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Randomized double-blind trial of oral essential amino acids for dialysis-associated hypoalbuminemia

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Background. Hypoalbuminemia is associated with substantial morbidity and mortality in dialysis patients.

Methods. Subjects with a mean three-month prestudy serum albumin of 3.8 g/dL or less and who demonstrated ≥90% compliance during a two-week run-in period were randomized to 3.6 g of essential amino acids (EAAs) or placebo three times daily with meals for three months. Randomization was stratified by dialysis modality and by severity of the hypoalbuminemia. The primary study outcome was change in the average of three monthly serum albumin measurements between baseline and follow-up.

Results. Fifty-two patients were randomized; 47 patients (29 hemodialysis and 18 peritoneal dialysis) met the predetermined primary analysis criteria. The mean compliance rates averaged 75, 70, and 50% at months 1, 2, and 3, respectively, and were similar for EAAs and placebo. Serum albumin in the hemodialysis patients, EAA versus placebo, improved [(mean ± SE) 0.22 ± 0.09 g/dL, $P = 0.02$]. Changes in peritoneal dialysis patients were not significant (0.01 ± 0.15 g/dL), but approached significance for the total study group (0.14 ± 0.08 g/dL, $P = 0.08$). Patients in the very low albumin strata (<3.5 g/dL) improved more than those in the low albumin strata (3.5 to 3.8 g/dL, $P < 0.01$). There was a significant correlation ($r = 0.83$, $P = 0.001$) within the hemodialysis EAA group between the baseline C-reactive protein level and improvement in serum albumin. Improvements were also seen in grip strength and SF-12 mental health score, but not in serum amino acid levels, SF-12 physical health score, or anthropometric measurements.

Conclusions. Oral EAAs induce a significant improvement in the serum albumin concentration in hemodialysis but not peritoneal dialysis subjects. Further study of their long-term effects on morbidity and mortality is warranted.

Hypoalbuminemia is a powerful predictor of poor outcome for patients on maintenance dialysis [1–5]. This association is evident not only for patients with subnormal serum albumin levels, but also for those whose levels are in the low normal range [1, 2, 4]. Several studies have reported the beneficial effects of amino acid supplements on a wide range of outcome measures. The interpretation of these results is limited, however, as only a minority of these studies were randomized [6–8], and in addition, none were double blind. We have previously reported the beneficial effects of essential amino acid (EAA) supplements to a very low-protein diet in preventing hypoalbuminemia at the onset of dialysis [9] and in improving survival on subsequent dialysis [10]. We therefore carried out the following investigator-initiated, industry-supported, randomized, double-blind, placebo-controlled study in order to examine whether oral EAA supplements could improve serum albumin levels in maintenance dialysis patients with hypoalbuminemia.

Key words: malnutrition, dietary supplement, aminogram, C reactive protein.

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METHODS

The study participants were selected from two urban, hospital-affiliated, dialysis units in Baltimore, Maryland, USA. For inclusion, subjects were required to be 18 years or older, not to have taken any investigational drug in the month prior to study entry, and to have a mean (−90, −60, −30-day prerandomization) serum albumin of 3.8 g/dL or less. Patients who, in the opinion of their primary nephrologist, were poorly compliant with medications or who were estimated to have a life expectancy of less than three months were excluded. The study protocol was reviewed by the institutional review boards of both participating centers. All patients gave informed written consent, which was obtained by a researcher other than the patient's primary nephrologist. All study subjects were volunteers and received no direct compensation, financial or otherwise, from their study involvement.

Patients underwent a two-week run-in period during which they took a placebo tablet on the same schedule

as for the actual study. At the end of the run-in period, compliance was assessed by weighing the pill container. The container held additional tablets over that necessary for two weeks of full compliance. Patients who demonstrated less than 90% compliance were excluded.

A computer-derived, stratified randomization scheme was prepared by the study sponsors prior to the start of the study. Study investigators were blinded to the randomization scheme and to the randomization block size. Patients were stratified prerandomization by dialysis modality (hemodialysis or peritoneal dialysis) and by albumin level (very low serum albumin, <3.5 g/dL, or low serum albumin, 3.5 to 3.8 g/dL). Patients were randomized to take five Aminess N® tablets or placebo three times a day with meals for three months. The study tablet contained 720 mg of amino acids, including nine L-EAAs (histidine 45 mg, isoleucine 60 mg, leucine 90 mg, lysine 65 mg, methionine 90 mg, phenylalanine 70 mg, threonine 65 mg, tryptophan 25 mg, valine 135 mg) and 75 mg of L-tyrosine. This preparation was based on a modification of the recommended daily intake suggested by Rose, as outlined by Alvestrand et al [11]. It is formulated specifically to address the disturbances in amino acid metabolism seen in uremia. The placebo tablets used in our study were identical in appearance to the EAA tablets. To help improve the quality of the mask, especially on first opening the pill container, a small quantity of methionine was added to the canister of drying agent in the placebo containers. Tablets were dispensed monthly. As with the run-in period, the returned pill containers, with the unused pills, were weighed to assess compliance. During the course of the study, subjects were blinded to their serum albumin results. No new dietary supplements were prescribed, and no other amino acid preparations were used. The patients' dialysis and routine medical care continued to be supervised by the patients' primary nephrologist without the involvement of the study investigators.

Outcome measures

At randomization (day 0) and at days 30, 60, and 90, postrandomization serum albumin, transferrin, and prealbumin levels were measured. To explore possible mechanisms leading to an improvement in serum albumin levels, an additional serum sample was obtained and frozen at −70°C. All laboratory tests were nonfasting and in the hemodialysis patients were performed prior to commencing dialysis. The primary study outcome measure was the difference between the follow-up mean (30-, 60-, and 90-days postrandomization) serum albumin and the baseline mean (−60, −30, 0-day prerandomization) serum albumin level. When one of the follow-up blood tests was missing, the available results were used. Blood tests measured during hospitalizations were not used.

At study entry and monthly thereafter, study subjects had anthropometric measurements, grip strength, and quality-of-life assessments performed. They were additionally questioned regarding the development of any adverse events or hospitalizations. Anthropometric measurements consisted of triceps skin fold thickness and midarm muscle circumference and were measured using a standard protocol [12]. Midarm muscle area (MAMA) was calculated from this formula:

$$\text{MAMA} = \text{midarm circumference} - (3.14 \cdot \text{triceps skin fold thickness}) \quad [13]$$

Anthropometric measurements were made after dialysis and were performed on the right side, unless a functioning access was present there. Grip strength measurements were performed on the dominant hand using a Jamar hydraulic dynamometer (Sammons Preston, Bolingbrook, IL, USA). The Short Form 12 (SF12) instrument was used to assess quality of life. Results were analyzed as outlined by Ware, Kosinski, and Kelly [14], with the calculation of a summary mental and physical health score. Values were standardized to the U.S. population, in which the population mean is 50 and the standard deviation is 10 [14].

A 24-hour dietary history was taken from patients at the beginning and end of the study by an experienced renal dietitian. The patient's 24-hour energy and total protein intake was calculated using the "Nutritionist 4" software package (N Squared Computing, Salem, OR, USA). The dietary assessment excluded the study medication. Urea kinetic studies were performed monthly for hemodialysis patients, using a two-blood urea nitrogen (BUN) sample and a variable-volume, single-pool urea kinetic model [15], and quarterly for peritoneal dialysis patients using total Kt/V measured using a standardized method [16]. Routine dialysis laboratory tests (basic chemistry, metabolic panel, and full blood count) were also monitored.

Laboratory assays

Blood tests from the two clinics were analyzed in their usual laboratories. Thus, each patient had all of his/her blood tests analyzed in the same laboratory. Albumin was assayed using the bromocresol green method for which the coefficient of variation in both laboratories during the study period was under 2.5%. Prealbumin, transferrin, and C-reactive protein (CRP) levels were measured by nephelometry. Serum amino acid levels were analyzed using ion exchange chromatography with a Beckman analyzer, model 6300. Serum free tryptophan was measured as previously described [17]. Free indoxyl sulfate was measured by the same high-performance liquid chromatographic (HPLC) method. Serum nitrates

and nitrites were measured as described by Everett et al, and their sum was calculated [18].

Statistical analysis

Data were independently double entered and electronically compared. The internal consistency of the data was assessed using time trend analysis and by examining outliers. The analysis plan was outlined in the original study protocol and prepared in detail prior to release of the randomization code. Normality was assessed using the Shapiro-Wilks test and with quartile-quartile plots [19]. The primary analysis was an intention-to-treat analysis of subjects who were alive, on dialysis, and had at least one serum albumin level measured at 45 or more days after randomization. Our primary analysis was an analysis of variance, with change in mean serum albumin as the dependent variable adjusted for dialysis center, dialysis modality, and albumin strata. We a priori hypothesized the possibility of interaction between the change in serum albumin and dialysis modality and/or the degree of hypoalbuminemia at baseline.

Estimates of the magnitude of change in mean serum albumin were obtained using least-squares linear regression. Routine diagnostic testing of the model included examination of the distribution of residual values and investigation of points with high leverage and/or influence [19]. In addition, the analysis was repeated using the Huber-White robust variance estimator [20]. Other study variables were examined using independent sample *t*-test, Mann-Whitney *U*-test, or chi-square test as appropriate. The correlation between CRP levels and albumin levels was examined using the nonparametric Spearman rank correlation coefficient. Analyses were performed using SPSS Base 7.5 (SPSS Inc., Chicago, IL, USA) and Stata release 5 (StataCorp, College Station, TX, USA).

RESULTS

Baseline data

Of the two clinics combined, approximately 174 out of a total of 393 patients met the study's entry criteria. Seventy-nine of these patients entered the study run-in phase, of which 52 patients (31 hemodialysis and 21 peritoneal dialysis) were subsequently randomized. However, five of these patients (4 active and 1 placebo) failed to meet the prespecified protocol requirements for inclusion in the primary analysis. The ineligibility of these patients was established in accordance with the study protocol and before the release of the randomization code. Three of these patients had no follow-up albumin at 45 or more days postrandomization because of transfer to another dialysis unit (1 subject), receipt of a cadaveric renal allograft (1 subject), and patient death (1 subject). The two remaining patients (1 active and 1 placebo) lacked the required follow-up outpatient serum albumin measurements, as they became ill within the first month of the study and underwent prolonged hospitalizations, which eventually proved fatal.

The study population was broadly representative of our inner-city dialysis population, with a predominance of black subjects. The mean age of the study group was 65 years, and the mean duration of dialysis was 3.4 years. Twenty-six patients were affiliated with the Good Samaritan Hospital (19 treated with hemodialysis and 7 with peritoneal dialysis) and 21 with the Johns Hopkins Hospital (10 treated with hemodialysis and 11 with peritoneal dialysis). The etiology of the renal failure was diabetes mellitus in 38%, hypertension in 38%, glomerulonephritis in 19%, and miscellaneous causes in 4% of subjects. Among the hemodialysis patients, 41% were dialyzed with a cellulose triacetate membrane, 31% with an AN69 membrane, 14% with a polysulfone membrane, and 14% with a cellulose acetate membrane. Only the cellulose triacetate membrane was reused, with a mean of 20 reuses. An arteriovenous access (either a graft or a fistula) was the predominant form of angioaccess used in the hemodialysis group, with an average blood flow of 450 mL/min, an average treatment duration of four hours and bicarbonate-based dialysate. Fifty-five percent of the peritoneal dialysis subjects were treated with continuous cycler peritoneal dialysis and 45% with traditional continuous ambulatory peritoneal dialysis; all patients used standard glucose-based dialysate. There were no significant differences in the underlying cause of renal failure, type of hemodialysis membrane used, or peritoneal dialysis modality between the EAA and the placebo groups.

The stratified randomization scheme resulted in an equal allocation of Aminess N® and placebo. Twenty-nine hemodialysis patients (15 in the very low albumin strata and 14 in the low albumin strata) and 18 peritoneal dialysis patients (14 in the very low and 4 in the low albumin strata) were randomized. The baseline demographic and laboratory results are shown in Tables 1 and 2. The study included nine patients with a body mass index of greater than 30; seven of these subjects were hemodialysis patients, of whom six were randomized to EAA treatment. As a result, the median (intraquartile range) body mass index for the hemodialysis active group was 30.6 ± 13.5 kg/m^2, while that for the placebo group was 24.6 ± 4.2 kg/m^2 ($P = 0.05$). Skin fold thickness and MAMA varied with body mass index, and therefore, these parameters were also significantly larger in the hemodialysis active as compared with the placebo group. Mean values of skin fold thickness and MAMA for hemodialysis EAA versus placebo subjects were 18.0 versus 12.4 mm ($P = 0.02$) and 29.8 versus 25.7 cm ($P = 0.05$), respectively. At baseline, there was no significant difference in grip strength between the EAA (mean grip strength 20.7 kg force) and the placebo group (mean

Table 1. Baseline demographics, serum chemistry and hematocrit

	Hemodialysis			Peritoneal dialysis		
	Aminess N®	Placebo	P[a]	Aminess N®	Placebo	P[a]
Number %	14 (48%)	15 (52%)	NS	9 (50%)	9 (50%)	NS
Age *years* [mean SD][b]	61.7 (17.7)	61.5 (16.6)	NS	60.7 (11.0)	55.8 (12.9)	NS
Gender *male/female*	3/11	7/8	NS	5/4	5/4	NS
Race *black/white*	12/2	10/5	NS	6/3	7/2	NS
Duration of ESRD[b] *months* [median (IQR[b])]	24.3 (43.3)	26.0 (58.0)	NS	30.9 (32.1)	38.12 (36.9)	NS
Baseline albumin[c] *g/dL* [median (IQR)]	3.57 (0.43)	3.47 (0.50)	NS	3.43 (0.42)	3.0 (0.63)	NS
Prealbumin *mg/dL* [mean (SD)]	29.79 (9.21)	25.67 (7.39)	NS	31.33 (11.88)	34.50 (10.37)	NS
Transferrin *mg/dL* [median (IQR)]	161 (34)	167 (72)	NS	170 (38)	153 (104)	NS
Bicarbonate *mmol/L* [mean (SD)]	22.2 (3.3)	21.5 (2.6)	NS	24.1 (4.9)	25.4 (2.9)	NS
Urea nitrogen *mg/dL* [mean (SD)]	59.2 (13.1)	55.4 (15.2)	NS	33.9 (10.3)	46.0 (19.6)	NS
Creatinine *mg/dL* [mean (SD)]	9.0 (3.1)	9.1 (2.1)	NS	9.7 (3.0)	11.1 (4.9)	NS
Phosphate *mg/dL* [mean (SD)]	5.2 (1.0)	5.0 (1.6)	NS	4.4 (1.8)	4.6 (1.6)	NS
Hematocrit % [mean (SD)]	33.9 (5.1)	32.6 (4.7)	NS	34.7 (5.1)	33.7 (2.4)	NS

[a] Two-tailed P value using independent sample Student's t-test, Mann-Whitney U-test or chi square test as appropriate
[b] Abbreviations are: NS, not significant; SD, standard deviation; IQR, intraquartile range; ESRD, end-stage renal disease
[c] Mean (−60, −30 and 0 days prerandomization) serum albumin

Table 2. Baseline body mass index, urea kinetic data and 24-hour dietary recall

	Hemodialysis			Peritoneal dialysis		
	Aminess N® (N = 14)	Placebo (N = 15)	P[a]	Aminess N® (N = 9)	Placebo (N = 9)	P[a]
Actual body weight *kg*	84.3 (30.0)	65.1 (13.2)	0.03	72.2 (10.2)	67.9 (14.5)	NS
Body mass index kg/m^2 [median (IQR[b])]	30.55 (13.5)	24.60 (4.23)	0.05	24.28 (6.13)	22.61 (10.11)	NS
Kt/V [mean (SD)]	1.41 (0.59)	1.47 (0.36)	NS	1.79 (0.3)	1.96 (0.35)	NS
PNA *g/kg/day* [median (IQR)]	1.03 (0.48)	1.01 (0.54)	NS	0.53 (0.17)	0.76 (0.69)	0.02
Total energy intake *kcal/day* [mean (SD)]	1338 (411)	1435 (473)	NS	1321 (161)	1507 (512)	NS
Total energy intake *kcal/kg ABW*[b]*/day* [mean (SD)]	18.6 (11.5)	23.7 (8.8)	NS	20.6 (4.6)	21.1 (8.2)	NS
Protein intake *g/day* [mean (SD)]	60.3 (16.4)	71.1 (23.8)	NS	57.0 (12.6)	64.0 (23.9)	NS
Protein intake *g/kg ABW/day* [mean (SD)]	0.94 (0.57)	1.13 (0.39)	NS	0.91 (0.05)	0.93 (0.31)	NS

[a] Two-tailed P value using independent sample Student's t-test or Mann-Whitney U-test, as appropriate
[b] Abbreviations: NS, not significant; SD, standard deviation; IQR, intraquartile range; ABW, actual body weight; PNA, protein nitrogen appearance

grip strength 23 kg force). In the peritoneal dialysis group, there was no significant difference for any of these baseline parameters.

Compliance

Compliance, measured as a percentage of the prescribed number of pills absent from the returned pill containers, averaged 75, 70, and 50% during the first, second, and third months of the trial. Compliance rates were almost identical for the Aminess N® group and placebo, but were approximately 10% lower for peritoneal dialysis than for hemodialysis patients (Fig. 1).

Change in serum albumin

The primary analysis revealed a significant qualitative interaction (P = 0.02) between the effects of treatment allocation and dialysis modality (Fig. 2). For the hemodialysis group, the change in mean serum albumin (95% CI) from baseline to follow-up, adjusted for center and albumin strata, was 0.22 (0.03, 0.40) g/dL higher in the EAA than the placebo group (P = 0.02). The peritoneal dialysis group failed to demonstrate any improvement with a mean difference (95% CI) between EAA and placebo of 0.01 (−0.31, 0.34) g/dL. The effect of treatment on the total study group (both modalities combined) reflects the larger representation of hemodialysis subjects and approached but did not reach significance, with an adjusted mean serum albumin change (95% CI) of 0.14 (−0.02, 0.30) g/dL higher in the EAA than in the placebo arm (P = 0.08). A nonparametric analysis using a robust variance estimator yielded similar results (data not shown). In a secondary analysis, the significant treatment effect for hemodialysis patients persisted (P = 0.04) after adjustment for baseline body weight. The mean serum albumin values at each time point are shown in Figure 3.

The mean ± SD improvement in serum albumin for the total study population, in EAA versus placebo subjects, was 0.21 ± 0.12 g/dL for subjects in the very low albumin strata, as compared with 0.05 ± 0.10 g/dL for those in the low albumin strata, interaction $P < 0.01$. A similar result was present for hemodialysis patients: very low stratum (mean ± SD), change in serum albumin, 0.31 ± 0.17 g/dL, low albumin stratum (mean ± SD)

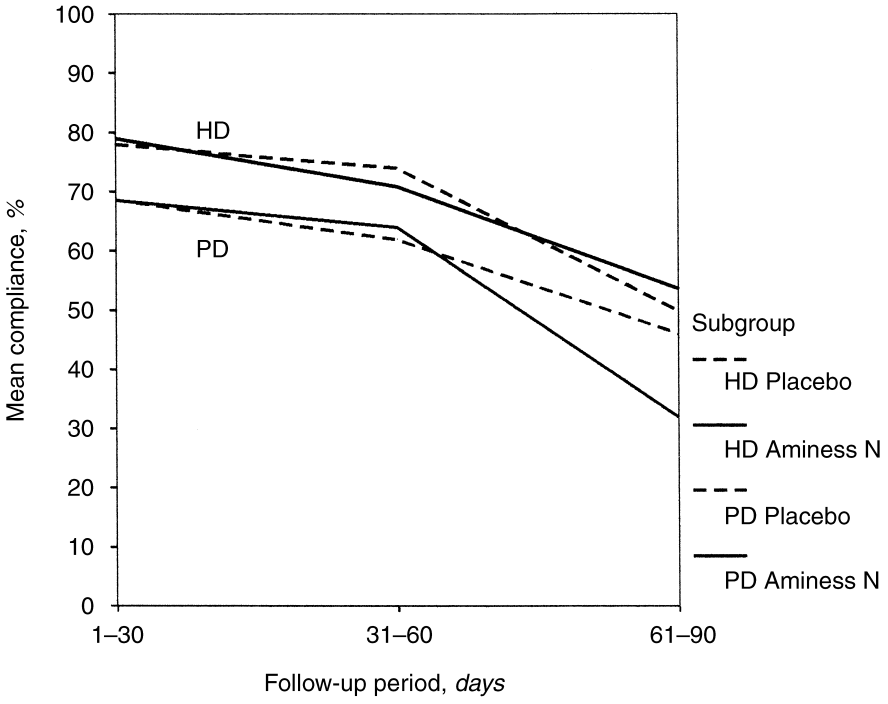

Fig. 1. Mean monthly pill compliance as a percentage of the prescribed number of pills by dialysis modality and treatment assignment.

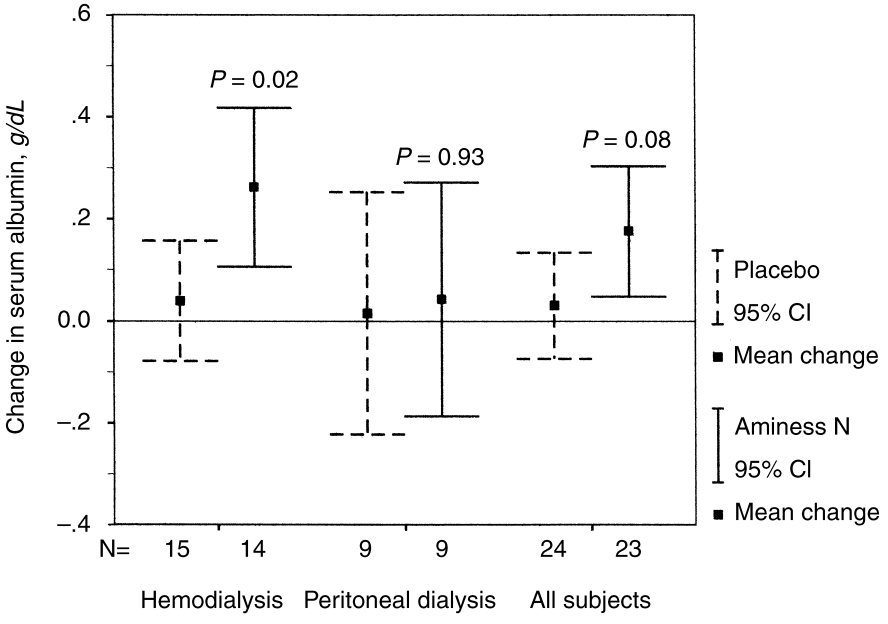

Fig. 2. Change in serum albumin from the mean baseline to the mean follow-up level adjusted for study center and strata.

change, 0.14 ± 0.1 g/dL, interaction $P = 0.05$. Our ability to examine this effect in peritoneal dialysis patients was limited by the relatively small numbers of subjects in this group (4 subjects in the peritoneal dialysis low albumin stratum). Nevertheless, a similar trend for an increased degree of improvement in the very low albumin stratum was also seen in the peritoneal dialysis group.

Secondary outcomes

There was no significant change over the study period in either serum transferrin or prealbumin levels nor in the potential confounding laboratory or dietary recall variables (Table 3). The other routinely measured laboratory parameters similarly showed no significant difference with treatment (data not shown). There was a bor-

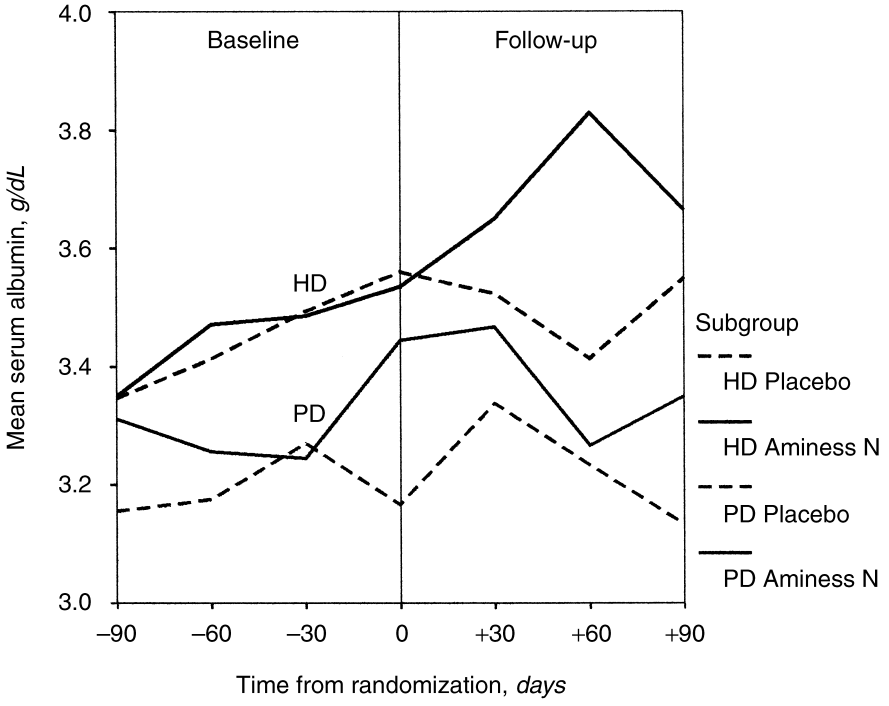

Fig. 3. Mean serum albumin levels at individual time points.

Table 3. Unadjusted change (follow-up minus baseline) in study outcome measures and covariates by dialysis modality and treatment type

	Hemodialysis			Peritoneal dialysis		
	Aminess N® (N = 14)	Placebo (N = 15)	P[a]	Aminess N® (N = 9)	Placebo (N = 9)	P[a]
Primary outcome						
Change in albumin *g/dL* [mean (SD)][b]	0.26 (0.27)	0.04 (0.21)	0.02	0.04 (0.30)	0.02 (0.31)	NS
Secondary outcomes						
Change in prealbumin *mg/dL* [median (IQR)][b]	−0.17 (5.00)	−0.33 (5.00)	NS	1.75 (8.88)	3.58 (11.83)	NS
Change in transferrin *mg/dL* [mean (SD)]	4.19 (17.65)	0.12 (28.50)	NS	8.31 (32.60)	−9.96 (24.53)	NS
Change in BMI kg/m^2 [median (IQR)]	0.00 (0.42)	−0.12 (0.54)	NS	−0.20 (0.97)	−0.01 (0.40)	NS
Change in grip strength *kg force* [mean (SD)]	2.45 (3.67)	−0.28 (2.38)	0.05	−0.06 (2.73)	0.98 (2.76)	NS
Change in MAMA, *cm* [median (IQR)]	−0.54 (1.2)	−0.66 (1.36)	NS	−0.90 (1.36)	−0.39 (1.92)	NS
Change in SFT *mm* [median (IQR)]	0.17 (2.32)	0.15 (2.24)	NS	−0.83 (1.42)	1.72 (4.63)	0.04
Change in SF12 mental health score [mean (SD)]	2.9 (8.1)	2.6 (6.6)	0.07	−0.1 (3.5)	3.9 (6.6)	NS
Change in SF12 physical health score [mean (SD)]	−3.3 (5.0)	−0.2 (4.8)	NS	−1.2 (5.4)	−0.0 (6.4)	NS
Covariates						
Change in serum bicarbonate *mmol/L* [mean (SD)]	−1.8 (4.0)	−0.2 (3.2)	NS	0.4 (2.5)	0.4 (3.1)	NS
Change in Kt/V [mean (SD)]	−0.12 (0.55)	0.05 (0.30)	NS	0.29 (0.44)	0.11 (0.29)	NS
Change in PNA *g/kg/day* [median (IQR)]	0.06 (0.54)	−0.15 (0.60)	NS	0.00 (0.10)	0.07 (0.25)	NS
Change in total energy intake *kcal/day* [mean (SD)]	151.6 (253.0)	232.0 (527.3)	NS	202.0 (193.5)	179.3 (245.9)	NS
Change in protein intake *g/day* [mean (SD)]	5.81 (28.36)	0.84 (28.6)	NS	7.51 (13.44)	−6.28 (35.27)	NS

[a] Two tailed P value for the difference between Aminess N® and placebo using independent sample Student's t-test or Mann-Whitney U-test, as appropriate
[b] Abbreviations: NS, not significant; SD, standard deviation; IQR, intraquartile range; MAMA, mid arm muscle area; SFT, skin fold thickness

derline significant improvement in grip strength in the hemodialysis group, with a mean (95% CI) change, EAA vs. placebo, of 2.27 (0.0, 5.5) kg force. There was no significant change in the other anthropometric measurements in the hemodialysis group. The only significant change among these parameters in the peritoneal dialysis group was a decrease of 2.55 mm in the median skin fold thickness of the EAA versus placebo subjects ($P = 0.04$). Among the nine obese subjects in the study, the mean (intraquartile range) change in actual body weight between baseline and follow-up was 0 (1.0) kg.

The mean SF12 mental health score at randomization was similar for both dialysis modalities and averaged 52. The mean (95% CI) difference in change in mental health score between EAA and placebo participants for all study subjects was 5.0 (0.8 to 9.2, $P = 0.02$). The mean (95% CI) difference for change in hemodialysis patients was 5.5 (CI, −0.4 to 11.4, $P = 0.07$) and for

Table 4. Baseline levels and change post-treatment in serum amino acids, free indoxyl sulphate, nitrate plus nitrite and C-reactive protein in the hemodialysis active subgroup ($N = 14$)

	Baseline median (IQR[a])	Change median (IQR)	
	Plasma amino acid μmol/L		P[b]
Essential			
Histidine	127 (43)	−19 (38)	NS
Isoleucine	108 (55)	−22 (83)	NS
Leucine	213 (194)	−63 (260)	NS
Lysine	321 (229)	−40 (273)	0.06
Methionine	37 (18)	0 (43)	NS
Plenylalanine	116 (90)	−19 (114)	NS
Threonine	185 (85)	0 (117)	NS
Valine	153 (150)	22 (75)	NS
Free tryptophan	9 (4)	0 (4)	NS
Total essential	1299 (623)	−137 (1006)	NS
Nonessential			
Alanine	491 (289)	−57 (188)	NS
α-Amino butyric acid	7 (21)	0 (13)	NS
Arginine	324 (371)	−68 (319)	0.05
Asparagine + glutamic acid[c]	452 (340)	−61 (225)	NS
Aspartic acid	78 (120)	−4 (143)	NS
Citrulline	82 (44)	7 (60)	NS
Cystine	8.5 (15)	−3 (17)	NS
Glutamine	232 (252)	27 (219)	NS
Glycine	458 (234)	−106 (229)	NS
Hydroxyproline	0 (93)	0 (54)	NS
1-Methyl histidine	46 (35)	−2 (31)	NS
3-Methyl histidine	24 (20)	−1 (14)	NS
Ornithine	100 (47)	5 (88)	NS
Proline	349 (168)	−60 (147)	NS
Serine	210 (170)	−46 (177)	0.06
Tyrosine	85 (47)	−19 (94)	NS
Total nonessential	2852 (1318)	−520 (915)	NS
Ratio essential:nonessential	0.47 (.10)	0.03 (.23)	NS
Additional assays			
Free indoxyl sulphate *μmol/L*	9.1 (9.4)	0.8 (7.6)	NS
Nitrate plus nitrite *μmol/L*	94.5 (88)	−40.7 (77.7)	NS
C reactive protein[d] *mg/dL*	1.4 (2.1)	0.3 (2.3)	NS

[a] Abbreviations: NS, not significant; IQR, intraquartile range
[b] Mann-Whitney U-test
[c] Assay was unable to accurately separate elevated asparagine levels from glutamic acid levels, and combined levels are therefore reported
[d] Reference range 0–1 mg/dL

peritoneal dialysis was 3.8 (−2.2, 9.8, $P > 0.1$; Table 3). The mean physical health score at baseline was 36. There was no significant difference in physical well being between the EAA and the placebo arms, at either baseline or at follow-up.

Aminograms

Serum aminograms were measured at both randomization and follow-up for the 14 hemodialysis patients who received Aminess N® treatment. Baseline aminograms revealed high levels of the measured serum amino acids except valine, tyrosine, glutamine, cystine, and amino-N-butyric acid. There was no significant change in the ratio of essential to non-EAA during the study (Table 4). Serum valine levels increased, although not significantly, from a mean baseline level of 165 to 205 at follow-up, while the mean glycine level decreased from 469 to 388. However, there was no correlation between either the change in the ratio of valine to glycine or of essential to non-EAAs and the change in serum albumin.

Post hoc analyses

Using the remaining frozen serum from the 14 hemodialysis patients treated with Aminess N®, we measured the concentration of free indoxyl sulfate, nitrate plus nitrite, and CRP. Neither serum levels of indoxyl sulfate nor nitrate plus nitrite rose significantly in hemodialysis patients on Aminess N®. The change in these levels also did not correlate with change in albumin concentration. Sufficient serum for CRP measurement was available in 12 of these 14 patients. Five patients had undetectable CRP levels, the lower limit of detection for the assay used being 1 mg/dL. The median (intraquartile range) of the CRP level at baseline was 1.35 (2.08). The change from baseline to follow-up was 0.28 ± 2.25 (mean ± SD; $P > 0.1$). There was no correlation between the changes in CRP levels and change in serum albumin during the course of the study. There was, however, a strong positive correlation between baseline CRP levels and the change in serum albumin levels (Spearman's $r = 0.83$, $P = 0.001$; Fig. 4). This correlation was particularly strong for the six obese subjects for whom data were available (Spearman's $r = 0.93$, $P = 0.003$).

Patient morbidity

In general, Aminess N® treatment was well tolerated. There were 20 nonelective hospitalizations during the course of the study, 10 in the EAA arm and 10 in the placebo arm. Two peritoneal dialysis patients developed peritonitis. The first was hospitalized during the first month postrandomization and subsequently died. He was excluded from the primary analysis, as discussed previously. The second was hospitalized seven weeks after study entry and made an uneventful recovery. An additional 12 adverse events, which did not require hospitalization, occurred, five in the EAA group and seven in the placebo group. As expected, the combined hospitalization/adverse event rate was higher in the very low-albumin strata (22 of 32 total advents) as compared with the low-albumin strata. Three patients died within three months of randomization; two were on active treatment, and one was on placebo. The reported causes of death were trauma, myocardial infarction, and septicemia, respectively. In no case was there any evidence of a contribution of study involvement to the development of any adverse event, hospitalization, or death.

DISCUSSION

Hypoalbuminemia is a powerful predictor of poor outcome in patients on maintenance dialysis. In a large

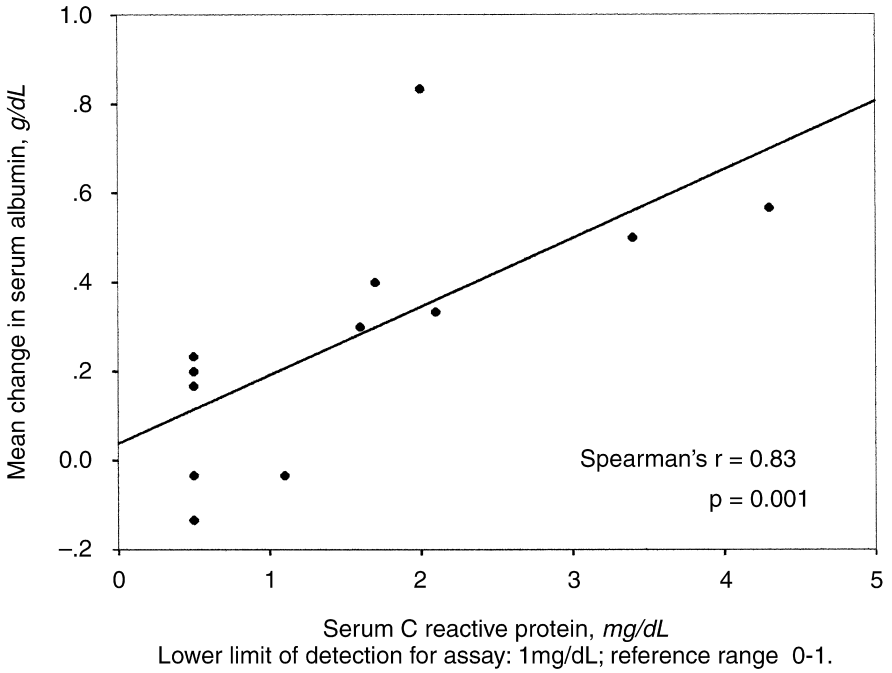

Fig. 4. Scatterplot of change in serum albumin from mean baseline to mean follow-up level with serum C-reactive protein level at randomization.

retrospective study, Lowrie and Lew demonstrated a twofold increased risk of dying for patients with a serum albumin of between 3.5 and 4.0 g/dL as compared with a reference group with albumin levels of 4.0 to 4.5 g/dL [1]. Several other large studies have supported these results in both hemodialysis [3, 4, 21] and peritoneal dialysis [2, 5]. Hypoalbuminemia is not only a powerful predictor of outcome. It also is highly prevalent among dialysis patients. The Health Care Financing Administration's core indicators project conducted in 1997 found, using the bromocresol green method, that 43% of peritoneal dialysis and 16.5% of hemodialysis patients had serum albumin levels below 3.5 g/dL [22].

The etiology of hypoalbuminemia in dialysis patients is both complex and multifactorial. It is contributed to by uncontrolled acidosis [23–25], by protein losses in the dialysate [26, 27], and at least in some cases, by a chronic inflammatory state [28–30]. This combination of factors contributes to the increased protein requirement needed to maintain a neutral nitrogen balance in these patients [31, 32]. At the same time, dialysis is associated in many patients with moderately severe anorexia. This anorexia possibly results from the combination of inadequate dialysis, the ingestion of multiple medications, the presence of comorbid problems such as diabetic gastroparesis, as well as more general psychosocial problems, such as poverty and depression [33]. The result is that dialysis patients frequently have an inadequate oral intake of both total protein and energy. Moreover, even though energy requirements in dialysis patients remains normal [34, 35], the frequent failure of patients to meet these requirements results in the further diversion of consumed protein from anabolic to catabolic pathways. Thus, dialysis-associated anorexia underlies the inability of many dialysis patients to increase their oral intake voluntarily, despite intensive instruction and supervision by renal dietitians. The use of amino acid supplements, by either the enteral or parenteral route, may help overcome this shortfall, while avoiding the provision of additional toxins, such as excess potassium or phosphate.

Several studies, most of them from the late 1970s and early 1980s, have examined the effect of oral EAA on serum amino acid levels of hemodialysis patients [8, 36–41]. Interpretation of these results is complicated by the different amino acids formulations used and by the omission of relevant clinical information, particularly regarding the achieved dialysis dose and the degree of acid-base control. In the majority of these studies, subnormal serum concentrations of EAAs were improved by the administered supplement [8, 36, 38, 40, 41]. In addition, Philips, Havard, and Howard, in a nonrandomized though controlled study, reported a mean increase in serum albumin levels of 0.2 g/dL ($P = 0.02$) [36]. An advantage of the enteral, as compared with the parenteral, route of supplementation is that it is relatively inexpensive. The Aminess N® supplement, at the dose prescribed in our study, costs approximately $5 a day or $450 for the total three-month course of treatment.

Intradialytic parenteral nutrition (IDPN) was widely used in the United States in the past. It had the advantage of convenience, bypassing the need for active patient compliance. The popularity of IDPN appears to have

rested more on this convenience than on the oral route of administration, having been convincingly shown to be ineffectual. Approximately 28 studies have examined the effects of IDPN in dialysis patients [reviewed in 42], only three of which were randomized. Blondin and Ryan, in an additional recent report describing the application of a standardized nutritional assessment and intervention program, reported an improvement in serum albumin of 0.2 g/dL with six months of IDPN therapy, at an estimated cost of $20,000 per patient [43]. There was an associated decrease in the mean ± SD number of hospitalizations from 2.7 ± 1.7 to 1.9 ± 2.0 and of duration of hospitalizations from 20.2 ± 18.9 days to 12 ± 12.6 days in the IDPN-treated patients. The inherent limitations of such observational studies, the great cost of IDPN, and its extremely restricted reimbursement potential have all greatly limited the current use of this approach.

There have been fewer published studies examining the use of amino acid-based dialysate in peritoneal dialysis patients. A study by Kopple et al using a 1.1% amino acid-based dialysate showed a net improvement in nitrogen balance [44]. A prospective study of 15 patients showed a significant improvement in serum albumin levels (mean change 0.24 g/dL) [45], while a randomized trial of 105 subjects showed a significant improvement at one month but not at two or three months of follow-up [46].

Our study is the first clinical trial, of which we are aware, to examine the potential benefits of oral amino acid supplements in hypoalbuminemic dialysis patients in a rigorous fashion, using a prospective, randomized, placebo-controlled, double-blind methodology. We chose to examine albumin as our primary outcome variable since its measurement is objective, and it has a proven association with patient outcomes in a wide range of studies for both peritoneal and hemodialysis patients. The serum albumin levels in our two participating centers were measured in different laboratories. However, both laboratories used the same methodology for measuring albumin levels, and our primary outcome was the within-individual change in serum albumin, which therefore could not be influenced by any systemic bias between the two laboratories. In addition, our primary analysis was adjusted for study center to control for any nonrandom differences between the two study centers and their associated laboratories.

It is possible that some peritoneal dialysis patients may have lost a substantial proportion of the ingested supplement into their dialysate. We did not measure peritoneal dialysate amino acid concentrations and so were unable to investigate this possibility in our study.

As a result of the smaller number of peritoneal dialysis patients enrolled in the study, their lower compliance rates and the greater variance in their serum albumin levels, as compared with the hemodialysis patients, our study's power to determine a significant effect of amino acid supplements in peritoneal dialysis was markedly attenuated. As can be seen by the wide confidence intervals for the mean difference in serum albumin between the peritoneal dialysis EAA and placebo (−0.31, 0.34), we can neither confirm nor dismiss the possibility of a beneficial effect of oral supplements in this group.

While compliance rates deteriorated considerably during the course of the study, compliance was similar for the EAA and placebo groups, suggesting that this suboptimal compliance was not a consequence of treatment with amino acids per se. We instead believe that the progressive deterioration in compliance over the course of the study resulted primarily from the large number of pills the patients were requested to take, and that the study was placebo-controlled but lacked any formal incentives to help maintain subject compliance. The mean study compliance of 9 to 10 tablets per day may form the basis of a more realistic dosing strategy in future studies.

We were unable to demonstrate a significant improvement in our secondary outcomes, with the exception of improvements in grip strength and the mental health score. We had limited power to detect a change in either serum transferrin or prealbumin levels due to the wide variance of these measurements in comparison with the level of improvement expected with this limited course of treatment. In addition, serum transferrin levels may have been influenced by intravenous iron therapy. The reproducibility of the standard anthropometric measurements may vary considerably, even with the use of standardized measurement techniques [13]. Furthermore, these anthropometric parameters may only slowly respond to patients' altered nutritional status. The lack of change in the physical health score may similarly reflect the limited follow-up period of the study and the lack of any formal associated physical rehabilitation program. The observed borderline improvements in grip strength are in keeping with the recent report by De Bisschop et al of improved muscle metabolism, as measured by P^{31} spectroscopy, following amino acid supplementation [47].

In contrast to previous published studies, we did not find subnormal amino acid levels in our hemodialysis subjects at baseline. This may partly be the result of better acid-base control and the relatively high achieved dialysis dose as compared with historical studies, or be influenced in part by the fact that our samples were taken from nonfasting subjects. Similarly, while alterations in the concentrations of free tryptophan [17], free indoxyl sulfate [48] and nitrate plus nitrite [49] have been associated with chronic renal failure and dialysis, and we did not find any correlation between changes in these substances and change in the serum albumin.

Several patients in our study had a body mass index of greater than 30 but were nonetheless hypoalbuminemic. Random assignment resulted in these patients being

over-represented in the Aminess N®-treated hemodialysis group. There is, however, a considerable prevalence of obesity in the dialysis population. In a recent study by Fleischmann et al, 24.4% of 1346 patients studied had a body mass index of 30 or greater [50]. Although obesity has been shown to be protective when compared with a low body mass index in dialysis patients [3, 50], the prognostic significance of hypoalbuminemia in this obese subgroup of individuals is not well established. While it is possible that some of the hypoalbuminemia seen in this group may have been dilutional in origin, caused by the difficulty in establishing accurate dry weight in obese patients, when we adjusted our primary analysis for the patient's body mass index, the study results were unchanged. Similarly, median actual body weight was unchanged among the obese subjects over the course of the study. Both of these observations argue against the possibility of a reduction in the patient's target dry weight, with a resultant reduced dilutional effect, as leading to the observed improvement in their serum albumin levels.

The presence of elevated CRP levels among the obese study patients supports a role for chronic inflammation as contributing to their hypoalbuminemia, while the improvement in serum albumin levels seen with treatment suggests that amino acid supplements may at least partly ameliorate the nutritional consequences of chronic inflammation in these patients. The absence of any correlation between change in serum albumin and change in CRP over the course of the study argues against a decrease in chronic inflammation as leading to the improvement in serum albumin.

The current study lacked both the number of subjects and the length of follow-up required to the address the question of the impact of amino acid supplements on patient hospitalization or survival. The study by Owen et al found a 25% decrease in mortality in association with a 0.2 g/dL increase in serum albumin [4]. However, the observational nature of their study limits determination of whether the relationship is causal. Therefore, while it is likely that the effective treatment of hypoalbuminemia will exert a beneficial effect on patient outcome, this hypothesis remains to date unproved by a rigorously conducted clinical trial.

We have previously reported that a very low-protein diet, supplemented by EAAs, results in a low prevalence of hypoalbuminemia at the onset of dialysis [51] and may in some patients bring about the remission of nephrotic range proteinuria and hypoalbuminemia [9]. This paradoxical improvement in protein nutrition resulting from the combination of severe protein restriction with an EAA supplement remains unexplained. The possibility that these same effects could be seen in dialysis patients on such a regimen has not been explored. The assumption that higher dietary protein intake will improve serum albumin levels in dialysis patients is generally accepted but has not been proven. In this connection, it is interesting to note that no change in serum albumin concentration was observed by Blumenkrantz et al in severely hypoalbuminemic peritoneal dialysis patients fed either 0.98 or 1.44 g/kg of protein for two to four weeks, despite a pronounced improvement in nitrogen balance on the latter diet [31].

In summary, we demonstrate a modest though significant improvement in serum albumin levels among hemodialysis patients treated with oral EAAs. The mean increase (0.2 g/dL) is of the same order as has been described with several other interventional studies using either intravenous or intraperitoneal supplements. Treatment with oral supplements, such as Aminess N®, however, costs only a fraction of the cost of these parenteral supplements. While the observed improvements in serum albumin are small, they are potentially of major clinical significance, especially as the improvement was greatest for patients in the very low serum albumin strata, patients who are at the greatest risk of developing complications. We believe that oral amino acid supplements may provide both a cost-effective and safe method for improving the nutritional intake of those hemodialysis patients who are unable to meet their daily protein requirements despite maximal conservative measures. However, before such a treatment strategy can be advocated, the potential benefits of oral supplements on patients' long-term outcome should be objectively assessed in a randomized, double-blind, placebo-controlled clinical trial.

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