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# THE RELATIONSHIP BETWEEN DIETARY PROTEIN INTAKE AND THE POSTPRANDIAL DIGESTIVE BALANCE OF PLASMA ALANINE, GLUTAMATE AND GLUTAMINE IN RATS AFTER SHORT AND LONG TERM TREATMENT

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*The effects of different amounts of dietary protein /0% (I), 5% (II), 15% (III), 50% (IV) casein in the diet/ on postprandial portal-systemic plasma level differences, i. e. digestive balance (DB), of alanine (Ala), glutamate (Glu) and glutamine (Gln) were estimated after 7 and 21 days in rats. The results showed specific plasma patterns of these amino acid with a positive DB for Ala in all groups, for Glu in the hyperprotein groups (IV-7, IV-21) and for Gln at 7 days (groups III-7, IV-7). In contrast, a negative DB for Glu was obtained in the (I-7, II-7, II-21) and in one normoprotein group (III-7). A tendency for the level of amino acids and/or DB to decrease from 7 to 21 days of treatment, was noted in both portal and systemic plasmas, except for Gln in groups 1-7 and I-21.*

*Key words: protein intake, amino acids, plasma, digestive balance*

## INTRODUCTION

There is growing evidence that the concentrations of amino acids in systemic plasma, including alanine (Ala), glutamate (Glu) and glutamine (Gln) change in many nutritional states, especially when the protein supply is reduced or excessive (Hutson and Harper, 1981; Remesy and Demigne, 1982). However, there are not sufficient data for other vascular beds, such as the portal circulation, although the digestive tract plays a major role in the metabolism of these amino acids, particularly during the postprandial period (Windmueller and Spaeth, 1980; Felig, 1981). It was computed (Remesy et al., 1978) that the increase in Ala concentration in portal plasma was almost exactly equal to the decrease in sum of Asp + Glu + Gln in both low and high protein diets. In order to examine the specific interrelations of Ala, Glu and Gln at the intestinal level at low and high dietary protein intakes over different periods, we estimated

the postprandial digestive balance (Demigne et al., 198 ) of some amino acids in rats fed different amounts of casein in the diet for 7 and 21 days, respectively. The digestive balance (DB) is the difference in amino acid concentrations between the portal and systemic plasma.

## MATERIAL AND METHODS

A total of 80 male Wistar rats, weighing 180-220 g, was fed on isocaloric diets (Roger and Harper, 1968) containing 0%, %, 1 % and 0% casein *ad libitum* for 7 and 21 days, respectively. The casein replaced an equivalent weight of potato starch and sucrose (2:1). After that, the rats were anesthetized with ether between 8 and 9 h a.m., during the period of maximal absorption of nutrients (postprandial state). Following laparatomy, 2 ml of blood was taken from the clamped portal vein and the same from the inferior vena cava (systemic plasma). To measure amino acid concentrations, plasma was deproteinized with sulphosalicylic acid immediately before analysis by ion-exchange chromatography utilizing a 119 CL Beckman Amino Acid Analyzer. Two-way analysis of variance with the least significant difference (Plochinski, 1970) was used in the analysis of our data.

## RESULTS AND DISCUSSION

The postprandial digestive balance, namely, the differences in portal (P) and systemic (S) plasma levels of Ala, Glu and Gln in rats fed different amounts of protein for 7 and 21 days are presented in table 1. As can be seen, a significantly positive DB (higher P than S levels) was obtained for Ala in all experiimental groups at both time intervals, except in the group fed 0% casein at 21 days (I-21). The DB for Glu in both hyperprotein groups (IV-7 and IV-21) as well as for Gln in groups III-7 and IV-7 were also significantly positive. In contrast, a negative DB (higher S than P levels) was shown in the protein deficient groups for Glu (I-7,II-7) and for Gln (I-7,II-7,II-21) and in the normoprotein group (III-7) only for Glu. It is well known (Felig, 1981; Remesy and Demigne, 1982) that the gut metabolises the nitrogen of amino acids mainly into Ala, resulting in its level being higher in portal plasma than in systemic plasma and consequently a positive DB for Ala in all groups (table 1). In contrast to that, the amount of Glu added to the portal plasma was relatively small compared with its content in casein or systemic plasma (Yamamoto et al., 1974; Suzić, 1989). It is possible that the intestine has a protective role in preventing the toxic effects of an excess of Glu and Asp on peripheral tissues (Remesy and Demigne, 1982). Concerning Gln, it is an important respiratory substrate for cells in the small intestinal mucosa and the intestine is a major site for metabolism of Gln released into the circulation by other tissues in the rat (Windmueller and Spaeth, 1980), as well as in sheep (Wolf et al., 1972) and man (Felig, 1981). Our findings indicate (Table 1) that in the protein deficient groups (I,II) removal

of Glu and Gln from systemic plasma by the intestine was significantly higher after 7 days than after 21 days, but through the longer period the difference was maintained only for Gln. We could not explain with certainty the extremely high plasma levels of Gln in this condition (21 days of a protein free diet) but it is possible that degradation of tissue proteins and/or synthesis of Gln by liver and muscle play an important role as well as impaired utilization (Bender, 1985). In contrast, in rats fed high protein diets (IV) the fractional recovery of Glu and Gln (7 days) and only of Glu (21 days) in portal blood was higher, resulting in a positive DB, presumably because the enzymic capacity for their metabolism in the intestine had been exceeded (Remesy et al., 1978). Stimultaneously, it can also be seen that the differences in DB of the estimated amino acids were smaller after 21 days than after 7 days treatment (Table 1). These differences are more clearly expressed in table 2, where differences in plasma levels of Ala, and Glu as well as Gln between 7 and 21 days of treatment (the 7 day values minus the 21 day values) are shown for each circulation. The levels, especially of Ala and Glu in all groups but with some exceptions, significantly and dramatically declined. These results may be explained by the induction of key enzymes involved in the catabolism of amino acids in the liver (Anderson et al., 1968) and/or skeletal muscle (Bondy, 1987) as well as consequences of metabolic changes at the intestinal level (Suzić et al., 1993). In contrast, significantly higher levels of Glu in both plasms were observed after 21 days iin groups consuming 0% casein. This might suggest net release of Glu from the liver, the intestine and other extrahepatic tissue which intensified during treatment in protein deficient condition. It is well known that Glu is a major constituent of the intracellular free amino acid pool. Following starvation, trauma and sepsis intracellular Glu concentration falls but in plasma it is increased (Bessey and Wilmore, 1988).

Table 1. Effect of protein intake on portal (P) and systemic (S) plasma levels *(μmol/1)* of ala, glu, gln and their digestive balances (DB) at 7 and 21 days (diets: I-0% casein; II-5% casein; III-15% casein; IV-50% casein

| Amino acid/Diets | | $I_7$ | $I_{I7}$ | III7 | IV7 | I21 | II21 | III21 | IV21 |
|---|---|---|---|---|---|---|---|---|---|
| Ala | P | 539 | 763 | 690 | 1.300 | 439 | 450 | 415 | 579 |
| | S | 398 | 616 | 352 | 456 | 345 | 317 | 278 | 271 |
| | DB | $161^b$ | $147^b$ | $338^c$ | $844^c$ | 95 | $133^c$ | $137^c$ | $308^c$ |
| Glu | P | 185 | 156 | 152 | 494 | 148 | 84 | 76 | 141 |
| | S | 234 | 253 | 198 | 204 | 156 | 90 | 60 | 82 |
| | DB | $-48^a$ | $-97^b$ | $-46^a$ | $290^c$ | -8 | -6 | 15 | $60^b$ |
| Gln | P | 225 | 432 | 341 | 815 | 361 | 322 | 258 | 381 |
| | S | 342 | 635 | 257 | 426 | 429 | 471 | 275 | 339 |
| | DB | $-117^b$ | $-203^c$ | $84^a$ | $389^c$ | -68 | $-149^b$ | -17 | 42 |

*Note.* Values for portal and systemic plasma levels are significantly different at $^a/p<0.05$, $^b/p<0.01$, $^c/p<0.001$

Table 2. Effects of protein intake on the differences of plasma levels of ala, glu and gln between 7 and 21 days (7 day minus 21 day values) in systemic and portal circulations (I-IV diets-see table 1)

| Amino acid/Diets | Systemic | | | | Portal | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | I | II | III | IV |
| Ala | 53,7 | 298,9$^{c}$ | 74,3 | 184,8$^{b}$ | 120,2$^{a}$ | 313,2$^{c}$ | 275,1$^{c}$ | 720.7$^{c}$ |
| Glu | 77,7$^{b}$ | 163,0$^{c}$ | 137,5$^{c}$ | 121,9$^{c}$ | 37,2 | 72,1$^{b}$ | 75,8$^{b}$ | 352,7$^{c}$ |
| Gln | -86,8$^{a}$ | 164,3$^{c}$ | -17,9 | 87,0$^{a}$ | -136,3$^{b}$ | 110,6 | 83,4 | 434,1$^{c}$ |

*Note*. Values for differences in plasma levels of amino acids between 7 and 21 days are significantly different at $^{a/}p<0.05$, $^{b/}p<0.01$, $^{c/}p<0.001$.

Our results (table 1 and 2) indicate clearly that a specifically positive DB for Ala is maintained in all groups through the whole experimental period as well as for Glu in the high protein group. On the contrary, the negative DB for Glu and Gln which was obtained in protein deficient groups, disappeared with continuation of the treatment for Glu but not for Gln. Accordingly, it is obvious that the adaptation to high and/or low protein diet shifts the intestinal metabolism of the estimated amino acids (from removal and utilization to production and/or protection). This is probably mediated in different ways by numerous still inadequately studied adaptation mechanisms.

A c k n o w l e d g m e n t

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## POVEZANOST PROTEINSKOG UNOSA I POSPRANDIJALNOG DIGESTIVNOG BILANSA ALANINA, GLUTAMATA I GLUTAMINA U PLAZMI PACOVA POSLE KRAĆEG I DUŽEG DIJETETSKOG TRETMANA

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SADRŽAJ

Ispitivani su efekti unosa različitih količina proteina /0% (I grupa), % (II grupa), 1 % (III grupa), 0% kazeina (IV grupa) u dijeti/, na koncentracije alanina (Ala), glutamata (Glu) i glutamina (Gln) kao i njihova razlika u sistemskoj i portnoj plazmi - digestivni bilans (DB) pacova posle 7. i 21 dana tretmana. Rezultati su pokazali specifični odnos kretanja nivoa ovih aminokiselina u plazmi, sa pozitivnim DB za Ala u svim grupamma, za Glu u hiperproteinskim grupama (nakon kraćeg i dužeg tretmana (IV-7, IV-21) i za Gln u grupama III-7 i IV-7. Nasuprot ovome, negativni DB je dobijen za Glu i Gln u grupama sa proteinskim deficitom /Glu (u I-7, II-7 i II-21)/, kao i za Glu (III-7) u normoproteinskoj grupi. Zapažena je tendenca snižavanja nivoa odnosa DB ispitivanih aminokiselina u obe (portalnoj i sistemskoj) cirkulacije, izuzev za Gln u grupama I-7 i I-21.