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Relationships among inflammation nutrition and physiologic mechanisms establishing albumin levels in hemodialysis patients

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Relationships among inflammation nutrition and physiologic mechanisms establishing albumin levels in hemodialysis patients.

Background. Serum albumin concentration is a balance among its synthesis rate, fractional catabolic rate (FCR), distribution, dilution in the plasma pool and external loss. The physiologic bases for establishing the level of serum albumin in hemodialysis patients have not been defined despite the association of hypoalbuminemia with excess mortality. Albumin concentration is associated with the levels of several acute phase proteins (APPs), C-reactive protein (CRP), α1 acid glycoprotein (α1 AG), or ceruloplasmin, and with nutritional markers, such as normalized protein catabolic rate (nPCR).

Methods. To establish the relationship among parameters that regulate albumin levels and markers of nutrition and inflammation, we injected [^{125}I]-albumin, into 64 hemodialysis patients enrolled in the HEMO study to measure albumin distribution, synthesis and FCR. These variables were related to the levels of acute phase proteins (APPs), nPCR, body mass index (BMI), external albumin loss as well as demographic variables. Albumin distribution, synthesis and FCR were calculated from kinetic modeling, as was the initial plasma volume (PV). Serum albumin, transferrin, CRP, ceruloplasmin and α1 AG were measured weekly. Dialysate was collected during one dialysis each week to measure albumin loss. Results were analyzed by multiple linear regression.

Results. Albumin concentration correlated with its synthesis rate and FCR, but not with PV or its distribution between the vascular and extravascular pools. Albumin concentration also correlated with nPCR and α1 AG. However, albumin synthesis was directly related most strongly to PV and BMI (or nPCR), but not to levels of APPs. By contrast, albumin FCR correlated positively with both α1 AG and ceruloplasmin.

Conclusion. Albumin concentration in dialysis patients changes with inflammation and nutritional status through their effects on albumin catabolism and synthesis, respectively. Within the range of albumin levels in these patients, nutritional variables primarily affected albumin synthesis while inflammation caused hypoalbuminemia by increasing albumin FCR. Albumin synthesis also increased in proportion to PV. The result of this is that PV expansion does not contribute to hypoalbuminemia.

Key words: albumin synthesis, fractional catabolic rate, plasma volume, acute phase, C-reactive protein, ceruloplasmin, α1 acid glycoprotein, nutrition, body mass index, nPCR.

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Hypoalbuminemia is a powerful predictor of mortality in dialysis patients [1, 2]. Inflammation itself alters albumin levels. Low albumin levels and mortality correlate with markers of inflammation, either cytokines [3], or acute phase proteins [4, 5]. Several investigators have proposed that the process is a reduced rate of albumin synthesis [6] based on the finding that albumin mRNA and synthesis in the liver is reduced following trauma [7] or administration of either cytokines [8] or endotoxin [9, 10].

The finding that the absolute rate of albumin synthesis is decreased in the presence of inflammation or trauma by no means, however, has been uniform. Ruot et al noted that albumin mRNA and albumin synthesis relative to total hepatic mRNA and total hepatic protein synthesis decreased in the livers of rats with sepsis, although the absolute rate of albumin synthesis did not decrease [11]. The rats developed hypoalbuminemia, but the investigators concluded that the mechanism was not one of decreased albumin synthesis. Instead, fractional albumin synthetic rate increased, suggesting that the primary cause of hypoalbuminemia was an increase in albumin FCR. Von Allmen, Hasselgren and Fischer reported that albumin synthesis in rats was increased by four days after sepsis induced by celiac puncture [12], and Koj and McFarlane found that albumin synthesis was actually increased following endotoxin injection [13]. The rate of

albumin synthesis also has been reported to be increased in patients following head trauma, despite a decrease in serum albumin concentration [14]. Similarly, albumin synthesis was increased in hypoalbuminemic cancer patients [15]. Thus, the conclusion that trauma- or sepsis-induced hypoalbuminemia is due to suppression of albumin synthesis is controversial.

The concentration of any protein in plasma is a function of its rate of synthesis, its fractional catabolic rate (FCR) plus external loss and its volume of distribution. We previously established that hypoalbuminemic hemodialysis patients had a decreased rate of albumin synthesis when compared to those with a normal albumin concentration [16]. Those results were obtained in two small groups of patients, six each, selected from the lowest and highest quartiles of albumin concentration in the available population. While there was no difference in dietary calorie or protein intake between these two groups there was evidence for activation of the acute phase response in those with hypoalbuminemia. To investigate the relationships among albumin synthesis and catabolism and serum concentration in dialysis patients more completely and to identify determinants controlling albumin synthesis in hemodialysis patients, we measured albumin distribution and turnover rate in 64 hemodialysis patients who were participating in the National Institutes of Health sponsored hemodialysis (HEMO) study of morbidity and mortality in dialysis patients [17]. The patients reported here were sequentially recruited to an ancillary study to measure albumin synthesis and markers of inflammation longitudinally and survived the six week period following injection of [^{125}I] albumin in order to permit accurate kinetic modeling of albumin turnover and distribution.

METHODS

Patient selection

The individual Institutional Review Boards at each institution approved the protocol and informed consent was obtained from each patient. Sixty-four patients enrolled in the National Institutes of Health HEMO Study at the University of California Davis (Sacramento, CA), at Beth Israel Medical Center (New York, New York) and at Emory University (Atlanta, GA) were recruited to participate in a longitudinal study of the relationship between the levels of acute phase proteins and serum albumin concentration. All patients who had been randomized into one of the four HEMO treatment groups were eligible for enrollment in this study. During the initial six weeks albumin turnover rate was measured as previously described [16].

The 64 patients had been on dialysis for a mean time of 6.44 $\pm$ 3.45 years (range 1.32 to 21.56 years; median 6.40 years; 25th percentile, 3.73 years; 75th percentile, 7.68 years). Thirty-two patients were African American, and 38 patients had diabetes mellitus. Renal failure was caused by hypertension in 14 patients, 9 patients had glomerulonephritis, 2 had adult polycystic kidney disease, one had systemic lupus, one had chronic pyelonephritis, one had acute renal failure, one had analgesic nephropathy, one had been nephrectomized because of renal cell carcinoma, and the remainder had renal failure of uncertain etiology. Thirty-one patients had a subcutaneous polytetrafluoroethylene (PTFE) graft, 25 had arteriovenous fistulas and eight had a semi-permanent transcutaneous access (Permcath).

Nine patients were dialyzed using a F80 B (Fresenius Medical Care AG, Bad Homburg Germany), 12 with a F80 A, 26 using a F 8 (Fresenius) one with a F 6 (Fresenius), five using a CA210 dialyzer (Baxter Healthcare Corporation, Deerfield, IL, USA), seven using a CT190G dialyzer (Baxter), and three using CT 190, (Baxter) dialyzer. All of these dialyzers were subjected to reuse with bleach/formaldehyde or hot citric acid. One patient was dialyzed with a Filtral 20 dialyzer (Hospal, Boca Raton, FL, USA) without reuse. All dialysis was performed using bicarbonate-based dialysate at a delivered bicarbonate concentration of 39 mEq/L.

Method of measuring albumin turnover

Approximately 10 μCi of [^{125}I] albumin was injected into a peripheral vein and blood samples of about 3 mL obtained periodically until plasma [^{125}I] levels decreased to 5% of original counts (in practice this required sampling for between 600 and 900 hours, that is, 25 to 38 days). Initially blood was sampled at 15, 30 and 60 minutes, then at three and five hours. Thereafter blood was drawn prior to and following each hemodialysis for the 600 to 900 hour period. Three 400-μL aliquots of from the sample were then counted in a gamma counter (Searle, Analytics, Des Plaines, IL, USA) for 10 minutes. The plasma radioactivity disappearance curve was integrated by use of the numerical model using SAAM II [18].

Prior to counting, serum was precipitated using 10% trichloroacetic acid (TCA) to remove non-protein bound ^{125}I and then redissolved in 1 N NaOH.

The average value for serum albumin concentration measured during the first day of the study (taken prior to dialysis) did not vary from the average pre-dialysis albumin values obtained throughout the six week period. However, the average serum albumin concentration between the pre- and post-dialysis values for individual patients was significantly different. Serum albumin concentration increased, sometimes by a considerable amount because of hemoconcentration during dialysis. The specific radioactivity of albumin, however, did not change between the pre- and post-dialysis values. Because albumin concentration was different at the beginning and at the end of hemodialysis, we were concerned that the clear-

ance of albumin would be either overestimated, by using only the predialysis albumin concentration, or clearance underestimated by using only the post-dialysis concentrations of albumin for the integration. Furthermore, it was not clear which average plasma albumin value should be used in the calculation of albumin turnover once the albumin clearance had been established. We used specific radioactivity rather than absolute counts because the use of specific radioactivity does not require the assumption that plasma albumin remains constant and all albumin measurements can be used. Instead of obtaining a clearance term, the integration yielded a rate for albumin turnover in mg · h^{-1} directly.

$$\text{Total albumin turnover} = \frac{D}{\int_0^\infty \frac{1}{A} \cdot C \cdot dt} \qquad \text{(Eq. 1)}$$

where D is the total number of counts injected at time = 0 (dose), A is albumin concentration in mg/mL at each time point, and C is the trichloroacetic acid (TCA) precipitable counts/mL of serum. Specific radioactivity is C/A at each time point. Unit analysis reveals that counts/min cancel and we are left with total albumin turnover/unit time.

Recognizing that specific radioactivity is counts/min/mg of albumin:

$$\text{Total albumin turnover} = \frac{D}{\int_0^\infty SA \cdot dt} \qquad \text{(Eq. 2)}$$

where SA is counts/min/mg of serum albumin (specific radioactivity).

Plasma volume was calculated by isotope dilution: the counts of [^{125}I] obtained during the first 60 minutes after the injection were extrapolated to t = 0. Plasma albumin mass (PAM) is the product of initial plasma albumin concentration (the average of serum albumin concentration from each time point during the first hour) and plasma volume.

Similarly, specific radioactivity was used to directly calculate total albumin mass, rather than first calculating a steady state volume of distribution (VDss) [19, 20], so as to utilize all albumin values measured during kinetic analysis rather than an average value for serum albumin concentration. Total albumin mass was calculated directly, without calculating its volume of distribution first:

$$\text{TAM} = D \cdot \frac{\left(\int_0^\infty \frac{t}{A} \cdot C \cdot dt\right)}{\left(\int_0^\infty \frac{C}{A} \cdot dt\right)^2} \qquad \text{(Eq. 3)}$$

where D is the total counts injected (dose), A is the plasma albumin concentration at each time point, t is time since injection in hours, and C is TCA precipitable counts/mL of plasma. Since a concentration term is included at each time point integrated, this relationship yields a mass term rather than a volume term.

Recognizing that specific radioactivity is counts/unit mass of albumin:

$$\text{TAM} = D \cdot \frac{\left(\int_0^\infty t \cdot SA \cdot dt\right)}{\left(\int_0^\infty SA \cdot dt\right)^2} \qquad \text{(Eq. 4)}$$

where SA is specific radioactivity of serum albumin in counts/min/mg of albumin.

Method of measuring trans-dialyzer albumin loss

We also determined the effect of trans-dialyzer albumin losses on albumin homeostasis. Since collection of all dialysate from a given treatment is cumbersome, the value obtained was compared by proportionally splitting the stream throughout a treatment (this technique collected 1% of the total dialysate) versus collecting spot samples and then estimating total albumin losses from that concentration and the dialysis flow rate [16].

Albumin fractional catabolic rate (FCR) was calculated as the percent of the plasma albumin pool removed per day, assuming a steady state minus measurable albumin losses. Since these patients were anuric and there were no urinary albumin losses, only the average daily dialysis losses through the dialyzer were used for this calculation. For this value we used the average loss during each dialysis session times 3/7, to account for the fact that dialysis was only delivered three days a week.

$$\text{FCR} = (\text{Turnover rate} - \text{measured albumin losses})/\text{PAM} \qquad \text{(Eq. 5)}$$

Laboratory methods

Serum albumin obtained at each point on the day of injection of [^{125}]I albumin, and then prior to and following each dialysis for the six week period was measured in duplicate using bromcresol green. All other proteins (transferrin, ceruloplasmin and α1 AG) were measured initially and then weekly by rate nephelometry using a Beckman Array automated nephelometer (Beckman Instruments, Fullerton, CA, USA) [21]. Albumin in dialysate was also measured using nephelometry, with a lower limit of detection of 2 mg/L. All nephelometric measurements were made in duplicate in each of two optical systems. The average of these values was used for calculations. The intra-assay coefficient of variation for albumin was 0.22%, with an inter assay coefficient of variation ranging from 2.42% to 3.16% over the usable range of the assay. The intra-assay coefficient of variation for α1 acid glycoprotein was 0.14%, with an inter-assay coef-

ficient of variation ranging from 3.07% to 5.50% over the usable range of the assay. The intra-assay coefficient of variation for ceruloplasmin was 0.27%, with an inter-assay coefficient of variation ranging from 4.44% to 9.18% over the usable range of the assay. The intra-assay coefficient of variation for transferrin ranged from 0.026% to 0.07%, with an inter-assay coefficient of variation ranging from 2.56% to 4.38%.

Normalized PCR was measured monthly using a double pool method [22].

Summary of the statistical analysis for predicting albumin synthesis

To determine the factor that principally determined albumin concentration, first, multiple regression analysis was performed using the initial value of serum albumin concentration in our cohort of 64 hemodialysis patients as the dependent variable and a function of albumin synthetic rate (turnover /1.73 m^2), fractional catabolic rate (FCR; % of the plasma pool that was removed per day after subtraction of the amount lost through the dialysis membrane), plasma volume as % body weight (PV) and distribution of albumin between the vascular and extravascular compartment established kinetically (that is, plasma albumin mass/total albumin mass; (PAM/TAM). The initial albumin concentration was calculated as the first six-week average for albumin after injection of [^{125}]I albumin. All other averages for the other longitudinally collected proteins (and nPCR) were also the first six-week average value.

Specific biochemical and demographic variables (levels of acute phase proteins, age, cause of renal failure) also could influence baseline albumin concentration, and so a separate regression model was constructed for baseline serum albumin concentration as a function of these variables.

The next goal was to fit a regression model for albumin turnover rate to results from the cohort. To achieve this, we examined a number of potential predictors including CRP: α1 AG, ceruloplasmin, transferrin, nPCR, initial plasma volume percent (expressed as % body weight), BMI, age, vascular access (fistula, AV graft, or transcutaneous access), ethnicity (African American, Caucasian, Hispanic, Asian), gender, and the presence or absence of diabetes. There were insufficient numbers of patients having other causes of renal failure to perform meaningful analysis based on other causes of renal failure. All non-African American patients were combined for statistical analysis because of the low numbers of other ethnic groups.

The albumin synthesis rate correlated most strongly with plasma volume and with either nPCR or with body mass index (BMI). If both the latter were entered into the regression model, BMI eliminated nPCR as a predictor of albumin synthesis rate. Significantly and surprisingly, of the acute phase proteins only α1 AG correlated with albumin turnover and the correlation coefficient was positive.

The relationships between albumin concentration and that of acute phase proteins and nPCR that had been previously reported also were evaluated [23, 24]. We found that this relationship remained significant for both albumin and CRP (after log transformation; Fig. 1) or for α1 AG (Fig. 2) or ceruloplasmin. Having established this, we then analyzed whether the concentration of any of the acute phase proteins interacted in any way with any of the determinants of serum albumin concentration other than albumin synthetic rate.

Once the albumin fractional catabolic rate (FCR) was established as one determinant of initial albumin concentration, further analysis was performed to determine the relationships between albumin FCR and acute phase protein and serum albumin concentration.

A number of variables used are normalized to adjust for differences in body mass and composition. Albumin turnover was adjusted for body surface area [BSA (m^2) = $0.007184 * kg^{0.425} * cm^{0.725}$], BMI was a measure of body composition by adjusting weight for height (kg/m^2), and plasma volume is adjusted for body size as percent body weight. To assure that our results were not a consequence of artifactual coupling of these parameters the results also were analyzed using total albumin turnover, absolute plasma volume, absolute protein catabolic rate and body weight as variables.

RESULTS

Table 1 shows the baseline characteristics of patients that were included in the model, plus the average concentrations of CRP, transferrin, ceruloplasmin, and α1 AG during the six week period of observation. There was no significant difference between the average plasma albumin concentration averaged over the entire period of the turnover study and the initial serum albumin concentration so the average albumin value was shown. Albumin turnover rate, plasma mass and total mass were normalized by body surface area and plasma volume as percent body weight.

Significant albumin losses could only be detected in 7 of the 64 patients and these were primarily related to use of CT 190 dialyzers (even without reuse) or F80B dialyzers after reuse with bleach (albumin loss did not begin before reuse 4). By contrast, albumin losses could be detected with the CT 190 dialyzers even with the first use led to losses of 0.38 ± 0.086 g/dialysis treatment. Reuse with bleach, but not with other methods increased the amount of albumin lost by either CT 190 or F80B dialyzers. The maximum loss recorded with the CT 190 occurred with the 20th reuse bleach (4.24 g/dialysis). The maximum loss with the F80B was only 1.15 g (on the

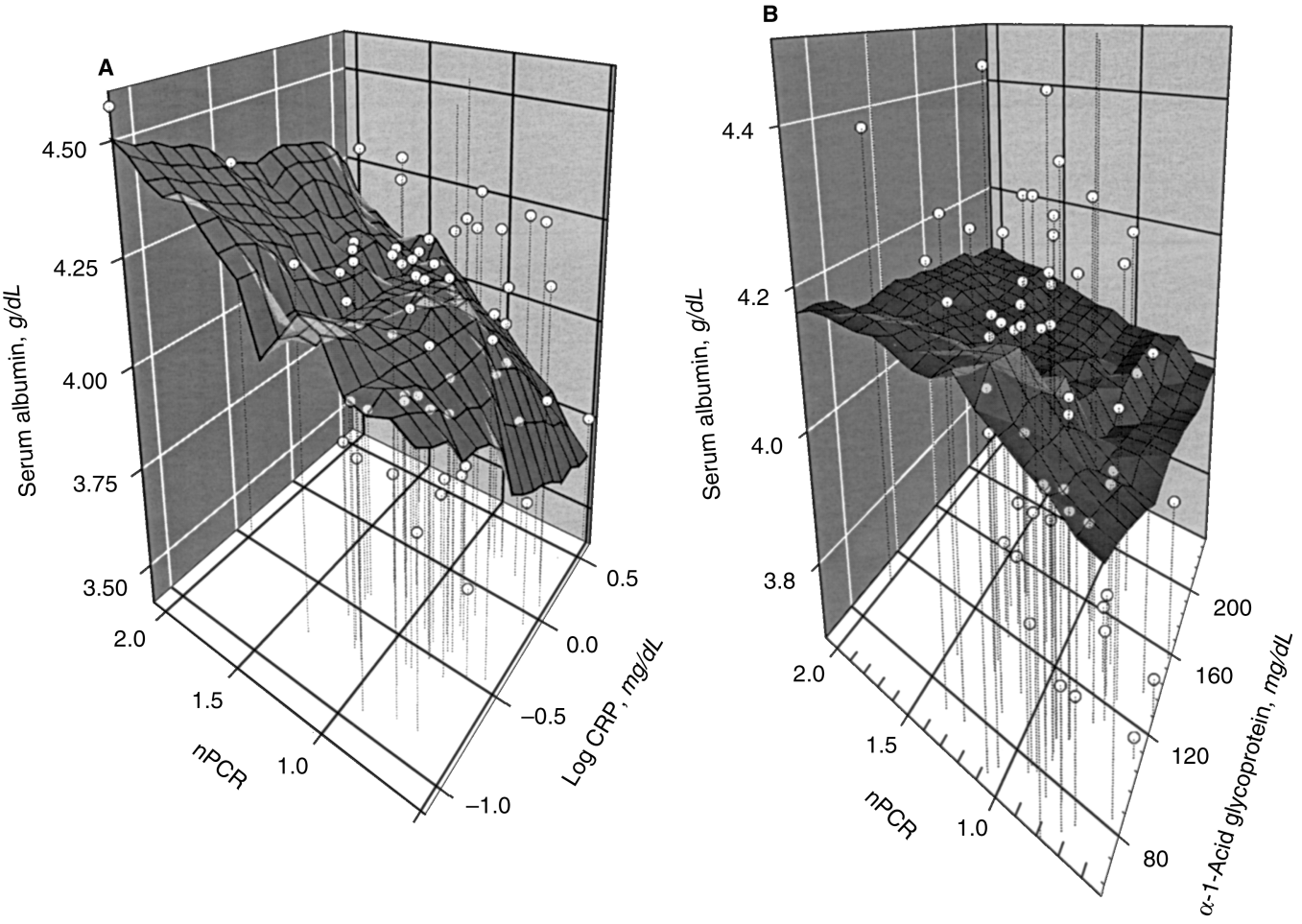

Fig. 1. (*A*) Relationship between serum albumin concentration (g/dL), nPCR and C-reactive protein (CRP; after log transformation). Albumin = 3.785 − (0.185 * Log CRP) + (0.258 * nPCR); $r^2 = 0.145$; log CRP, $P = 0.047$, nPCR $P = 0.04$. The surface is a best fit model extrapolated using the method of least squares. The symbols represent values obtained from the individual patients. The vertical line descending from each symbol is to orient the symbol to the XY plane. (*B*) Relationship between serum albumin concentration, nPCR and α1 acid glycoprotein. The surface and symbols are as described in panel A. Albumin = 4.109 − (0.00205 × α1 AG) + (0.229 × nPCR); $r^2 = 0.135$; α1 acid glycoprotein, $P = 0.026$; nPCR, $P = 0.023$.

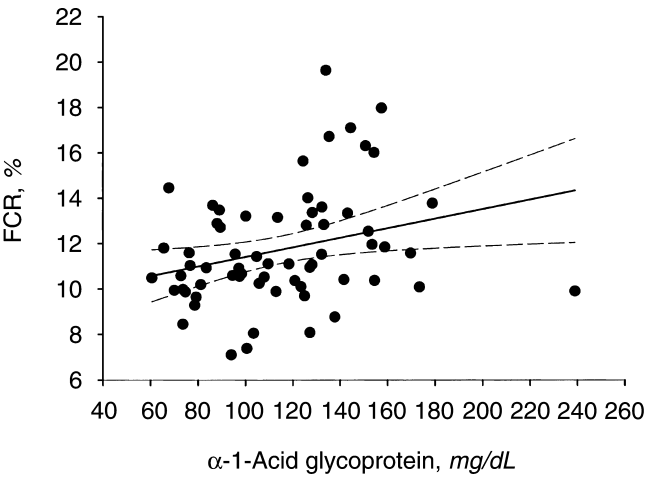

Fig. 2. Relationship between albumin fractional catabolic rate (FCR) and α1 acid glycoprotein (α1 AG). FCR = 9.309 + (0.0209 × α1 AG); $P = 0.023$. The regression is presented as the least squares fit (solid line) with 95% confidence levels (dashed lines).

19th reuse); and during the 20th reuse, it was 0.69 ± 0.30 g/treatment with this dialyzer. The maximum average albumin loss observed by any patient, however, was only 1.8 g in one patient, and among the 6 in whom any loss was measured the mean loss was 0.532 g/treatment. Thus, extracorporeal albumin losses played no significant role in establishing serum albumin concentration in this group of patients.

The age of the patients ranged from 19 to 81 with a median value of 58.5 years. The 25th percentile was at 49.5 years and the 75th percentile at 69 years. There were 37 males and 27 females in the population. We first examined the relationship between serum albumin concentration and the demographic and laboratory variables: α1 AG, ceruloplasmin, CRP (after log transformation), transferrin, nPCR, BMI (body weight/height^2), age, gender, ethnicity (African American vs. other ethnicity), presence of diabetes, and type of vascular access.

Table 1. Mean values for all parameters

Parameter	Mean	SD	Maximum	Minimum	Median	25%	75%
Albumin *g/dL*	4.10	0.254	4.77	3.62	4.13	3.89	4.27
CRP *mg/dL*	0.992	1.008	4.30	0.075	0.51	0.316	1.61
α1 AG *mg/dL*	115	34.2	239	60.6	114	88.3	135
Ceruloplasmin *mg/dL*	38.4	8.22	56.5	21.4	38.1	32.0	41.6
Transferrin *mg/dL*	180	30.594	241	112.85	182.167	157.67	205
nPCR *g/kg/day*	1.00	0.313	2.09	0.521	0.923	0.8	1.23
Surface area m^2	1.77	0.23	2.25	1.228	1.77	1.61	1.95
Synthesis *g/1.73* m^2*/day*	15.1	3.372	23.9	8.551	14.779	12.819	16.9
PAM *g/1.73* m^2	130	30.141	212	76.334	127.186	108.938	146
TAM *g/1.73* m^2	341	90.581	693	178.58	332.081	289.626	379
PAM/TAM	0.392	0.0711	0.622	0.200	0.388	0.346	0.437
Dialysate albumin loss *g/1.73* m^2	0.0269	0.109	0.774	<0.10	<0.10		
FCR *%/day*	11.7	2.504	19.6	7.105	11.066	10.1127	13.2
BMI kg/m^2	23.7	4.45	3.33	12.0	23.3	20.9	27.3

Abbreviations are: CRP, C-reactive protein; α1 AG, α1 acid glycoprotein; nPCR, normalized protein catabolic rate; PAM, plasma albumin mass; TAM, total albumin mass; FCR, fractional catabolic rate; BMI, body mass index.

Table 2. Demographic, inflammatory and nutritional determinants of serum albumin concentration

Coefficients:	Value	Standard error	*t* value	*P*
(Intercept)	4.287	0.233	18.368	<0.00001
α1 AG	−0.002	0.001	−2.318	0.024
nPCR	0.208	0.096	2.177	0.034
Age	−0.004	0.002	−1.618	0.111
Gender	0.118	0.06	1.955	0.055

Potential predictors used for this regression were: log.CRP, α1 AG, ceruloplasmin, transferrin, nPCR, BMI, age, gender, ethnicity (analyzed as black vs. other ethnic groups), cause of renal failure (analyzed as diabetes vs. other cause), and access as fistula vs. AV graft vs. transcutaneous access. One outlier was removed from model due to a serum albumin less than 2 SD below the mean value, $N = 63$. Abbreviations are in Table 1. Residual standard error for the regression was 0.231 with 58 degrees of freedom. Multiple R^2 was 0.226. F-statistic: 4.23 on 4 and 58 degrees of freedom, with a *P* value of 0.00448.

Table 3. Relationship between albumin synthesis, catabolism and distribution and serum albumin concentration in hemodialysis patients

Coefficients:	Value	Standard error	*t* value	*P*
(Intercept)	4.77	0.392	12.188	<0.001
Synthesis rate	0.039	0.016	2.447	0.017
PV %	−0.067	0.040	−1.695	0.095
FCR	−0.055	0.023	−2.402	0.020
PAM/TAM	−0.749	0.523	−1.433	0.157

Potential predictors were albumin synthesis rate (Synthesis rate), plasma volume expressed as percent body weight (PV %), albumin fractional catabolic rate (FCR), the ratio of plasma albumin mass to total albumin mass (PAM/TAM). One outlier was removed from model because of reduced albumin concentration (same patient as in model from Table 2). Residual standard error for the regression was 0.245 on 58 degrees of freedom. The multiple R^2 for the regression was 0.128. The F-statistic was 2.13 on 4 and 58 degrees of freedom, with a *P* value of 0.088.

One patient had a serum albumin concentration that was greater than 2 standard deviations from the mean of the group and was not included in this analysis for this reason. Therefore, results from 63 patients were used for these analyses. Of these variables, the ones that were included in the final model (maximum adjusted r^2) were age, gender, nPCR and α1 AG; only α1 AG and nPCR reached statistical significance (Table 2).

Next, the relationship between serum albumin concentration and only these variables was evaluated (Table 3). Serum albumin concentration correlated positively with its rate of synthesis and inversely with its FCR. While the distribution of albumin between the plasma and extraplasma compartment (PAM/TAM) did not significantly affect serum albumin concentration, there was a tendency for serum albumin concentration to decrease when adjusted for the rate of albumin synthesis and FCR.

The factors that controlled the two principal determinants of albumin concentration—FCR and albumin synthetic rate—were analyzed. Albumin FCR is a function of α1 AG, a marker of inflammation, BMI, a nutritional marker, age, and serum albumin concentration (Table 4). Of these, the influence of serum albumin concentration did not achieve statistical significance. When only the relationship between FCR and any of the acute phase proteins were examined by linear regression analysis, both α1 AG ($P = 0.023$) and ceruloplasmin ($P = 0.013$) correlated positively with FCR (Figs. 3 and 4). Notably, this was not true for CRP, as its relationship with FCR either with or without log transformation was not significant ($P = 0.127$). The only demographic variable that correlated with either CRP or α1 AG was age ($P = 0.0046$ for α1 AG and $P = 0.0495$ for CRP).

Albumin synthetic rate was most powerfully controlled by plasma volume and BMI (Table 5), although age, nPCR and α1 AG also entered into the model that was found to have the highest adjusted r^2 value. If BMI was excluded from the regression model, then albumin synthesis was predicted by plasma volume and nPCR ($P = 0.0046$).

As PV increased so did the rate of albumin synthesis, and as BMI (or nPCR) increased so did albumin synthesis. We suspected that there was an interaction between

Table 4. Determinants of albumin fractional catabolic rate

Coefficients:	Value standard	Error *t*	Value	*P*
(Intercept)	−3.507	4.3	−0.816	0.418
Alb	1.135	0.859	1.321	0.191
α1 AG	0.03	0.009	3.447	0.001
BMI	0.173	0.061	2.823	0.006
Age	0.053	0.02	2.631	0.011

Potential predictors used were serum albumin concentration (Alb), log CRP, α1 AG, ceruloplasmin, transferrin, nPCR, BMI, age, gender, ethnicity, as in Table 2, diabetes, access. No outliers removed from model, $N = 64$. Final model variables and results: Residual standard error: 2.17 on 59 degrees of freedom. Multiple R^2, 0.298. F-statistic, 6.27 on 4 and 59 degrees of freedom, with a P value < 0.0001.

both nPCR and BMI since both variables are related to protein energy status; nPCR reflects short-term (recent dietary intake of protein) while BMI reflects long-standing energy stores.

When absolute albumin turnover was analyzed as the response variable using total PCR, absolute plasma volume and weight as variables the essential relationships were unchanged (data not shown), suggesting that mathematical coupling within the variables was not responsible for the relationship to the response variable, albumin synthesis. The absolute rate of albumin synthesis was correlated with body weight ($r = 0.507$, $P < 0.001$), but this is anticipated because larger organisms would need to maintain a greater steady state rate of synthesis of plasma components in order to maintain normal plasma composition in the presence of increased body size.

When the interactions among albumin synthesis/1.73 m^2, plasma volume (% body weight) and nPCR were examined, they were highly significant (Fig. 4A). Similarly, albumin synthesis correlated independently with PV and BMI (Fig. 4B). The relationship between the absolute rate of albumin synthesis and absolute plasma volume was unchanged when weight was used as a variable as was the relationship between albumin synthesis and PCR unadjusted for body weight. When both weight and BMI are used as variables BMI, both variables are no longer significant predictors for (un-normalized) albumin synthesis rates. The correlation between BMI and albumin synthesis/1.73 m^2 ($P < 0.001$) with weight excluded from the model is stronger than is the relationship between the un-normalized rate of albumin synthesis and weight ($P = 0.023$) with BMI excluded from the model.

Plasma volume (4.47 ± 0.27 vs. 5.28 ± 0.23%; $P = 0.0126$) and nPCR (0.90 ± 0.58 vs. 1.10 ± 0.49; $P = 0.0111$) were significantly lower in African Americans, possibly explaining the lower rate of albumin synthesis in this cohort of patients.

Albumin synthesis was significantly increased in diabetic patients (16.12 ± 0.716 vs. 14.25 ± 0.592 g/1.73 m^2/day $P = 0.354$) and in those with AV fistulas compared to those with other types of vascular access ($P = 0.0092$).

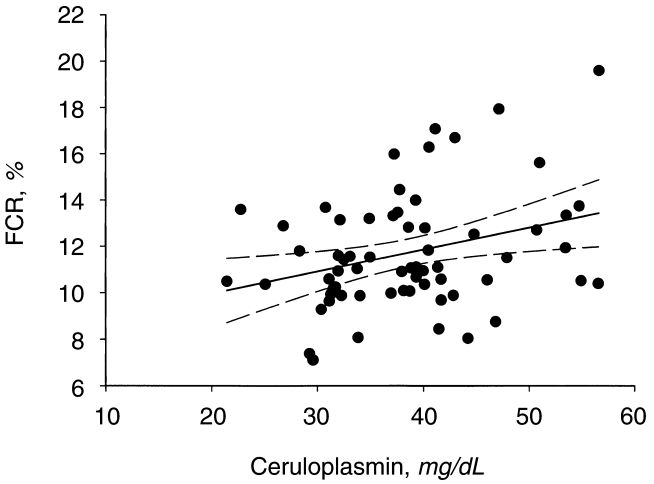

Fig. 3. Relationship between albumin FCR and ceruloplasmin. FCR = 8.058 + (0.0949 × ceruloplasmin); $P = 0.013$. The regression is presented as the least squares fit (solid line) with 95% confidence levels (dashed lines).

DISCUSSION

The reason for studying the mechanisms that change serum albumin concentration in dialysis patients is the close correlation between hypoalbuminemia and morbidity and mortality [1–5]. A low serum albumin has been linked to markers of nutrition [16, 25–28] and inflammation [21, 22] but this link does not detail mechanisms causing the low serum albumin level. While much attention has been focused on the regulation of albumin synthesis, alterations in albumin FCR also have been identified as playing an important role in sustaining albumin concentration during protein restriction [29, 30]. As albumin concentration declines, the normal homeostatic response is a decrease in albumin FCR [27]. We did not find this response in hemodialysis patients but did find that inflammation played an important role in regulating albumin FCR in this population. This is in agreement with observations in rheumatoid arthritis and systemic lupus [31]. These data are consistent with the conclusion that inflammation decreases serum albumin concentration in part by increasing its FCR and by suppressing the normal homeostatic relationship between serum albumin concentration and FCR.

It is of interest that while both ceruloplasmin and α1 AG correlated with albumin FCR, CRP did not. In cross sectional studies CRP has been correlated with albumin concentration measured contemporaneously, yet it has no predictive effect on future albumin levels [21, 22]. By contrast, both ceruloplasmin and α1 AG predict future albumin concentration [32], suggesting that whatever process is reflected by ceruloplasmin and α1 AG, the influence of this process with albumin concentration is different than the process that is reflected

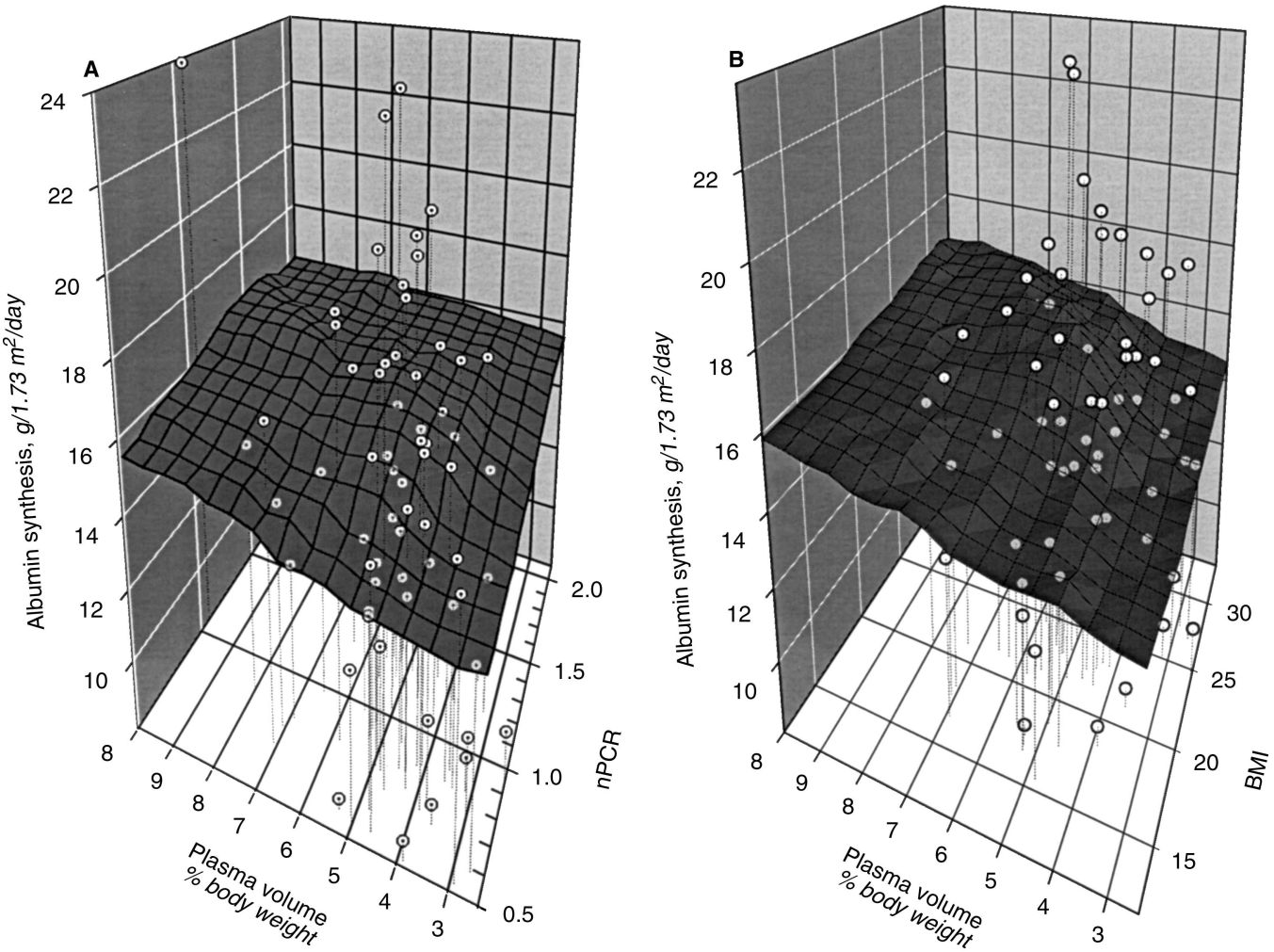

Fig. 4. (*A*) Relationship between albumin synthesis in g/1.73 m^2/day, plasma volume in percent body weight (PV%) at the time of the initial injection of [^{125}I] albumin and normalized protein catabolic rate in g/kg (nPCR). The surface is a best fit model extrapolated using the method of least squares. The symbols represent values obtained from the individual patients. The vertical line descending from each symbol is to orient the symbol to the XY plane. Synthesis/1.73 m^2/day = 7.292 + (0.867 * PV(%) + (3.444 * nPCR); $r^2 = 0.259$; PV %, $P = 0.005$; nPCR, $P = 0.007$. (*B*) Relationship between albumin synthesis in g/1.73m^2/day, plasma volume in percent body weight (PV%) at the time of the initial injection of [^{125}I] albumin and body mass index (BMI). Synthesis/1.73 m^2 = 2.812 + [1.803 * PV(%)] + (0.380 * BMI); $r^2 = 0.328$ * BMI; PV, $P < 0.001$; BMI, $P < 0.001$.

by a change in CRP level. While the processes regulating ceruloplasmin and α1 AG act over a long period of time, processes affecting CRP levels may have a more immediate but less long acting effect.

One mechanism whereby inflammation acts to cause hypoalbuminemia is to increase albumin FCR. Since the half-life of albumin is approximately 14 days, an effect on catabolism would require a prolonged period of time to be reflected by a change in serum levels. It is possible that we failed to find an effect of CRP levels on albumin FCR because of the high short-term variation in CRP levels even within the six-week period of observation, reducing its statistical power. The value used was the average obtained over the six-week period and thus the variability in CRP may have limited its statistical power.

Our measurements produced an average value for albumin FCR, synthesis and distribution of albumin over a six-week period. The methods were not designed, nor could they detect rapid shifts of albumin between pools. It is possible that the effect of the process reflected by CRP is rapid, such as a shift of albumin from the vascular to extravascular pool. If that is the case, then the measurements performed in this study would not detect these changes.

Age may increase albumin FCR, and contribute to hypoalbuminemia in elderly, putatively well-nourished elderly patients [33]. This is relevant because we indeed found that age was positively and significantly correlated with albumin FCR.

Albumin synthesis (adjusted for body surface area)

Table 5. Determinants of albumin synthesis

Coefficients	Value	Standard error	*t* value	*P*
(Intercept)	−7.216	3.874	−1.863	0.068
PV %	1.476	0.328	4.5	<0.001
α1 AG	0.022	0.011	2.075	0.043
nPCR	1.85	1.182	1.565	0.123
BMI	0.339	0.094	3.61	0.001
Age	0.045	0.025	1.815	0.075
Ethnicity	−1.203	0.518	−2.324	0.024

Potential predictors were PV (%), log. CRP, α1 AG ceruloplasmin, transferrin, nPCR, BMI, age, gender, ethnicity, as in Table 2, diabetes, access. No outliers removed from model, $N = 64$. Final model variables and results: Residual standard error: 2.64 on 57 degrees of freedom. Multiple R^2, 0.472. F-statistic, 8.48 on 6 and 57 degrees of freedom, with a P value < 0.0001.

correlated positively with BMI (a nutritional marker) and if this factor was eliminated, then it correlated with nPCR. The absolute rate of albumin synthesis correlated positively with total PCR (the product of body weight and nPCR) after adjusting statistically for body weight. These relationships would be expected from the effect of nutritional state on protein synthesis [34]. A more difficult relationship to understand was the powerful (and previously unknown) relationship between plasma volume and albumin synthesis rate: higher plasma volumes were related to higher albumin synthetic rates. This relationship was not due to artifactual mathematical coupling [35], since the relationship between albumin synthesis and plasma volume remained even when analyzed without adjustment for body size or weight. Perhaps the wide range of plasma volumes found in dialysis patients has uncovered a relationship that was obscured by the small range of plasma volumes present when kidney function is intact.

We measured albumin turnover rate kinetically by following disappearance of injected [^{125}I] albumin and plasma volume was measured using the same data. Both are based on knowledge of the dose injected, so an error in the dose administered will cause a change in the same direction of both the rate of albumin turnover (synthesis) and plasma volume. It is unlikely that such an error occurred, since the dose administered was counted on several occasions both by dilution of an aliquot of the material that was used for injection and by counting a dose sample vial supplied by the manufacturer. However, this relationship must be recognized as a potential shortcoming of the technique. It is reassuring that Giordano et al directly measured albumin synthetic rate using stable isotope methods and reported that albumin synthesis was increased in dialysis patients having normal serum albumin concentrations [36]. The reason that albumin concentration was not increased in his patients was that PV was increased, thus diluting a significantly expanded plasma albumin pool. Similarly, Imoberdorf et al found that albumin synthesis increased in subjects at high altitude in proportion to the plasma volume expansion that accompanied adaptation to altitude [37]. Of interest, although the absolute albumin synthetic rate increased significantly, albumin concentration did not. Thus, plasma volume expansion may well simulate albumin synthetic rate as part of a homeostatic mechanism.

Another unexpected relationship was the significantly reduced rate of albumin synthesis in African American patients. A possible mechanism is that the African American patients studied here also had a significantly lower nPCR.

A powerful regulator of albumin synthetic rate is long-term nutritional status with respect to energy and protein; either BMI or nPCR powerfully and independently controlled the rate of albumin synthesis (Fig. 4), while nPCR alone significantly controlled serum albumin concentration as did nPCR combined with a marker of inflammation, log CRP (Fig. 1) or α1 AG (Fig. 2). This latter observation is a corroboration of earlier observations [21, 37]. Second, measures of the activity of the acute phase response that is either reflected in the levels of short-lived (CRP) or long-lived (α1 AG) proteins strongly affected baseline serum albumin concentration independently of nutritional variables.

While both the acute phase proteins CRP and α1 AG are inversely correlated with serum albumin concentration we were unable to demonstrate any statistically significant interaction between the levels of any of the acute phase proteins, either individually or in combination, with the rate of albumin synthesis. We previously reported that albumin synthesis was significantly less in a group of hemodialysis patients having serum albumin levels <3.5 g/dL compared to those with a serum albumin concentration >4.0 g/dL [16]. In that study the levels of acute phase proteins also were significantly increased in those having the lower albumin concentration. Serum albumin concentration in this study was significantly greater than that reported by us previously [16]. Thus, it is possible that inflammation also reduces albumin synthesis in hypoalbuminemic patients.

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