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PROTEIN METABOLISM IN ADULT RABBITS FED DIETS WITH DIFFERENT PROTEIN LEVELS : PLASMA FREE AMINO ACID CONCENTRATION

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INTRODUCTION

It is well known that low protein intakes affect plasma amino acid according to a fairly regular pattern : there is a fall in most of the essential amino acids, particularly the branched-chain amino acids, while several non essential amino acids tend to increase. However direct relationships between dietary and plasma amino acids are sometimes obscured by many factors which influence the levels and patterns of plasma amino acids.

Different responses of plasma amino acids to dietary protein levels may be related to differences in species. Fuijta et al. (1979) found that the increase of total non essential amino acids in the plasma of protein-deficient young men was mainly due to an increase in alanine whilst the plasma level of serine was little affected. In adult rats on the contrary serine rather than alanine increased when animals were given low-protein diets (Fuijta et al. -1981).

The effects of dietary protein on plasma amino acids has been studied till now mostly in growing animals submitted to protein deficiency.

Reports dealing with the effects of high protein intakes upon plasma amino acids are scarce.

Thereafter in the present study we examined the effects of low and high protein intakes upon plasma amino acids in adult rabbits.

MATERIALS AND METHODS

Twenty adult male NZW rabbits were used. After adaptation on a standard diet (STD), the animals were weight-sorted in four group of five, 15 animals were transferred to three experimental diets : the 'high protein' diet (HP) had a Crude Protein content of 27.7% (dry matter), the ' moderate low protein'diet (MLP) a CP content of 8.1% and the 'low protein' diet (LP) a CP content of 4.8%. The 'control' group continued on the STD diet (18.3%, CP). Details of the animals, diets composition and food intakes are given in the preceding study.

The experimental diets were fed for a 40 days period. At the end of this period blood samples were obtained from six hours fasted rabbits by cardiac puncture. Blood specimens were collected into Li-heparinized tubes. Plasma samples with norleucine added were deproteinized with sulphosalycilic acid and delipidized with n-heptane. Concentrations of individual amino acids were determined with a CARLO ERBA 3A28M automatic amino acid analyzer according to Spackman et al. (1958). Tryptophane was determined phluorimetrically by the method of Denkla e Dewey (1967) with modifications suggested by Tagliamonte et al. (1973). Statistical analysis of the differences between means was performed by Duncan's Multiple Range Test.

RESULTS

As shown in Table 1 total essential amino acids (EAA) were significantly reduced in the plasma of animals given the MLP and the HP diets.

Total plasma non essential amino acids (NEAA) appeared inversely proportional to the dietary protein levels (X%). The regression could be expressed by the following equation : $Y = - 0.094 X + 4.613$, $n = 20$, $r = - 0.850$ - $p < 0.001$-, where Y is expressed as µmoles per ml of plasma. The ratio of EAA to NEAA (E/N) was directly proportional to the dietary protein levels. The regression could be expressed by the following equation where Y% is the E/N ratio and X% the protein content of the diet : $Y = 0.009 X + 0.155$, $n = 20$, $r = 0.843$ - $p < 0.001$.

When the concentration of each single amino acid was studied (Table 2) it was shown that valine and methionine plus cystine were the main essential amino acids significantly reduced in altered nutrition. When compared to the controls isoleucine and leucine were significantly reduced in the plasma of rabbits given the MLP diet and tryptophane in that of animals given the LP diet. Among NEAA arginine, exibited the most sensitive response to the protein intake (°), followed by alanine and citrulline plus alfa-aminobutiric acid. Histidine, taurine and tyrosine plasma levels were little if any affected by feeding different quantities of protein.

DISCUSSION AND CONCLUSIONS

Essential amino acids. The present results show that the branched-chain amino acids and valine in particulary are prominently affected by the protein intake in rabbits. This is in agreement with reports in man (Adibi-1976). Anderson(1968) reported that the concentration of leucine, unlike the other amino acids, was elevated by feeding an excess of dietary protein. He suggested that in animals fed high protein diets the degrading enzymes for branched-chain amino acid respond more slowly than the degrading enzymes for other amino acids. However in the plasma of the rabbits given the HP diet the concentration of branched-chain amino acids as well as the sum of EAA, were reduced in comparison with the controls.

(°) the regression could be expressed by the following equation : $Y = 254 - 6.66 X$, $r = -0.823$, $n= 20$ -$p < 0.001$ where X% is the protein content of the diet and Y is arginine concentration expressed as nanomoles per ml plasma

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It has been reported that plasma lysine is conserved or even elevated in protein-calorie malnutrition (Munro-1969, Grimble and Whitehead-1971, Badger-1974). Yamashita and Hashida (1971) found that in adult rats an homeostatic mechanism for lysine blood levels is effective but did not found such a mechanism for threonine. Threonine is one of the main essential amino acid which is significantly affected by protein intakes not only in young rats (e. g. Salem et al. -1973) but also in adult rats (Fuijta et al. -1981). Our findings however show that threonine too was conserved in the plasma of rabbits given the low protein diet. Since lysine and threonine do not parti cipate in transamination reactions this could reflect poor amino acid utilization for protein synthesis. The high concentration of lysine in the plasma of animals fed the LP diet support this suggestion.

The fall in tryptophane which is the least abundant amino acid in the free amino acid pool (Munro-1969) we observed in the LP group but not in the MLP, indicates depletion of the amino acid pool in the body of the rabbit given the low protein diet. Under the circumstances of the present study, tryptophane seems to become the limiting amino acid for protein synthesis. These results confirm the validity of plasma tryptophane as a reliable index of protein nutritional status as far as severe alterations are concerned (Smith et al. -1974).

Non essential amino acids. Our findings agree with the rise of total NEAA observed in the plasma of adult rats receiving low protein diets (Fuijta et al. -1981). Such a rise has not been observed in growing rats (Coward et al. 1977, Lunn and Austin-1983). In adult rats serine is the main amino acid that is significantly elevated when low protein diets are administered while alanine shows to be slightly affected and arginine is not affected at all. Our data show that in the rabbit plasma NEAA could be affected according to a different pattern : serine is little if any increased in the plasma of animals given the low protein diets whereas urea cycle amino acids and alanine exibit a large increase. Such results add evidence about species differences in the response of plasma amino acids to dietary protein. The sensitiveness of urea cycle amino acids is of peculiar interest. Since plasma urea cycle amino acids are likely to reflect urea cycle activity, the present data suggest that urea cycle enzymes and in particular arginase activity could respond more sensitively in rabbits than they do in rats. The effect of varying protein intake on arginase and urea cycle enzymes activity has been demonstrated in rats under conditions of extreme protein deficiency and supplementation (Schimke-1962).

The accumulation of NEAA in the plasma of rabbits given low protein diets indicates that the amino acid catabolism in greatly reduced when the protein intake is impaired. On the contrary the low concentration of gluconeogenic and urea cycle amino acids in the plasma of rabbits fed the HP diet indicate a great increase of amino acid catabolism. The increased ability to catabolize amino acids under conditions of high protein intakes has been observed by Chanez et al. -1979, Peret et al. 1981.

The ratio of essential to non essential amino acids. The ratio E/N is a reliable index of protein nutritional status. It responds in opposite directions to energy and protein deficiency. Yap and Hafkensheid(1982) found that in starved adult rabbits the E/N ratio is elevated because plasma EAA levels rise above those of well fed animals. In the present study the E/N ratio, as well as total plasma NEAA, showed to be correlate with the dietary protein levels providing useful indexes of the protein nutritional status in adult rabbits.

SUMMARY

The effects of feeding for a 40 days period four diets containing 48, 81, 183 and 277 g of Crude Protein per kg of dry matter on plasma amino acid pattern and level were studied in adult rabbits. Significant correlations were found between the total non essential amino acids and the protein content of the diet (r = -0.85) and between the ratio of essential to non essential amino acids and the protein content of the diet (r = 0.84). Among essential amino acids valine and methionine plus cystine were the most affected, while arginine, followed by alanine, were the main non essential amino acids affected by varying the protein dietary level.

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RIASSUNTO

Gli effetti della somministrazione per un periodo di 40 giorni di quattro diete caratterizzate da tenori proteici (N x 6,25) del 4,8, 8,1, 18,3 e 27,7 % sul profilo ed i livelli degli aminoacidi plasmatici sono stati studiati in conigli maschi adulti NZW. Sono state trovate delle correlazioni significative tra il tenore proteico della dieta e la somma degli aminoacidi non essenziali (r = 0,85) nonchè tra il tenore proteico della dieta e il rapporto tra la somma degli aminoacidi essenziali e quella dei non essenziali (r = 0,84). Tra gli aminoacidi essenziali la valina e la metionina più cistina sono risultati particolarmente sensibili alla variazione del tenore proteico della dieta, tra i non essenziali l'arginina seguita dalla alanina.

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Table 1. Amino acid concentration in the plasma of adult rabbits 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	MLP	STD	HP
Total NEAA[1]	4.54 + 0.10[a]	3.46 + 0.28[b]	2.81 + 0.24[b]	2.13 + 0.21[c]
Total EAA[2]	0.92 + 0.02[ab]	0.77 + 0.05[a]	1.07 + 0.03[b]	0.87 + 0.09[a]
Grand Total[3]	5.46 + 0.12[a]	4.23 + 0.28[b]	3.88 + 0.22[b]	2.96 + 0.29[c]
E/N ratio (%)	20.46 + 0.56[a]	22.58 + 1.76[a]	31.36 + 2.78[b]	41.20 + 4.47[c]

1 Total EAA consists of Lys, Thr, Met+Cys, Phe, Trp, Val, Ile, Leu

2 Total NEAA consists of Gly, Ser, Ala, Glu+Gln, Asp, Asn, Arg, Ornithine, Citrulline + alfa-aminobutiric acid, Taurine, Pro, Tyr

3 Ratio of EAA to NEAA

Table 2. Amino acid concentration in the plasma of adult rabbits fed diets with different protein content : individual amino acids

Mean ± SEM expressed as nmoles per milliliter of plasma means in the same line and without common superscripts differ significantly for $p < 0.05$

	diet			
Essential	LP	MLP	STD	HP
Threonine	129 ± 10[ab]	100 ± 15[a]	135 ± 19[ab]	154 ± 12[b]
Valine	154 ± 16[a]	144 ± 10[a]	210 ± 11[b]	156 ± 18[a]
Methionine plus Cystine	100 ± 11[a]	93 ± 7[a]	158 ± 30[b]	80 ± 13[a]
Isoleucine	72 ± 11[ab]	60 ± 5[a]	100 ± 11[b]	69 ± 12[ab]
Leucine	97 ± 8[ab]	74 ± 7[a]	129 ± 16[b]	119 ± 19[b]
Phenylalanine	58 ± 6	75 ± 6	84 ± 9	67 ± 11
Lysine	287 ± 23	180 ± 23	223 ± 44	180 ± 48
Tryptophane (total)	27 ± 1[a]	42 ± 5[ab]	63 ± 10[b]	47 ± 4[b]
Non Essential				
Taurine	68 ± 14	61 ± 11	61 ± 11	95 ± 18
Aspartic acid	32 ± 5	8 ± 2	16 ± 1	26 (n=2)
Serine	225 ± 56[a]	185 ± 28[ab]	162 ± 22[ab]	138 ± 23[b]
Asparagine	122 ± 47	55 ± 11	86 ± 9	62 ± 8
Glutamic acid plus Glutamine	812 ± 136[a]	512 ± 49[b]	375 ± 38[b]	358 ± 28[b]
Proline	416 ± 68	230 ± 115	137 ± 43	38 (n=1)
Glycine	1423 ± 76[a]	991 ± 165[b]	873 ± 108[b]	785 ± 81[b]
Alanine	886 ± 131[a]	567 ± 27[b]	451 ± 86[bc]	250 ± 59[c]
Citrulline plus alfa-ABA	105 ± 25[a]	82 ± 9[ab]	47 ± 6[bc]	35 ± 4[c]
Tyrosine	62 ± 6	64 ± 5	83 ± 9	71 ± 16
Ornithine	218 ± 33[ab]	310 ± 35[a]	253 ± 75[ab]	112 ± 21[b]
Histidine	114 ± 21	102 ± 6	119 ± 11	84 ± 14
Arginine	223 ± 22[a]	195 ± 31[ab]	137 ± 12[bc]	67 ± 9[c]