Amino Acids (1991) 1: 319–329

Amino
Acids

Effects of a new amino acid supplement on blood AA pools in patients with chronic renal failure

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Summary. We designed a new formula for AA supplement in order to correct blood pools of AA in chronic renal failure (CRF). This supplement was given to 5 patients with CRF and its effectiveness was tested during long term (12–24 weeks) administration. The patients had previously been on a diet providing 0.6 g of protein and 34–36 kcal/kg/day. The diet was then modified to one providing the same caloric content but only 0.3 g/kg high biological value protein per day with the addition of the AA supplement (0.3 g/kg). The new diet corrected most of the abnormalities in blood AA pools. After 1 month of treatment Val, Leu, Thr, Ser and Tyr levels rose and became normal throughout the study. Ratios Tyr/Phe, Ser/Gly and Val/Gly also improved. During the treatment no side effect or toxicity was observed, and serum albumin, transferrin and nutritional anthropometric parameters persisted to be normal. It is concluded that this specially designed AA supplement added to a hypoproteic diet is an acceptable regimen which can quite completely correct the imbalance in blood AA pools in CRF.

Keywords: Amino acids – Diet – Amino acid supplement – Nutrition – Chronic renal failure

Introduction

It has been shown that patients with chronic renal failure (CRF), evaluated in the postabsorptive state, show abnormal levels of amino acids (AA) in the arterial blood [1, 2]. Typical alterations are higher levels of Cit, Pro, Cys and His and lower levels of Asp, total Trp, Tyr, Ser, Val, and Thr. Changes in blood AA profile occur in well nourished patients with relatively preserved renal function and are therefore caused by renal failure per se [2]. Studies carried out in order to evaluate AA exchange across the organs which play a major role in AA metabolism show that changes in blood levels of many AA are the consequence of altered kidney [3] or muscle [4] metabolism. At the same time, abnormalities

in blood AA may affect AA utilization by the brain [5] and the hepato-splanchnic bed [6].

Additional alterations in AA metabolism have been observed after AA or protein ingestion [2, 7, 8]: total non essential AA (NEAA) increase in arterial blood much more than in controls, as a consequence of an exaggerated increase in Pro, Gln, Gly, Ser, Cys and Ala levels; total essential AA (EAA) increase to the same extent as in normal subjects. However, Thr, Phe, and His increase more than in controls, whereas Trp rises less. These postprandial abnormalities in arterial blood are the consequence of a defective utilization of ingested AA by the splanchnic organs which release large amounts of NEAA into the hepatic veins [8], contrarily to what happens under normal conditions. Taken together, these data demonstrate that in CRF, before the uremic stage, blood AA profile and interorgan flow of AA are altered both in the postabsorptive and protein-fed state. The imbalance in AA profile may be harmful for tissues, since it may affect tissue AA pools and protein synthesis [9, 10]. Moreover, the abnormal supply of AA to the kidney in the postprandial period may accelerate the progression of renal insufficiency by inducing hyperfiltration [11] or toxic effects.

Based on these premises, we designed a new formula for AA supplement in order to correct abnormalities in circulating pools of AA in patients with CRF. This new AA supplement was given to five patients with CRF on a very low (0.3 g/kg/day)protein diet and its effectiveness and tolerability were tested during long term administration.

Experimental procedures

Five patients (4 males and 1 female) with CRF, were studied after obtaining informed consent. Table 1 shows characteristics of these patients and the duration of treatment with the new experimental diet. The mean age was 47 $\pm$ 6 years. The glomerular filtration rate, estimated as the average of 24-hour urea and creatinine clearances, was 13.7 $\pm$ 2 ml/min. The patients had been for the previous 14–16 months on a diet providing 0.6 g/kg/day protein and 34–36 kcal/kg/day (conventional low protein diet). Phosphorus intake was about 600–700 mg/day. The diet was then changed to one providing the same calorie content but only 0.3 g/kg high biological value protein/day, with the addition of the new supplement (0.3 g of AA/kg/day) (experimental diet). Phosphorus intake was about 500–600 mg/day. During the study treatment with calcium and phosphate binders, if any, was not interrupted. Hypotensive drugs and furosemide which were prescribed as appropriate for each individual were also continued. Patients were also given supplements of folic acid (1 mg/day) and pyridoxine-Hcl (20 mg/day). The percent composition of the AA supplement (Genoamino, Bruschettini Industria Farmaceutica, Genova, Italy) is shown in table 2. The percent composition in Val and Leu are increased above the normal requirements, whereas those in Ile, Thr, Phe and Met are decreased. Moreover His, Ser and Tyr are added to the supplement. The supplement was taken by patients during meals as a cream dissolved in water or milk or spread on low-protein bread. Natural proteins were selected according to preferences of each patient. The supplemented diet, isonitrogenous with the 0.6 g/kg low protein diet, provided 6.7 g N/day.

Blood arterialized samples for AA and chemical determination were obtained from a dorsal hand vein [13] in the postabsorptive state. Three samples taken at intervals of 10 days in the month preceeding the new diet, during the conventional low protein diet, provided the initial values. Subsequently blood samples were taken monthly during the experimental diet.

Table 1. Initial characteristics of patients and duration of therapy

Patients	Sex	Age years	Diagnosis*	Glomerular filtration rate** ml/min.1.73 m2	Weeks of treatment
1	M	50	CGN	8.6	16
2	M	54	CGN	9.2	12
3	F	25	CPN	20.6	24
4	M	56	CGN	14.2	20
5	M	49	CGN	15.8	12

* Abbreviations: *CGN* chronic glomerulonephritis; *CPN* chronic pyelonephritis.
** Glomerular filtration rate was estimated as the average of 24 hr creatinine and urea clearances

Table 2. Composition and amounts of AA supplied daily with the new supplement* and estimated AA requirements for normal adults**

	Composition of the new AA supplement (g%)	AA supplied with the new AA supplement # (g/day)	Adult AA requirements # (g/day)
VAL	19.5	4.1	0.98
LEU	18.9	4.0	1.12
ILE	6.8	1.4	0.84
THR	4.7	1.0	0.56
MET	5.5	1.1	0.70
CYS	—	—	
PHE	4.0	0.8	1.12
TYR	11.0	2.3	
LYS	10.0	2.1	0.84
TRP	3.2	0.7	0.21
HIS	3.2	0.7	?
SER	13.2	2.8	—

* Genoamino (Bruschettini Industria Farmaceutica, Genova, Italy).
** Data from Ref. 12.
# Data are calculated for a 70 kg person

The methods employed for the preparation of samples for AA assay are reported elsewhere [3, 5]. AA were measured in whole blood by ion-exchange chromatography [5] (3A30 Amino Acid Analyzer, Carlo Erba Strumentazione, Milano, Italy), glutamine and glutamate enzymatically [14].

Nutritional assessment was evaluated monthly throughout the study. Height, weight, skinfold thicknessess (TSF) and mid-arm circumference (MAC) were measured. Assessment of body fat [15] was performed by the following formula:

$$\% \text{ FAT} = \frac{4.95}{\text{Density}} - 4.5$$

where Density = $1.1689 - 0.0793 \cdot X$ and X = log (triceps + biceps + subscapular skinfold thicknesses) The MAC was converted into a measure of body muscle [16] by the following formulas:

$$\text{Mid-arm muscle circumference (MAMC)} = \text{MAC} - (\pi \times \text{TSF})$$

and:

$$\text{Arm Muscle Area (AMA)} = \left(\frac{\text{MAC} - \pi \times \text{TSF}}{4\pi}\right)^2$$

Urea nitrogen appearance was determined according to Mitch [17] assuming that 60% of body weight is the volume of distribution of urea.

Serum chemistries were determined by routine clinical chemistry laboratory procedures. Urea was determined enzymatically [18]; creatinine was determined with a Beckman Creatinine Analyzer 2 (Beckman Instruments, California); phosphorus was determined by the method of Goldenberg and Fernandez [19].

Arterial blood pH and pCO2 were estimated at 37°C with pHM 72 BMS 3 apparatus (Radiometer Co; Copenhagen, Denmark). Blood bicarbonate levels were calculated by the Henderson Hasselbach equation.

Statistical analysis

Means and their relative confidence limits for whole blood AA in the postabsorptive state were obtained in 35 normal subjects (25 males and 10 females; mean age 39 ± 2 years). These data provided reference values for blood AA. A randomized block design was applied to analysis of variance for the paired data [20]. Regression equations and correlation coefficients were calculated by standard procedures [20]. Values are given as mean ± SEM.

Results

Patients compliance to the diet, including the AA mixture, was good. Gastrointestinal disturbances or other side-effects were not observed. Patients 1 and 2 with more advanced renal failure were free from uremic symptoms at the beginning of the new diet and remained symptom-free during the treatment. Under conventional low protein diet, patients showed blood levels of Val, Leu, Thr, Ser and Asp under the normal range and Tyr at its lower limits, whereas those of Pro, Gly, His, Cys and Cit were over the normal range. Moreover EAA/NEAA, Tyr/Phe, Ser/Gly and Val/Gly ratios were reduced. These data, obtained from arterialized whole blood samples are in accordance with findings obtained in previous studies in which AA were measured in arterial whole blood in patients with comparable degrees of renal failure [2].

The administration of the experimental diet was associated with an improvement of many abnormalities in blood AA profile. Total EAA increased significantly after 8 weeks and remained stable. Total NEAA did not change, and therefore remained elevated in patients (Fig. 1).

When individual AA are considered, Val and Leu reached normal values after 4 weeks of treatment and remained normal. Ser and Thr increased, reaching the upper limits of normal range, while Tyr, which was on the low border line before the AA diet, increased and reached levels which were slightly over the normal limit (Fig. 2). His and Gly decreased in two patients whereas Pro, Cys and Cit remained increased. Phe levels were unchanged. The experimental diet was associated with an improvement or a correction of representative AA ratios (Fig. 3). The Tyr/Phe ratio significantly increased and remained normal. Also the

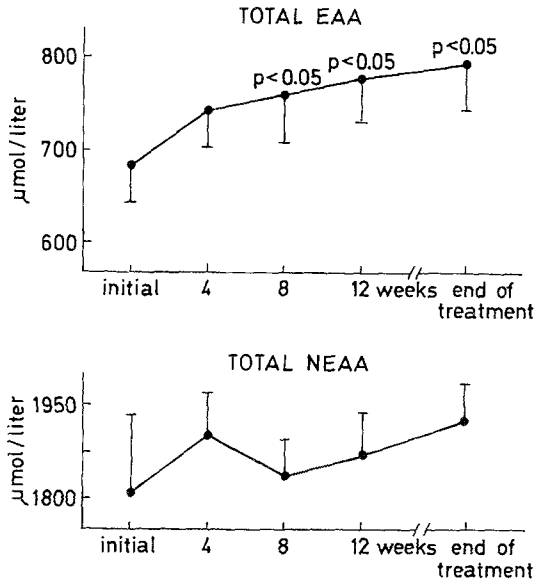

Fig. 1. Effect of the new experimental diet on whole blood total essential AA (*EAA*) and total non essential AA (*NEAA*) in 5 patients with chronic renal failure

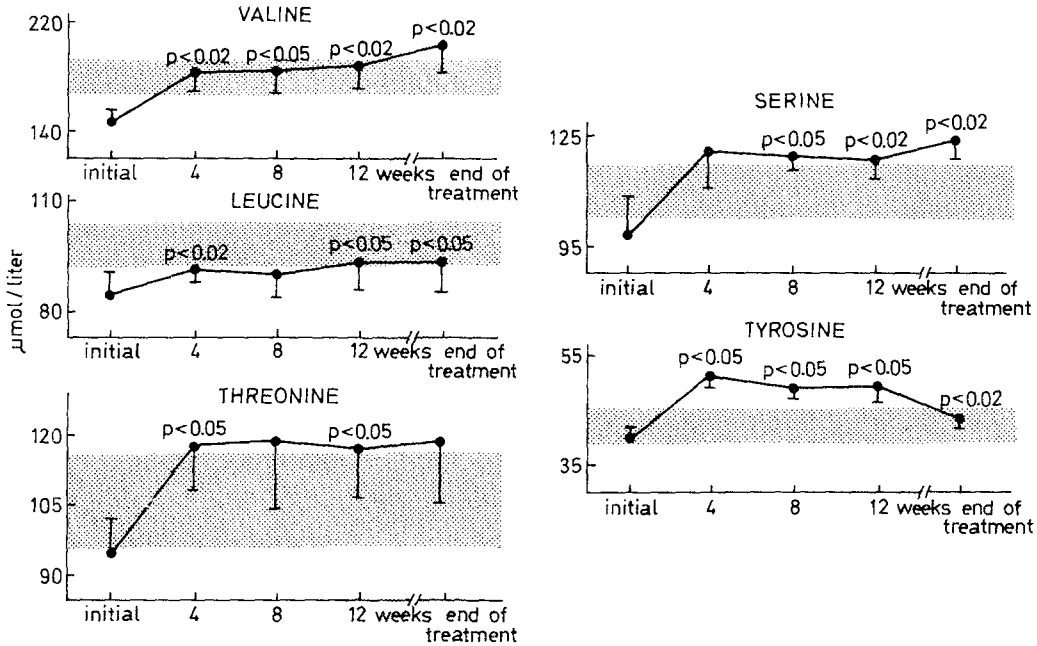

Fig. 2. Effect of the new experimental diet on whole blood levels of Val, Leu, Thr, Ser and Tyr in 5 patients with chronic renal failure. (⁝⁝⁝ confidence limits of the mean of values obtained in 35 normal subjects)

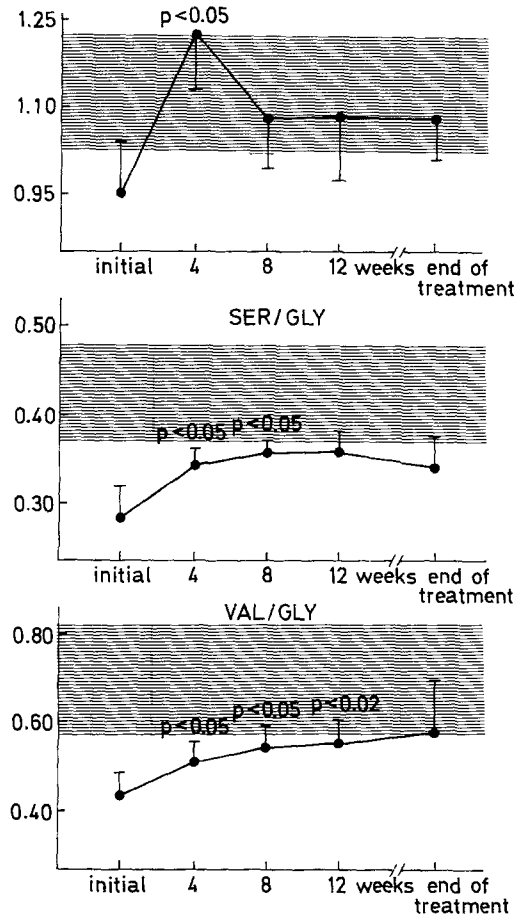

Fig. 3. Effect of the new experimental diet on Tyr/Phe, Ser/Gly, and Val/Gly ratios in 5 patients with chronic renal failure. (≡ confidence limits of the mean of values obtained in 35 normal subjects)

Ser/Gly and Val/Gly ratios increased, however their values continued to be slightly lower than in normal conditions.

Under conventional low protein diet, patients were in good nutritional conditions as demonstrated by anthropometric and biochemical data (Table 3 and 4). Their body weight was 101 + 4% of their ideal body weight (21) and anthropometric measurements were in the normal range (22). During the experimental diet, body weight did not change, and neither did the percent body fat. Arm muscle area increased slightly, even if not significantly, at the end of the study. Serum albumin, transferrin and pseudocholinesterase increased slightly, but not significantly, at the end of the treatment. A tendency towards a reduction in blood urea and serum phosphorus was found. Plama bicarbonates did not change significantly during the experimental diet. Urine nitrogen appearance did not change throughout the study, therefore confirming a good compliance with the diet.

Table 3. Anthropometry before and during the experimental treatment

	Initial	4	8	12	End of treatment (12–24 weeks)
		weeks			
% Ideal body weight	101 ± 4	99 ± 4	99 ± 4	100 ± 4	99 ± 4
Skinfold thickness (mm)					
Triceps	12 ± 0.7	12 ± 0.9	12 ± 1.0	12 ± 1.0	11 ± 0.5
Subscapular	12 ± 1.3	12 ± 1.5	11 ± 1.8	12 ± 2.0	12 ± 1.5
Lateral thoracic	15 ± 2.0	15 ± 2.5	15 ± 1.7	13 ± 1.1	13 ± 1.5
Arm muscle circumference (cm)	30 ± 1.9	30 ± 2.0	30 ± 2.0	31 ± 2.2	30 ± 2.1
Body fat (%)	20.2 ± 0.6	19.9 ± 0.7	19.5 ± 1.0	20.6 ± 0.9	20.0 ± 0.8
Arm muscle area (cm2)	54.2 ± 8.4	55.2 ± 8.7	56.1 ± 9.0	59.3 ± 9.8	56.6 ± 8.5

Table 4. Serum biochemical parameters before and during the experimental treatment

	Initial	4	8	12	End of treatment (12–24 weeks)
		weeks			
Albumin (g/dl)	4.0 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.4 ± 0.2	4.3 ± 0.2
Transferrin (mg/dl)	262 ± 29	250 ± 24	263 ± 29	282 ± 21	283 ± 17
C3 (mg/dl)	89 ± 4	93 ± 7	94 ± 9	93 ± 8	88 ± 8
Pseudocholinesterase (mU/ml)	4844 ± 732	5163 ± 536	5694 ± 311	5602 ± 367	5450 ± 308
Blood hemoglobin (g/dl)	11.4 ± 1.0	11.1 ± 0.9	11.5 ± 0.7	11.5 ± 0.6	11.8 ± 1.0
Phosphorus (mg/dl)	4.5 ± 0.8	4.4 ± 0.8	4.0 ± 1.1	4.0 ± 0.6	3.9 ± 0.6
Calcium (mg/dl)	9.4 ± 0.6	9.2 ± 0.9	8.7 ± 0.7	9.3 ± 0.6	9.2 ± 0.6
Creatinine (mg/dl)	4.9 ± 0.8	4.9 ± 0.9	4.7 ± 0.9	5.0 ± 1.2	5.2 ± 1.4
Urea (mmol/l)	15.4 ± 2.6	13.3 ± 2.6	12.9 ± 2.3	13.5 ± 2.8	14.9 ± 3.4
Urea Nitrogen Appearance (g/day)	4.6 ± 0.6	4.5 ± 0.1	4.1 ± 0.3	4.7 ± 0.2	4.4 ± 0.6

Discussion

The nutritional requirements for individual AA in patients with CRF are still unknown. Apart from the nine AA which are considered essential under normal conditions, it has been suggested that Tyr, Ser and perhaps Arg become essential in CRF [2, 23]. Previous studies [24, 25] suggested that EAA requirements, as proposed by Rose, cannot be applied to patients with CRF and do not permit adequate nutrition. Many studies have been performed using EAA supplements in patients with CRF [26], and the more detailed data have been carried out by Alvestrand et al [24, 25]. These authors experimented with a new supplement containing EAA in proportions which were specifically formulated to correct the abnormal plasma and muscle profile of AA in patients with advanced renal failure. This new supplement contained more Val and less Leu, Phe, Met and Lys, with respect to Rose's formula. Moreover, it also contained Tyr. In that study it was found that Val, Tyr and Thr but not Leu and Ser were corrected in plasma; Val pools were normalized in muscle.

The new AA supplement which has been evaluated in the present study differs from that previously used by Alvestrand et al. in that not only Val but also Leu are provided in increased amounts; moreover, Ile, Met, Thr, His, and Phe are reduced and considerable amounts of Ser are added. With this supplement, we obtained a more complete correction of blood AA levels, including Ser and Leu and representative AA ratios. Stability of blood levels of AA in patients under the new experimental diet, associated with constancy of urea nitrogen appearance, suggests that both absorption of supplemented AA and dietary compliance for protein was good and uniform during the study.

A major result which was obtained using the new AA formula relates to BCAA, taking into consideration their crucial role in protein metabolism [27]. Interestingly, Val and Leu have been corrected in blood simply by increasing the amounts administered. In CRF, in the postabsorptive state a number of alterations concerning BCAA metabolism have been described. The release of Val and Leu from the leg is decreased, likely accounting for their lower blood levels [4]. It has been suggested that the selective abnormalities in muscle BCAA metabolism follow an increased degradation due to an impaired glucose utilization [28, 29] and/or metabolic acidosis [30]. The importance of correcting BCAA levels is also evident if one considers that the reduced levels of VAL may be responsible for the reduced Val utilization by the brain in patients with CRF [5], and that BCAA, mainly LEU, can regulate protein breakdown in muscle [27]. It is commonly believed that protein synthesis requires a high quality AA pattern but in CRF the availability of EAA, mainly BCAA, from body pools may be insufficient. It has been pointed out that under normal conditions the rate limiting AA in the free AA pools are the BCAA, which are abundant in proteins, but maintained at very low concentrations in the free AA pool [31]. Thus, in CRF, BCAA could become rate limiting for protein synthesis. Accordingly, normalization of circulating BCAA, as obtained with the present study, could favorably influence protein turnover.

With the use of the new AA supplement we also observed a correction of blood Tyr and Tyr/Phe ratio. This result was obtained by substantially reducing

Phe and adding considerable amounts of Tyr to the supplement. Under normal conditions, Tyr and Phe are considered together in estimating nutritional requirements, since Phe is readily converted to Tyr. In CRF, different mechanisms seem to cooperate in causing a reduced conversion of Phe to Tyr. In the postabsorptive state a diminished renal synthesis of Tyr has been found, likely responsible for the reduced ciculating levels of this AA [3]. In the protein-fed state, a diminished conversion of Phe to Tyr, likely due to inhibition of PHE hydoxylase in the liver, has been suggested [8]. Results obtained from the present study in addition to previous data suggest that in CRF Tyr and Phe requirements are to be considered separately and that the requirement for Phe is lower, whereas an adequate amount of Tyr has to be provided.

The correction of levels of Thr found with the experimental diet have no explanation, if one considers that Thr was contained in the supplement in reduced amounts, following the observation of an abnormal splanchnic escape of Thr in the protein-fed state in CRF [8]. A correction of plasma levels of Thr has previously been demonstrated both with supplements according to Rose's formula and high Thr supplements [24, 25].

It is noteworthy that the new experimental diet, which provided increased amounts of Ser, was associated with normal blood levels of Ser and normal values of Ser/Gly ratio. Previous data have shown that blood levels of Ser are reduced in CRF mainly because of reduced synthesis of Ser by the kidney, which is the only source of this AA in the postabsorptive state [3]. Therefore, in renal failure, there may not be sufficient production and release of Ser into the circulation to satisfy the metabolic needs for this AA. Accordingly, Ser can be considered an essential AA and should be supplemented with the diet.

The experimental diet did not succeed in correcting blood levels of some NEAA, mainly Pro and Cys, which remained elevated even if the administered amount was reduced by reducing natural proteins. Further selection of natural protein is required in order to reduce the amount of some ingested NEAA, mainly sulphur AA and Pro.

A further support to the above mentioned assumption that in CRF nutritional requirements for individual AA are different from normal conditions, is given by the fact that slight improvements in anthropometric and biochemical nutritional parameters were found with the new experimental diet. It has also previously been observed [24, 25] that a partial correction of AA unbalance in plasma and muscle AA was associated with a slightly positive N balance. Contrarily, a negative N balance has been reported [24, 25] in patients receiving low protein diets supplemented with EAA according to Rose, and a significant reduction in muscle mass has been found [32] in patients on a low, mostly vegetable, protein diet supplemented with AA and keto-acids. These data support the hypothesis that in CRF tailoring AA supplements according to alterations in metabolism of AA may be followed by better nutrition.

In patients 1, 3 and 4 a significant reduction in the rate of change of serum reciprocal creatinine versus time was found; moreover, in patients 2 and 4 a significant increase in the same rate was observed when they resumed the conventional low protein diet. Similar results have been previously observed with hypoproteic diets supplemented with AA [33, 34]. Anyhow, it is uncertain if these results express any effect on the progression of renal failure [35].

In conclusion, our results show that in CRF the unbalance in AA profile can be greately improved by a specially designed AA supplement added to a hypoproteic regimen, while maintaining adequate nutrition. Further modification of nutritional treatment will be necessary in order to achieve a more complete correction of blood AA profile. An additional target will be to evaluate if the correction of blood AA profile has some influence on protein turnover and progression of renal failure.

Acknowledgements

This study was supported by grants from the Ministero della Pubblica Istruzione (assegnazione per la ricerca scientifica 40 and 60%) and from the Regione Liguria. We thank the Istituto Farmaceutico Bruschettini (Genoa, Italy) for technical support, preparation and supply of the amino acid supplement. The authors thank Mr. M. Bruzzone and Mr. M. Marchelli for their excellent technical assistance, and Mrs. F. Tincani for the preparation of the manuscript and figures.

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