the long term (62 ± 50.9, p < 0.05). At baseline, we found a significant positive linear correlation (p < 0.05) between TNFα and leptin (r = 0.52), TNFα and C-reactive protein [(CRP) r = 0.4], CRP and leptin (r = 0.49), fibrinogen and CRP (r = 0.78, p < 0.01), fibrinogen and leptin (r = 0.37), and leptin and body mass index [(BMI) r = 0.67].

In conclusion, rHuEPO induces a temporary, non inflammatory immune hyperactivity mediated by TNFα, without the adverse effects associated with that cytokine. By decreasing leptin, rHuEPO could increase food intake and improve the nutritional status of PD patients. At baseline, we confirm the existence of a chronic inflammatory process in uremia.

Key words
Recombinant human erythropoietin, inflammation, leptin, nutrition

Introduction
The introduction of recombinant human erythropoietin (rHuEPO) for treatment of uremic anemia has been an important advance, preventing complications frequently found at blood transfusion time. A decrease in cytotoxic antibody levels, improvement of anemia-derived symptoms and angina pectoris, exercise tolerance, nutritional status, and increase in libido and in immune response to infections and vaccines are some of the benefits of rHuEPO therapy (1).

The improvement of uremic immune deficiency has been associated with a direct effect of rHuEPO on the reticule—endothelial system, where it can induce an increase in cytokine secretion (2). The effect includes a rise in plasma levels of tumor necrosis factor α (TNFα), interleukin-1 (IL-1), IL-2, colony-stimulating factor, and interferon-γ (2,3). Most studies have analyzed the
Effect of rHuEPO on the immune system during the first months of treatment; long-term rHuEPO effects are less well known. The signs and symptoms of inappropriate uremic immune function and malnutrition in patients treated with rHuEPO and adequate dialysis still frequently persist (2—4). Moreover, the increase in TNFα production is a double edged-sword, because TNFα is an important mediator of systemic inflammation, which could be associated with uremic syndrome complications such as anemia, malnutrition, anorexia, hypertriglyceridemia, and acidosis (5,6). In the uremic pro-inflammatory state, several other substances (such as leptin) participate, with potential synergic action.

Our aim was to analyze the medium—long-term effects of rHuEPO treatment on plasma levels of inflammatory markers and to investigate the association of rHuEPO with the uremic systemic inflammatory process.

Patients and Methods

We studied 45 clinically stable peritoneal dialysis (PD) patients, 28 on continuous ambulatory peritoneal dialysis and 17 on automated peritoneal dialysis [13 on continuous cycling peritoneal dialysis (CCPD) and 4 on nightly peritoneal dialysis (NPD)]. The group included 20 men and 25 women, ranging in age from 25 to 86 years (mean 55.2 ± 13.4 years). The mean period on PD was 29.3 ± 28.6 months. No acute or chronic active disorders were present during the 3 months prior to the study. Patients with recognized endothelial disease (vasculitis, scleroderma, malignant hypertension), and those with immunosuppression were expressly excluded.

The patients were divided into two groups according their use of rHuEPO: the rHuEPO group (n= 24), 40—70 subcutaneous units/kg weekly of EPO; and the control group (CG, n= 21), no EPO. Both groups were matched according to clinical characteristics, biochemical data, and clinical atherosclerotic score (CAS), grade 1 being the lowest CAS, and grade 4 being the highest (7).

The rHuEPO group was analyzed over four different periods. Period 1 (rHuEPO-1) included 24 patients who had been using rHuEPO on a long-term basis (6—18 months). Period 2 (rHuEPO-2) included 21 of the original 24 patients at two months after rHuEPO withdrawal. Period 3 (rHuEPO-3) included 19 of the 21 patients from rHuEPO-2 at two months after restarting rHuEPO therapy. Period 4 (rHuEPO-4) included 17 of the 19 patients from rHuEPO-3 at four months on rHuEPO therapy. The controls were studied by two analytical determinations two months apart.

The following parameters were determined:

1. Dialysis adequacy was assessed by weekly urea Kt/V and equivalent of protein nitrogen appearance (PNA).
2. The measured markers of nutrition were serum albumin by the colorimetric method [Hitachi 704: Boehringer Mannheim, Mannheim, Germany (normal range: 210—390 mg/dL)]; transferrin and prealbumin by the immunonephelometric method [Behring Nephelometer Terminal S.A.: Behring-werte AG, Marbus, Germany (normal range for dialysis patients: >30 mg/dL)]; vitamin B12 (normal range: 150—750 pg/mL) and folic acid (normal range: 2—10 ng/mL) by radioimmunoassay; ferritin and iron by automated analyzer (Hitachi 911: Boehringer Mannheim).
3. Inflammatory marker TNFα was measured by Easia Medgenix Diagnostics SA, Fleurus, Belgium (normal range: 3—20 pg/mL) (5). C-reactive protein (CRP) was determined by the immunonephelometric method [ELISA Vectastain: Vector Laboratories, Burlingame, CA, U.S.A. (normal range< 0.5 mg/dL)]. Fibrinogen was determined by Clauss’A method (normal range: 200—400 mg/dL). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters, squared.
4. Leptin (body fat marker and component of inflammatory processes) was measured by radioimmunoassay (RIA) using a polyclonal antibody, raised in rabbits, against highly purified recombinant human leptin (Linco Research, St. Louis, MO, U.S.A.). The sensitivity limit was 0.5 ng/mL, and the linearity was 100 ng/L. Inter-assay and intra-assay variation were 7.2 ± 0.4 ng/mL and 25.6 ± 0.9 ng/mL respectively. The normal range in 115 healthy volunteers was 1—7.8 ng/mL.

Statistical Analysis

Results are given as medians and ranges. Comparisons between data groups were performed using a nonparametric test, the Mann—Whitney rank sum µ-test. We also used the linear Spearman
regression analysis and Student t-test for paired and non paired data. A p’ value less than 0.05 was considered statistically significant.

Results
Table I shows the general data for both groups. They were similar, except for a significantly higher serum ferritin and transferrin saturation index (TSI) in the control group. The treatment group showed lower values of residual renal function [as creatinine clearance (CCr)].

These changes were seen after rHuEPO withdrawal and reintroduction:

After rHuEPO withdrawal, a rapid decrease in the hemoglobin (Hb) level was noticed [11±1.8 g/dL (rHuEPO-1) vs. 9.2±1.5 g/dL (rHuEPO-2), p’<0.05]. After 2’months of rHuEPO re-introduction, the Hb value reached 10.6±1.5 g/dL (rHuEPO-3), p’<0.05. Two months later, Hb continued to increase to 11.1±1.4 g/dL (rHuEPO-4), p’<0.05.

In relation to the iron kinetic study, we found that ferritin and TSI showed significant increases. Ferritin levels in rHuEPO-1 and rHuEPO-2 were 429.6±422.5 ng/mL and 557±633 ng/mL respectively (p’<0.05), and a decrease was seen from rHuEPO-2 to rHuEPO-3 (412±431.7 ng/mL, p’<0.05). The mean ferritin in the rHuEPO-4 period was 403±320.7 ng/mL. The TSI also underwent a modification between rHuEPO-1 and rHuEPO-2 (19.6%±5.5% vs. 28.5%±16.7%, p’<0.05). The means of the TSI in the following periods were 20.2%±8.3% (rHuEPO-3) and 21.8%±7.5% (rHuEPO-4). Intravenous iron was administered in the various periods to achieve a TSI level above 20%.

Inflammatory markers
After re-starting rHuEPO in the patients, we found a progressive increase in levels of plasma TNFα, serum albumin, and PNA, and a decrease in the level of leptin. Nonetheless, at rHuEPO-4, albumin returned to a value similar to that at baseline (Table II).

After patients had been on rHuEPO treatment for two months, we found no differences in TNFα levels among patients with the lowest CAS as compared with the other groups (86.2±27 pg/mL vs. 73.3±24.2 pg/mL, NS). Notably, plasma leptin levels decreased in the rHuEPO-3 and rHuEPO-4 periods, but that decrease was not accompanied by a BMI change (Table’II).

### Table I
Baseline characteristics of the control and study [recombinant human erythropoietin (rHuEPO)] groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>rHuEPO</th>
<th>p Value</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53±11.2</td>
<td>55±13.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>131.6±12.6</td>
<td>143.8±20</td>
<td>&lt;0.05</td>
<td>&lt;140</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>80±10.1</td>
<td>79.8±8.7</td>
<td>NS</td>
<td>&lt;90</td>
</tr>
<tr>
<td>Diabetes (Y/N)</td>
<td>3a/16</td>
<td>11b/15</td>
<td>a&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>CAS (1/2/3/4)</td>
<td>8/8/2/1b</td>
<td>6/9/2/9b</td>
<td>b&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>219.1±42</td>
<td>206.5±35.4</td>
<td>NS</td>
<td>90—200</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>161.7±52.3</td>
<td>195.7±135.4</td>
<td>NS</td>
<td>40—170</td>
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<tr>
<td>CCr (mL/min)</td>
<td>2.7±2.3</td>
<td>1.02±1.47</td>
<td>&lt;0.01</td>
<td>Variable</td>
</tr>
<tr>
<td>Urea Kt/V</td>
<td>2.3±0.6</td>
<td>2.13±0.32</td>
<td>NS</td>
<td>&gt;1.8</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>60.2±19.2</td>
<td>80.8±45</td>
<td>&lt;0.1</td>
<td>59—145</td>
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<tr>
<td>Ferritin (ng/mL)</td>
<td>133.8±112</td>
<td>557.5±634</td>
<td>&lt;0.01</td>
<td>50—250</td>
</tr>
<tr>
<td>Transferrin (mg/dL)</td>
<td>274.3±57.3</td>
<td>246.8±56.7</td>
<td>NS</td>
<td>210—390</td>
</tr>
<tr>
<td>TSI (%)</td>
<td>18.4±5.5</td>
<td>28.5±16.7</td>
<td>&lt;0.05</td>
<td>20—40</td>
</tr>
<tr>
<td>Folic acid (ng/mL)</td>
<td>7.6±1.8</td>
<td>7.7±2.2</td>
<td>NS</td>
<td>2—10</td>
</tr>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>936.4±472</td>
<td>781.6±323.8</td>
<td>NS</td>
<td>150—750</td>
</tr>
</tbody>
</table>

NS’= nonsignificant; Y/N’= yes/no; CAS’= clinical atherosclerotic score (1’= better, 4’= worse); CCr’= creatinine clearance; TSI’= transferrin saturation index.

rHuEPO and Inflammation in Peritoneal Dialysis
The other markers of inflammation and nutrition, such as CRP, fibrinogen, prealbumin, or transferrin, showed no significant changes. Both groups (rHuEPO and CG) showed the following positive, significant linear correlations at baseline: TNFα and leptin (r = 0.52, p < 0.05), TNFα and CRP (r = 0.4, p < 0.05), CRP and leptin (r = 0.49, p < 0.05), fibrinogen and CRP (r = 0.78, p < 0.01), fibrinogen and leptin (r = 0.37, p < 0.05), leptin and BMI (r = 0.67, p < 0.05), and PNA and Cr (r = 0.38, p < 0.05). Significant negative linear correlations were found between TNFα and Cr (r = −0.4, p < 0.05), TNFα and prealbumin (r = −0.4, p < 0.05), and PNA with leptin (r = −0.52, p < 0.05). Markers of nutrition also correlated positively: prealbumin and albumin (r = 0.59, p < 0.05), cholesterol and albumin (r = 0.43, p < 0.05), and albumin with transferrin (r = 0.44, p < 0.05). In relation to the rHuEPO-3 and rHuEPO-4 periods, TNFα lost all of its statistical relationships with the remaining markers of inflammation and nutrition, maintaining positive linear correlation only with albumin in rHuEPO-3 (r = 0.37, p < 0.05) and rHuEPO-4 (r = 0.35, p < 0.05).

**Discussion**

The most interesting finding of this study is the short—medium-term increase in TNFα plasma levels that was induced by rHuEPO. That phenomenon was not associated with a worsening of uremic inflammatory status.

The beneficial effects of uremic anemia treatment with rHuEPO are unquestionable. One of those effects is improvement in immune response through increase in cytokine secretion (2,3), B’ cell and T’ cell function (2), vaccine responses (1), and phagocyte glycolytic activity (8). Those studies suggest that the improvement in the humoral immune response was due to an increase in TNFα, interferon-γ, IL-1, and IL-2 production (2,3), because TNFα has a central role in the immune response.

Nonetheless, we recently observed a close association between high plasma TNFα levels and many negative systemic effects which may be part of uremic syndrome, including anorexia, acidosis, hypertriglyceridemia, malnutrition, anemia, and uremic neuropathy (5,6). Here we confirm some of those adverse effects, as demonstrated by the negative baseline relationship between TNFα and markers of nutrition. According to our findings, the increase in TNFα level was not associated with an increase in other inflammatory markers such as CRP, fibrinogen, prealbumin, or transferrin. We thus conclude that rHuEPO-induced TNFα production is not associated with an inflammatory systemic response. On the contrary, we might interpret our findings as a temporary benefit of the rHuEPO effect, at least in the immune response. Nevertheless, we consider that TNFα may also be produced by damaged endothelial cells (9).

In addition, we recently demonstrated a negative effect of rHuEPO on endothelium, including a decrease in the fibrinolytic capacity, an increase in endothelial cell turnover measured by an increase in thrombomodulin (a marker of dead cells), and an increase in nitric oxide release (10). We therefore suggest that rHuEPO-induced TNFα production may be due to endothelial damage by direct effect of rHuEPO. The high TNFα levels found in patients with high CAS scores in this study, the high TNFα levels in uremic patients with endothelial dysfunction (11), and endothelial cells of aged rats (9) support our idea. Further studies are needed to establish the significance of our findings.

If the temporary elevation of TNFα associated with rHuEPO has a role in the correction of uremic...
immune deficiency, it may be mediated by activation of NADPH oxidase metabolism activation in neutrophils. That hypothesis is supported by in vitro experiments, which have shown that rHuEPO interacts with IL-3, IL-1, granulocyte/macrophage colony-stimulating factor and thrombocytopenias-stimulating factor (8). That effect is still controversial, however, because transfusions also improve the immune deficiency of uremia (1), and the decrease in iron overload associated with rHuEPO treatment also improves granulocyte phagocytosis capacity (12). It could thus speculated that the best moment for vaccine administration is immediately after correction of anemia.

Leptin is a 16-kDa protein synthesized by adipocytes in proportion to fat stores. Its physiologic effect is to inhibit hypothalamic synthesis and release of theorepeptide Y (NPY), the most potent orexigen known. Leptin is cleared by the kidneys, and plasma levels are elevated in hemodialysis and PD patients, in whom it could play a role in uremic anorexia (13). Treatment with rHuEPO has been associated with improvement of nutritional status, possibly due to an increase in appetite and correction of metabolic uremic anemia—related disorders (14). A decrease in leptin levels has recently been observed in 15’hemodialyzed patients after rHuEPO treatment (15). Our results confirm that finding, showing an improvement in nutritional status, as well as increases in albumin and PNA (Table II). We therefore could suggest that an increase in food intake is the mechanism by which rHuEPO improves nutritional status, although the effect seems to be a medium—long-term one.

According to the data of Kokot et al (15), leptin suppression by rHuEPO occurs over 12’months of treatment. Leptin and TNFα may have a synergistic role in malnutrition in dialysis patients, because both are inflammatory proteins able to induce anorexia (16). Our data confirm that association (baseline linear correlation). Recently, Stenvinkel et al (17) defined an interesting association between malnutrition, inflammation, and atherosclerosis (MIA syndrome). According to our data at baseline, the significant linear correlation between the various inflammatory parameters supports the MIA hypothesis. The use of rHuEPO was associated with changes in those relationships, possibly modifying the deleterious systemic metabolic context. Although inflammation plays an important role in the comorbidity of dialysis patients, evidence from longitudinal studies analyzing cytokine and CRP fluctuations is insufficient.

Conclusions
Treatment with rHuEPO is associated with a temporary elevation of plasma TNFα levels of non inflammatory character, because that elevation is not accompanied by the expected adverse effects of the cytokine. At baseline, we confirm data compatible with a chronic inflammatory process in uremic patients. Recombinant human erythropoietin could improve the nutritional status of PD patients through an increase in food intake mediated by a decrease in leptin production.

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References


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