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PLASMA FREE AMINOACIDS IN ADULT RABBITS WITH CHRONIC LIVER DAMAGE FED DIETS WITH DIFFERENT PROTEIN CONTENT

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INTRODUCTION

The protein content of the diet is a debated problem in patients with chronic liver disease. Normal or high protein intakes with the food may provoke encephalopathy in subjects with advanced cirrhosis (1), while a chronic low protein intake may induce a catabolic state, evidenced by low plasma levels of branched chain aminoacids (BCAA) (2), low urinary excretion of creatinine and high excretion of 3-methylhistidine (3, 4).

In order to better evaluate the metabolic effect of protein dietary intake in subjects with chronic liver disease, we determined plasma amino acids concentrations in rabbits with experimental chronic liver damage fed diets with different protein content.

MATERIALS AND METHODS

Twelve adulte male NZW rabbits were employed in the present study. They have been selected by a larger group of rabbits on the basis of the homogeneity of the body weight (3423 ± 195 g), of the daily food intake (107± 3 g/day) and of the growing rate (daily gain = 4.5 ± 1.3 g/day). The animals were submitted to a chronic carbon tetrachloride (CCl_4) intoxication according to Cameron and Karunaratne (5) through the intraperitoneal injection of CCl_4 diluted in corn oil (1:4) 2 ml/Kg body weight once weekly for 8 weeks.

At the end of the treatment the animals were maintained for 8 days at an adapting diet (STD = 18.3% of crude protein content and 3358 Cal/Kg) and then divided in three groups of four animals each which were submitted respectively to a low protein diet (LP, group a), a standard diet (STD, group b) and a high protein content diet (HP, group c). Details of the diet composition are given elsewhere (6).

The body weight and the daily food consumption were individually controlled every two days, and urine was collected twice weekly to determine creatinine excretion. After 40 days of treatment, the animals were sacrified under ethyl urethane anestesia. Both arterial and venous blood was obtained by abdominal aorta and cava. Liver specimens were taken for histology. Blood was collected into heparinized tubes, rapidly centrifuged and plasma taken to determine amino acids concentrations as already reported (7). Total and free tryptophan were determined by the method of Denkla and Dewey (8) according to the modifications of Tagliamonte et al. (9). Statistical analyses of the differences between means were performed by Duncan's Multiple Range Test.

RESULTS

After eight weeks of CCl_4 intoxication rabbits showed a mean body weight significantly lower when compared to that of normal pair-fed controls. Healthy control rabbits showed an increase of the body weight after either standard or hyperproteic diet, but a decrease after LP diet (6). On the contrary, rabbits with experimental chronic liver damage (CLD) showed increasing weight only during the HP diet (fig. 1). Creatinine urinary excretion paralleled the changes of the body weights, being maximal in animals with HP diet (fig. 2).

Plasma arterial amino acids concentrations were reported in table 1. Rabbits with CLD had reduced levels of essential amino acids (tab. 2) when compared to normal rabbits (6). Both in healthy control and in CLD rabbits the LP diet provoked a reduction of essential amino acids. Total and free tryptophan concentrations were normal only in control healthy animals fed STD diet. A significant decrease of this amino acid was observed after low

protein diet both in healthy and in CLD rabbits.

The arterio-venous difference of total tryptophan concentrations was positive in all control healthy rabbits (irrespectively of the type of the diet) and in CLD rabbits fed HP diet (+ 1.4 μg/ml) while it was negative in CLD rabbits fed STD (-0.5 μg/ml) or LP diets (-0.95 μg/ml).

Non-essential amino acids showed a significant increase after LP diet both in healthy and in CLD rabbits.

DISCUSSION

Patiens with chronic liver disease frequently show intolerance to protein reach food. Low protein diets, however, may be deleterious for these patients inducing a chronic state of negative nitrogen balance (3). Fischer et al.(10) showed that patients with cirrhosis are not intolerant to any amino acid: BCAA are well tolerated and the parentelar administration of large doses of these amino acids resulted to be beneficial in cirrhotic patients with encephalopathy and negative nitrogen balance (11).

The beneficial effect of long term oral amino acid consumption remains to be demonstrated in patients with chronic liver disease.

In our study, rabbits with experimental CLD obtained by repeated intraperitoneal administrations of CCl_4 showed lower plasma concentrations of essential amino acids (BCAA particularly) than healthy rabbits. A low content of protein in the diet produced a further depletion of plasma essential amino acids. This negative effect of LP diet seem to be more evident in CLD animals than in healthy controls.

Plasma tryptophan, a key amino acid in the regulation of protein syntesis and the precursor of the centrally active amine serotonin, showed similar concentrations in healthy and CLD rabbits. However, the arterio-venous difference of the concentrations of this amino acid were always positive in healthy rabbits but negative in CLD rabbits fed LP or STD diets.

High protein diet, which was scarcely effective in healthy controls, produced a significant increase of essential amino acids in CLD rabbits. In addition, animals with CLD fed HP diet had a positive arterio-venous differ-

ence for total tryptophan and increasing body weight. Urinary creatinine daily excretion, also, was significantly higher in CLD rabbits fed HP diet than in CLD rabbits fed STD or LP diets.

These data demonstrate that animals with chronic liver disease have a reduced body weight with derangement of the plasma amino acid pattern. Some essential amino acids as BCAA are decreased and so less available for peripheral tissues; other essential amino acids as tryptophan are normal but likewise little utilized because of a sustained release by peripheral tissue.

This pattern is worse when the animals are fed low or standard protein diets, while it is partly reversed if animals are fed a diet with high protein content.

Although the present study did not evaluate the effect of the different composition in amino acids, it can be postulated that a long-term diet reach in proteins is favorable to patients with chronic liver disease, determining a gain of the body weight, increase of plasma BCAA concentration and a better utilization of essential amino acids.

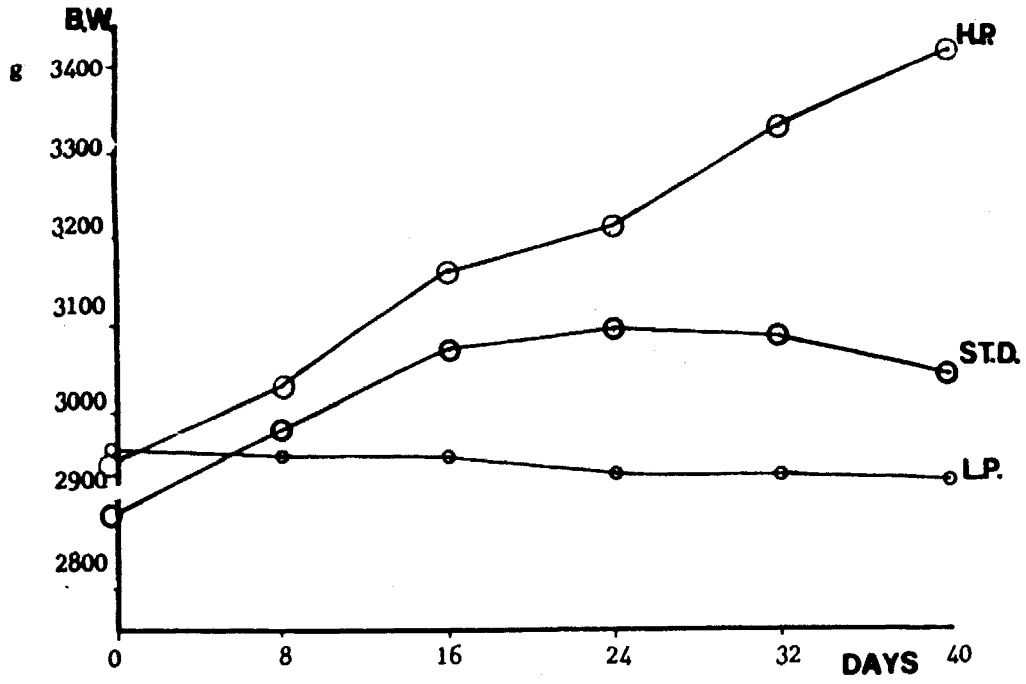

Fig 1 Behavior of the mean body weight of adult rabbits with CLD submitted to diets with different protein content: HP:high protein (27.7%), STD:standard (18.3%), LP:low protein (4.8%)

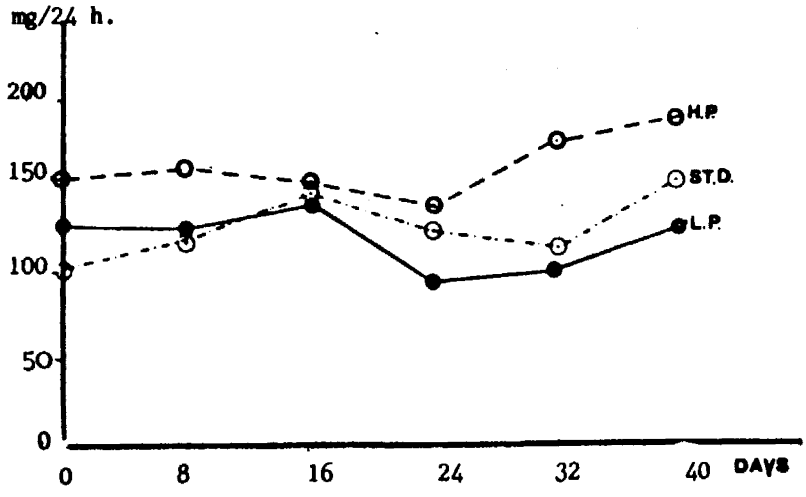

Fig 2 Urinary creatinine excretion in adult rabbits with CLD submitted to diets with different protein content (see fig 1 and ref 6)

Table 1 Aminoacid concentration in the plasma of adult rabbits with CLD fed diets with different protein content.
Mean ± SEM expressed as umoles per milliliter of plasma means in the same line and without common superscripts differ significantly for $p < 0.05$.

	diet		
	LP	STD	HP
Total NEEA	2.98 ± 0.48^{a}	1.86 ± 0.41^{ab}	1.67 ± 0.25^{b}
Total EAA	0.54 ± 0.06^{a}	9.68 ± 0.09^{b}	0.77 ± 0.15^{b}
E/N ratio %	18.5 ± 1^{a}	38.2 ± 9^{b}	49.8 ± 5^{c}

Table 2 Aminoacid concentration in the plasma of adult rabbits with CLD fed diets with different protein content: individual aminoacids Mean ± SEM expressed as nmoles/ml.

	diet		
Essential	LP	STD	HP
Threonine	62.5 ± 27.1^{a}	87.5 ± 17.1^{ab}	118 ± 36.8^{b}
Valine	100.7 ± 13^{a}	132.7 ± 31.6^{ab}	148.7 ± 54.8^{b}
Methionine plus Cystine	41.2 ± 11.6^{a}	64 ± 16.3^{ab}	119.2 ± 64^{b}
Isoleucine	66.2 ± 14.7	69.5 ± 17.3	74.5 ± 15.2
Leucine	78.2 ± 11.6^{a}	98 ± 33^{ab}	106.5 ± 33.5^{b}
Phenylalanine	56 ± 10.8	75.2 ± 16.6	68.5 ± 9.2
Lysine	116.7 ± 24^{a}	96 ± 19.6^{ab}	79.7 ± 8.4^{b}
Tryptophane (total)	27 ± 12.8^{a}	65.2 ± 11.2^{b}	58.1 ± 18.4^{ab}
Non Essential			
Taurine	88 ± 32.1	76.5 ± 50.8	55.7 ± 27.9
Serine	219.7 ± 47.9^{a}	140.5 ± 25^{b}	113.7 ± 28.9^{b}
Asparagine	95.7 ± 50.4	44.2 ± 18.8	57 ± 18.9
Glutamic acid plus Glutamine	545.9 ± 141.1^{a}	343.2 ± 56^{b}	275 ± 33^{b}
Glycine	969 ± 166.2	722 ± 302.7	764 ± 222
Alanine	735.2 ± 345.5^{a}	227.7 ± 94.3^{b}	137.2 ± 28.3^{b}
Citrulline plus alfa-ABA	46.6 ± 8.9	35.2 ± 13.2	39 ± 17
Tyrosine	53.2 ± 2.8^{a}	81.2 ± 16.3^{b}	60.7 ± 15.9^{ab}
Ornithine	66 ± 15.3^{a}	43.5 ± 13.8^{b}	30.7 ± 5.4^{b}
Histidine	84.7 ± 15.9	85.2 ± 10.3	81 ± 20.8
Arginine	121.6 ± 27.3^{a}	87.3 ± 12.5^{b}	72 ± 15^{b}

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SUMMARY

The effects of feeding for a 40 days period three diets containing 48, 183 and 277 g of Crude Protein per Kg of dry matter on the plasma amino acid pattern were studied in adult rabbits with experimental chronic liver disease. Essential amino acids (particularly BCAA) were significantly reduced in these animals when compared to control healthy rabbits, as long as body weight was lower, urinary creatinine excretion decreased and arterio-venous difference of tryptophan concentration negative. Low protein diet got these parameters worse, while high protein diet had a favorable effect. Thus, it can be postulated that the protein content of the diet may determine some feature of both the catabolic state and the deranged amino acid pattern of patients with severe liver disease.

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RIASSUNTO

Gli effetti della somministrazione per un periodo di 40 giorni di tre diete a differente tenore proteico (48, 183 e 277 g per Kg di peso secco) sul profilo aminoacido plasmatico sono stati studiati in conigli adulti maschi con danno epatico cronico sperimentale. Rispetto ad animali sani di controllo sottoposti agli stessi protocolli dietetici, i nostri animali mostrano una ridotta concentrazione di aminoacidi essenziali (in particolare BCAA), un ridotto peso corporeo, una ridotta escrezione urinaria di creatinina e una minore utilizzazione periferica di aminoacidi essenziali come il triptofano. La dieta a basso contenuto proteico peggiorò tutti questi aspetti, mentre la dieta ad alto contenuto proteico li migliorò. Si può dedurre, perciò, che il contenuto proteico della dieta sia in grado di influenzare lo stato catabolico e le alterazioni del profilo aminoacido plasmatico caratteristici dei pazienti con grave epatopatia.

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