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Inhibition of Visceral Protein Synthesis by Certain Amino Acid Supplements

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Summary

This study was focused on the effects produced by diets with different amino acid proportions on visceral protein synthesis (liver and jejunal mucosa). Eight groups of rats received an enteral modular diet supplemented with different amino acids (Group 1: Ile, Leu, Val; Group 2: Phe, Met, Gly; Group 3: Glu, Arg, Gly; Group 4: Gly, Group 5: Orn–Asp, Cys, Pro; Group 6: Lys, Ser, Thr; Group 7: Tyr, His, Ala). Group 8 was the control group. Rats were fed for four days. At the end of this period a flooding dose of ^{14}C-Leu was injected and animals were killed. Liver and jejunal mucosa were removed and protein synthesis rate was determined. Results show a decreased liver protein synthesis in group fed with aromatic amino acids (53.8±8.4 vs. 88.6±12.1) and Glu-Arg (68.6±10.9). In jejunal mucosa there was a decrease of protein synthesis in groups fed with aromatic amino acids (98.7±16 vs 160.5±49). These changes seem to be related to the intracellular amino acid pool size and its influence on protein metabolism.

Introduction

It is generally accepted that it is necessary to study in detail the mechanisms of biochemical and metabolic response to protein and amino acid intakes in order to calculate more accurately the qualitative requirements in different metabolic situations.

Rennie (1985) stated that changes in the synthesis and breakdown of body proteins which occur as a consequence of aggression, tend to be greater as the level of aggression increases. These changes affect metabolic adaptation and then may imply changes in relation to amino acid requirements and thus, to the amino acid pool and its relationship to amino acid intake. Schwartz *et al.* (1990) observed in fasted healthy fed and stressed fed rats that: 1. in each metabolic situation and at a given moment of the experiment, the flow of amino acids to a specific organ was reproducible; 2. of the sense of the change in amino acid flow did not determine the size of the global intracellular pool, or of each amino acid, but that these were reproducible; and 3 greater size of the global pool did not predict greater protein synthesis, but it may even be associated with its reduction. The question was whether protein synthesis could be modified by diets with normal protein content but changing qualitative amino acid composition.

Thus the aim of this work was to assess the effect of different amino acid supply in diets with normal nitrogen content on protein synthesis in the liver and jejunal mucosa.

Material and Methods

One hundred and twelve male Sprague-Dawley rats with the initial body weight of 150-170 g, were divided into 8 groups (7 experimental groups and a control group) and housed in metabolic cages with controlled temperature and 12-hour light-darkness cycle. Following a short period of adaptation (four days) the animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and post-surgical stress was produced by a fracture of the femur followed by the immediate insertion of a Kirschner pin. After the surgery rats received modular enteral diets with an invariable composition (14 % proteins, 40 % lipids and 46 % carbohydrates) for a period of four days. The medium-chain triglycerides/long-chain triglycerides ratio was 40/60. The nitrogen supplement was the same for all diets (0.104 gN/100 Kcal), with the qualitative composition of supplemented amino acids being the

only variable (group 1: Ile, Leu and Val; group 2: Phe, Met and Gly; group 3: Glu, Arg and Gly; group 4: Gly; group 5: Orn-Asp, Cys and Pro; group 6: Lys, Ser and Thr; group 7: Tyr, His and Ala). Group 8 was the control group, which received the modular diet without the supplement of amino acids. The control group received the same nitrogen content as the other diets. The quantitative amino acid composition of the diets is shown in Table 1. All diets were administered for four days after which the rats were killed.

Hepatic and jejunal mucosa protein synthesis was measured according to McNurlan *et al.* (1979). Briefly, a bolus injection of leucine was administered via a lateral tail vein of the rats (1-^{14}C Leucine, 50 mCi/mmol combined with unlabelled leucine to give 10 μCi and 100 μmol of leucine/ml). The specific activity was then 50 mCi/mmol. Animals were killed after 10 minutes. Organs were removed and immediately frozen in liquid nitrogen until analysis. The specific activity of free leucine and leucine bound to the protein was measured in order to calculate the fractional synthesis rate. The protein content was estimated by the Lowry method (Lowry et al. 1951). Statistical analysis was done by Student's t- and Mann-Whitney U-tests.

Table 1
Amino acid content of the diets

	Ile Leu Val	Phe Met Gly	Glu Arg Gly	Gly	Orn Cys Pro	Lys Ser Thr	Tyr His Ala	Control
Group	1	2	3	4	5	6	7	8
Ala	124	124	124	124	124	124	344	152
Arg	57	57	164	57	57	57	57	70
Asp	286	286	286	286	439	286	286	352
Cys	69	69	69	69	294	69	69	85
Glu	479	479	861	479	479	479	479	689
Gly	49	236	236	609	49	49	49	60
His	49	49	49	49	49	49	241	60
Ile	510	186	186	186	186	186	186	228
Leu	581	257	257	257	257	257	257	316
Lys	228	228	228	228	228	409	228	279
Met	62	430	62	62	62	62	62	76
Pro	193	193	193	193	407	193	193	237
Phe	127	835	127	127	127	127	127	156
Ser	143	143	143	143	143	402	143	175
Thr	231	231	231	231	231	526	231	284
Trp	57	57	57	57	57	57	57	70
Tyr	121	121	121	121	121	121	569	148
Val	470	181	181	181	181	181	181	222
Orn					153			

The results are given in mg of amino acids/100 Kcal

Results

Fig. 1 shows the hepatic protein synthesis which was similar in the experimental groups except of groups 2 and 3 in which a significant decrease compared to ontrols was observed (52.8±8.4 and 68.6±0.9 vs 88.6±12.1, respectively, $p<0.05$). Fig. 2 shows the protein synthsis in jejunal mucosa. Group 2, fed the aromatic amino acid supplement, exhibited a marked decrease in fractional synthesis rate values compared with the control group (98.7±16 vs 160.5±49, $p<0.05$). No statistically significant differences were observed in other experimental groups.

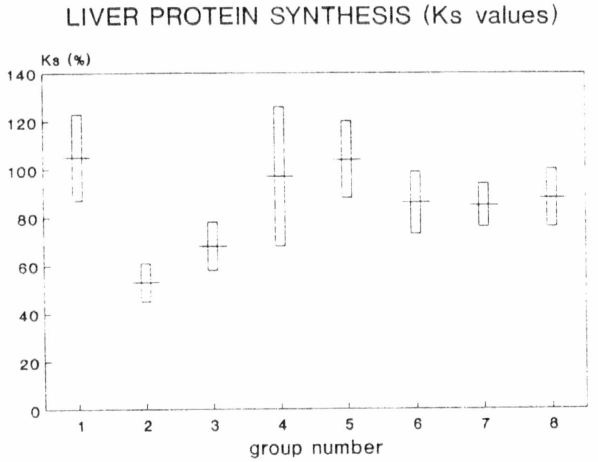

Fig. 1
Fractional protein synthesis rate in liver is given as means ± S.D.

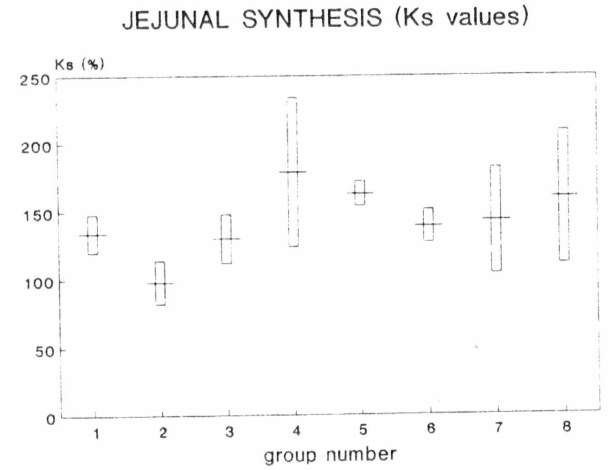

Fig. 2
Fractional protein synthesis rate in jejunum is given as means ± S.D.

Discussion

A contribution of this study might be that the qualitative variations in amino acid intake do not induce changes in visceral protein synthesis, except of cases in which supplemented amino acids affect relatively narrow therapeutic margins. This was clearly demonstrated (Harper *et al.* 1970) in diets with a low-protein content for aromatic amino acids which were supplemented in high doses in our study (Table 1). In fact, a direct toxicity produced by phenylalanine imbalance has been demonstrated. Our results indicate that at least part of the toxicity is produced at the level of protein synthesis. On the other hand, we have found certain differences between organs. In this context, there appears to exist an organ-specific effect which was already reported by Christensen (1986). In our study, the supplement of glutamic acid and arginine decreased protein synthesis in the liver but not in the jejunal mucosa, but values obtained in the liver were similar to the control values (68.8 ± 10.9 vs 88.6 ± 12.1). Thus, the toxicity of glutamic acid has been demonstrated (Christensen 1986). Moreover, improvement in muscular protein turnover induced by glutamine has been demonstrated (McLennan *et al.* 1988). This organ-specificity, with respect to both these adverse effects and the increase in protein synthesis, indicates that cellular and supracellular control mechanisms are involved. Furthermore, it should be noted that glycine supply in artificial nutrition formulae does not imply the adverse effects, considering the protein synthesis level which is a good indicator of cell function. Our results improve the strategy for designing new artificial nutritional formulae.

In this context, we have developed a new concept of cellular amino acid pool based on a close connection between the flow of amino acids into an organ, intracellular pool size and the capacity to modify of protein metabolism. In previous work, Schwartz *et al.* (1990) demonstrated that 1. the flow of amino acids into an organ was capable to modify the size of their intracellular pool, 2. the direction of the change in pool size did not depend on the direction of flow variations and 3. both the flow of amino acids and variations in the pool size depend on the organ studied. These findings suggested an organ-specific dependence of the cellular pool size with respect to the cellular uptake of amino acids. Thus we may postulate that each amino acid could be classified, in relation to the exogenous intake once the above mentioned conditions have been established, on three levels of intracellular pool: 1. A basal pool, the decrease of which would produce a decrease in protein synthesis by a lack of amino acids. This basically affects essential amino acids. 2. A useful pool in which the kinetics of protein synthesis of the first order are initiated and which are transformed into zero order kinetics as the pool size increases. 3. A futile pool in which the disproportion of amino acids that would produce a decrease in protein synthesis is reflected. The effect could be produced by toxicity, dysbalance or antagonism, and would affect both essential and dispensable amino acids. Artificial nutrition should permit us to attain the optimum value within a useful pool for each amino acid and organ to be studied. These variations should be analyzed in relation to different metabolic situations (stress, fasting, etc.) since variations in the flow of amino acids appear to depend on the metabolic status of the cell. These results show that the improvements in artificial nutrition formulae would permit us to reach a physiological pool. Further studies must therefore be conducted to determine the optimum qualitative variations of nitrogen intake with the aim of optimize protein synthesis from an organ-specific point of view.

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