EFFECT OF PROTEIN DEPRIVATION AND SUBSEQUENT REHABILITATION ON THE INTESTINAL TRANSPORT OF L-METHIONINE *IN VIVO*

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Summary : The effect of protein deprivation and subsequent rehabilitation on the intestinal transport of L-methionine was studied in albino rats of both sexes. The rats given diet containing no protein or 3 per cent maize protein for 28 days, lost their intestinal cell population by 50 and 20 per cent respectively. The net absorption rate of L-methionine was little affected, while absorptive capacity of intestinal cells was considerably enhanced in protein-deficient rats. The increase in absorptive capacity of intestinal cells was much higher in rats given protein-free diet than in those given maize diet.

The augmentation in absorptive capacity of intestinal cells of protein-deprived rats was a temporary adaptation to the conditions that prevent the formation of new cells. Rehabilitation of malnourished rat on diet containing 17 per cent casein, resulted in a rapid increase in intestinal cell population, return of the absorptive capacity of intestinal cells to normal, and augmentation in net absorption rates.

Key words: intestinal absorption protein deprivation L-methionine

INTRODUCTION

The study of changes in intestinal absorption associated with protein deprivation may provide useful information about control mechanisms regulating intestinal transport, and may have important nutritional implications. However, investigations of the effect of dietary manipulations on the absorption of amino acids have produced conflicting results. Using *in vivo* procedures, Adibi and Allen (1) and Lis *et al.* (17) demonstrated that protein deprivation of human volunteers and rats resulted in a decreased rate of intestinal absorption from amino acid mixtures. On the contrary, Kobatake *et al.* (13) showed that, from a mixture of 18 amino acids, the transport rate of each amino acid was higher in rats given no protein than in those given diet containing 20 per cent protein.

Using single amino acid in the test solution, some investigators have demonstrated an increase in the intestinal transport of some amino acids in experimental animals given nutritionally inadequate protein (14,21) or no protein in the diet (13,31). Lis *et al.* (16), however, concluded that 5 per cent protein in the diet had no effect, while complete protein deprivation of rats resulted in diminution in the rate of absorption of L-methionine *in vivo*. Wapnir and Lifshitz (28) concluded there exists a selectivity in the effect of dietary restriction on the intestinal absorption of amino acids *in vivo*. They observed a stimulatory effect on the absorption of L-tyrosine and L-leucine, no change in the transport rates of L-phenylalanine and glycine, and a decrease in case of L-lysine. This confusion has arisen due to different parameters used, by various investigators, to assess absorption.

The present investigation was undertaken to study the intestinal transport of L-methionine, by an *in vivo* procedure, in rats given no protein or low amounts of poor quality protein. The effect of rehabilitation of malnourished rats using a casein diet was also investigated. The suitability of the basis of expressing absorption rates has been discussed.

MATERIALS AND METHODS

Albino rats were obtained from the Small Animal House of National Dairy Research Institute, Karnal, India. The intestinal transport of L-methionine was studied in the following ten groups, each consisting of six male and six female rats:

Group N : Normal post-weanling rats weighing 50-55 g.

Group PF : Post-weanling rats given protein-free diet for 28 days.

Group PF-C5 : Post-weanling rats given protein free-diet for 28 days and then casein diet for 5 days.

Group PF-C21 : Post-weanling rats given protein-free diet for 28 days and then casein diet for 21 days.

Group M : Post-weanling rats given maize diet for 28 days.

Group M-C1 : Post-weanling rats given maize diet for 28 days and then casein diet for 1 day.

Group M-C3 : Post-weanling rats given maize diet for 28 days and then casein diet for 3 days.

Group M-12 : Post-weanling rats given maize diet for 28 days and then casein diet for 12 days.

Group N-C9 : Post-weanling rats given casein diet for 9 days.

The composition of the diets were as follows (*g*/100 *g* ration) :

Ingredients	*Protein-free diet*	*Maize diet*	*Casein diet*
Casein	—	—	20
Maize	—	27	—
Ground nut oil	8	8	8
Salt mixture*	4	4	4
Vitamin mixture*	1	1	1
Cellulose	1	1	1
Starch upto	100	100	100

*Salt and vitamin mixtures were preparad and mixed according to AOAC (2).

The commercial casein contained 13.32 per cent nitrogen as estimated by micro-kjeldahl method (2). The crude protein content of casein and maize diets was estimated to be 17.0 and 3.0 per cent respectively.

The procedure of Kershaw *et al.* (12) with some modifications was used for the study of intestinal absorption. The animal was fasted for 12-15 hours and then anaesthetised with sodium pentabarbital (0.3 *mg*/*g* body weight) given intraperitoneally. The abdomen was opened to expose gastrointestinal tract. The ileum was ligated about 1 cm proximal to the cecum. A solution of L-methionine (0.8%) in Krebs-Ringer bicarbonate buffer (pH, 7.4), sufficent to fill two-third of the intestine, was introduced from the duodenal end. The duodenal end was then tied with thread and the intestine returned to the abdominal cavity. During the whole procedure, care was taken to prevent repturing of any blood vessel. After the absorption had been allowed to take place for 10 min, the intestine was removed, its surface carefully and quickly washed to make it free from blood, its content drained out and analyzed for methionine by the procedure of McCarthy and Sullivan (19).

For extraction of DNA, 100 *mg* dry intestine was homogenized with 15 *ml* of 4.22 per cent ice cold perchloric acid and kept in cold for 30 min. The residue obtained after centrifugation was again washed with 4.22 per cent ice cold perchloric acid. DNA was

then extracted with 25 *ml* of 4.22 per cent perchloric acid in boiling water bath for 20 min and estimated by the procedure of Burton (3).

RESULTS

Rats given protein-free and maize diets for 28 days did not grow; rather they lost 42 and 21 per cent of body weight respectively. The diminutions in body weights were similar in both sexes. When rehabilitated on casein diet, the rats of protein-free and maize groups recovered the loss in body weight within 5 and 3 days respectively. PF and M rats reached 100 g body weight in about 21 and 12 days respectively, while normal post-weanling rats did so within 9 days, when given casein diet. Thus the growth rates were slow in rats previously fed protein-free diet.

The intestinal dry weight, protein and DNA contents were reduced to about half in both sexes when given protein-free diet for 28 days (Table I and II). The diminution in these constituents of the intestine were, however, much less in rats given maize diet for the same period.

There was an abrupt increase in the intestinal cell constituents, when PF rats were rehabilitated on casein diet. The losses in dry weight were recovered by 32 and 21 per cent in male and female rats respectively when given casein diet for 1 day. From second day onward there was a gradual increase in the intestinal cell constituents. The dry weight, protein and DNA contents increased by more than 50 per cent within 5 days of rehabilitation. Compared to PF rats, the increase in dry weight and protein deposition in the intestine of M rats on rehabilitation were very slow.

The rats given previously protein-free or maize diet were rehabilitated on casein diet till a body weight of 100 g was achieved. The levels of intestinal cell constituents in these two groups were compared with those of normal rats of same weight (N-C9). Table I and II show that the dry solids, protein and DNA constituents of three groups, that is, N-C9, PF-C21 and M-C12, were comparable in both sexes.

Table III and IV show the rates of intestinal absorption of L-methionine under different dietary treatments in male and female rats respectively. The net absorption as well as absorption per unit intestinal mass were about 50 per cent higher in males than in females. The absorption per unit intestinal mass increased by more than 120 per cent in both sexes, when given protein-free diet for 28 days. The net absorption was also significantly increased (about 10%) in rats given protein-free diet.

TABLE I : Changes in the fntestine of male rats.

Groups	Dry weight			Protein			DNA		
	mg	Difference(%)		mg	Difference (%)		mg	Difference (%)	
N	348±8			272±4			11.2±0.2		
PF	174±1	N vs PF	=-49.6	116±1	N vs PF	=-57.3	5.5±0.1	N vs PF	=-50.9
M	238±3	N vs M	=-31.0	161±2	N vs M	=-40.8	9.3±0.1	N vs M	=-20.0
PF-C1	229±3	PF vs PF-C1	= 31.6	139±2	PF vs PF-C1	= 19.8	6.3±0.0	PF vs PF-C1	= 14.5
PF-C5	261±1	PF vs PF-C5	= 50.0	178±1	PF vs PF-C5	= 53.4	9.7±0.0	PF vs PF-C5	= 76.4
M-C1	243±2	M vs M-C1	= 2.1	162±2	M vs M-C1	= 0.6	9.4±0.1	M vs M-C1	= 1.1
M-C3	253±2	M vs M-C3	= 6.3	174±2	M vs MC-3	= 8.1	11.5±0.1	M vs M-C3	= 23.6
M-C9	658±9			485±3			22.3±0.2		
PF-C21	640±2	N-C9 vs PF-C21	= 2.7	456±1	N-C9 vs PF-C21	= 6.0	23.4±0.1	N-C9 vs PF-C21	= 4.9
M-C12	647±3	N-C9 vs M-C12	= 1.7	476±2	N-C9 vs M-C12	= 1.8	25.7±0.1	N-C9 vs M-C12	= 15.2

Results expressed as Mean ± SEM.

TABLE II : Changes in the intestine of female rats.

Groups	Dry weight			Protein			DNA		
	mg	Difference (%)		n g	Difference (%)		mg	Difference (%)	
N	376±8			284±4			12.0±0.1		
PF	175±1	N vs PF	=-53.4	112±1	N vs PF	=-60.6	5.8±0.1	N vs PF	=-51.7
M	235±3	N vs M	=-37.5	161±3	N vs M	=-43.3	9.7±0.1	N vs M	=-19.2
PF-C1	218±4	PF vs PF-C1	= 24.6	137±2	PF vs PF-C1	= 22.3	6.1±0.1	PF vs PF-C1	= 5.2
PF-C5	261±1	PF vs PF-C5	= 49.1	180±1	PF vs PF-C5	= 60.7	9.7±0.1	PF vs PF-C5	= 67.2
M-C1	245±1	M vs M-C1	= 4.2	167±1	M vs M-C1	= 3.7	9.9±0.1	M vs M-C1	= 2.1
M-C3	250±2	M vs M-C3	= 6.4	172±2	M vs M-C3	= 6.8	11.4±0.1	M vs M-C3	= 17.5
N-C9	647±1			473±4			20.8±0.1		
PF-C21	633±2	N-C9 vs PF-C21	= 2.2	457±1	N-C9 vs PF-C21	= 3.4	23.6±0.2	N-C9 vs PF-C21	= 13.5
M-C12	647±2	N-C9 vs M-C12	= 0.0	483±1	N-C9 vs M-C12	= 2.1	26.2±0.1	N-C9 vs M-C12	= 26.0

Results expressed as Mean ± SEM.

TABLE III : Absorption of L-methionine in male rats per 10 minutes.

Groups	Net absorption (μmoles)	Difference (%)		μmoles/ g dry intestine	Difference (%)		μmoles/ 10 mg intestinal DNA	Difference (%)	
N	89.4±0.9			259.6±7.7			76.4±1.0		
PF	99.1±2.7	N vs PF	= 10.8*	569.6±16.0	N vs PF	= 119.7*	180.1±5.6	N vs PF	=135.7*
M	84.8±2.0	N vs M	=-5.1	348.4±9.5	N vs M	= 34.2*	89.9±1.6	N vs M	=17.7*
PF-C1	84.0±1.1	PF vs PF-C1	= 15.2*	367.8±7.3	PF vs PF-C1	=-35.4*	134.2±1.9	PF vs PF-C1	=-25.8*
PF-C5	92.6±1.1	PF-C1 vs PF-C5	= 10.2*	354.9±4.5	PF-C1 vs PF-C5	=-3.5	95.6±1.2	PF-C1 vs PF-C5	=-28.8*
M-C1	89.2±0.7	M vs M-C1	= 5.2	366.1±4.6	M vs M-C1	=5.1	90.6±1.1	M vs M-C1	= 0.8
M-C3	94.9±1.3	M-C1 vs M-C3	= 6.4	376.9±5.6	M-C1 vs M-C3	=2.9	82.6±1.6	M-C1 vs M-C3	= 8.8**
N-C9	101.6±2.6			154.9±3.0			45.5±1.0		
PF-C21	116.4±0.8	N-C9 vs PF-C21	= 14.6*	182.4±1.5	N-C9 vs PF-C21	=17.7*	49.9±0.3	N-C9 vs PF-C21	=9.7*
M-C12	118.3±0.7	N-C9 vs M-C12	= 16.4*	182.7±1.3	N-C9 vs M-C12	=17.9*	46.1±0.3	N-C9 vs M-C12	=1.3

Results expressed as Mean ± SEM.
*Differences are statistically significant ($P<0.01$).
**Differences are statistically significant ($P<0.05$).

TABLE IV : Absorption of L-methionine in female rats per 10 minutes.

Groups	Net absorption (μmoles)	Difference (%)		μmoles/ g dry intestine	Difference (%)		μmoles/ 10 mg intestinal DNA	Difference (%)	
N	61.0±1.5			161±5.5			52.4±0.7		
PF	66.4±1.1	N vs PF	= 8.8**	380±5.6	N vs PF	=136.0*	113.5±3.0	N vs PF	= 116.6*
M	69.7±2.7	N vs M	= 14.3*	297±12.6	N vs M	= 84.5*	71.4±2.8	N vs M	= 36.2*
PF-C1	58.8±1.2	PF vs PF-C1	=-11.4*	270±7.0	PF vs PF-C1	=-28.9*	95.5±3.0	PF vs PF-C1	=-15.8*
PF-C5	67.7±0.8	PF-C1 vs PF-C5	= 15.0*	259±4.9	PF-C1 vs PF-C5	=-4.1	70.2±0.8	PF-C1 vs PF-C5	=-26.5*
M-C1	67.7±0.9	M vs M-C1	=-2.9	275±4.6	M vs M-C1	=-7.4	68.2±1.3	Mvs M-C1	=-4.5
M-C3	75.7±7.1	M-C1 vs M-C3	= 11.8*	306±7.0	M-C1 vs M-C3	= 3.0	66.7±1.5	M-C1 vs M-C3	=-2.2
N-C9	104.5±2.6			160±4.0			50.0±1.1		
PF-C21	105.1±1.0	N-C9 vs PF-C21	= 0.6	165±0.3	N-C9 vs PF-C21	= 3.1	45.0±0.8	N-C9 vs PF-C21	= 10.0*
M-C12	107.5±0.6	N-C9 vs M-C12	= 2.9	166±0.9	N-C9 vs M-C12	= 3.7	40.9±0.3	N-C9 vs M-C12	= 18.2*

Results expressed as Mean ± SEM.
*Differences are statistically significant ($P<0.01$)
**Differences are statistically significant ($P<0.05$)

The net absorption of L-methionine increased significantly in females, while in males no significant alteration was observed after these animals were fed on maize diet for 28 days. The absorption per unit intestinal weight, however, incresed considerably in both sexes, the increase being more in females (84%) than in males (34%). The magnitudes of augmentation were much less when absorption rates were expressed on intestinal DNA basis. Thus the enhancement in the absorptive capacity of intestinal cells was less in rats given maize diet than those given protein-free diet.

An abrupt decline in net transport of L-methionine as well as absorption per unit intestinal mass was observed in PF rats when given casein diet for 1 day. The net absorption was decreased by 15.2 and 11.4 per cent and absorption per unit intestinal weight by 35.4 and 29.0 per cent in male and female rats respectively. From second day onward of rehabilitation on casein diet, there was a gradual increase in net absorption and decrease in absorption per unit intestinal mass.

The rats of M group showed a gradual increase in net absorption of L-methionine on rehabilitation. The absorption rates per unit intestinal mass were, however, little affected during first few days of rehabilitation and decreased thereafter.

The rats given previously protein-free or maize diets were rehabilitated on casein diet till they achieved a body weight of 100 g, and the intestinal absorption rates of L-methionine in these two groups were compared with those in normal rats of same weight group. The net absorption rates as well as absorption per unit intestinal weight were similar in malnourished - rehabilitated and normal female rats. In males, the absorption rates (both net absorption as well as absorption per unit intestinal weight) were significantly higher in malnourished - rehabilitated than in normal rats. When results were expressed on DNA basis, malnourished - rehabilitated female rats also absorbed L-methionine at higher rates than normal females.

DISCUSSION

The losses in the intestinal cells of rats given protein-free or maize diet are in agreement with the morphological and histological observations made by previous workers. Hooper and Blair (10) and Brown *et al.* (3) observed diminution in the mitotic figures in the crypt regenerative area with a reduction in the number of cells in the crypts and villi as well as definite cytological alterations, a few days (4 to 5 days) after withdrawal of food. Elimination of protein from the diet for prolonged periods resulted in mucosal atrophy, blunting and shortening of villi and microvilli of various experimental animals (6, 23, 27, 31). The villi were reported to be irregular in size and orientation, and fewer than in normal

cells (27). The rates of cell renewal and migration of epithelial cells to the villus tips were significantly reduced after protein depletion and starvation (3, 6, 10). Similar observations were also made for jejunal mucosa of malnourished human subjects (4, 26). Neonatal rats born to mothers deprived of protein, also had shorter and narrower intestines and fewer absorptive cells (20).

Intestinal structure is so susceptible to dietary conditions that the basis on which the absorption is expressed becomes crucial. Much confusion has arisen due to different modes of expression of absorption used by various investigators. The animals, in the present investigation, lost about 50 per cent of their intestinal cells when given protein-free diet for 28 days. Not only protein starvation for longer periods, but also complete starvation (20, 25) and semistarvation (18) for short periods are known to cause thinning of the intestinal wall, with reduction in size and number of villi and mucosal epithelial cells. Hence the results expressed on gut weight basis can not be compared with those expressed per unit intestinal length Some workers (16, 22) considered intestinal length to be more satisfactory for expressing "Physiological absorption" capacity of the intestine of malnourished animals, since intestinal length is little affected by dietary alteration (16). But it will be fallacious to assess absorption simply in terms of net absorption i.e. on the basis of intestinal length. Intestinal transport mechanisms adapt to protein deprivation by preferentially maintaining the functional activities of the epithelial cells or by increasing their activity - this fact gets over looked when transport is expressed in absolute terms. Therefore, the transport capacity of the intestine should be expressed in terms of net absorption as well as on the basis of intestinal weight or intestinal DNA.

In previous studies (13, 14, 16, 17, 21, 28, 31), for evaluating the effect of protein deficiency on the intestinal transport of amino acids, comparison used to be made between animals given protein-deficient diet and those given normal protein diet for the same period. Since the number of epithelial cells and intestinal weight are increased in animals given protein diet and decreased in those given no protein diet, any adaptive increase in the absorptive capacity of intestinal cells of protein deficient rats gets over looked because of higher rates of net absorption due to higher numbers of intestinal cells in animals given normal protein diet. Therefore, it was considered to be useful to compare the transport capacity of the intestinal cells of animals before and after giving protein deficient or protein free diet.

The increase in absorption per unit gut mass in rats given maize protein or no protein diet is in agreement with reports for enhanced transport of L-histidine in rats given low protein (21) or no protein diet (13); for tyrosine and L-leucine in rats given low protein diet (28) and for L-methionine, L-lysine and DL-theonine in rats given protein free diet

(31). Our results are in contrast with the observations of Lis *et al.* (16). Using *in vivo* procedure, they reported reduction in L-methionine absorption in rats given protein-free diet for 41 days. This conclusion was, however, drawn by expressing the absorption rates per unit intestinal length. When expressed per unit gut weight, they observed no difference in the absorption rates, between rats given normal protein or no protein diet for 15 or 41 days. Adibi and Allen (1) and Lis *et al.* (17) also expressed the absorption rates per unit gut length and reached the conclusion that protein deprivation results in decreased intestinal absorption of amino acids. The conclusion of Wapnir and Lifshtiz (i.e., there exists a selectivity in the effect of dietary restriction on the intestinal absorption of amino acids) is also based on their results expressed per unit gut length (28).

Though the absorption rates per unit intestinal mass increased markedly on protein deprivation, the net absorption of L-methionine was little affected. This was due, largely, to decrease in intestinal cell population. Kobatake *et al.* (13) also observed that although the absolute amount of L-histidine transported across the intestine was similar in rats given 5 per cent, 20 per cent or no protein diet, expressing the results on gut weight basis gave greater absorption in rats given no protein diet. Similar observations have been made for L-leucine, L-histidine, glycine, proline and L-alanine in 3-day starved rats (15). It is, therefore, concluded that dietary restriction or protein deprivation result in enhanced absorptive capacity of intestinal cells, but net transport rates do not change or show relatively small changes.

The augmentation in absorptive capacity of intestinal cells of protein deprived rats is, perhaps, a temporary adaptation to the condition that prevents the formation of new cells. As soon as the malnourished animals are provided a casein diet, the formation of new cells starts which is accompanied by return of the absorptive capacity of intestinal cells to normal.

For a number of reasons, the augmentation of active transport by protein deprivation does not seem to be due to thinning which the intestine undergoes during the period of dietary restriction. First, Dowling *et al.* (7) induced increased glucose and water absorption by rat jejunum by feeding a high-bulk low-calorie diet (by using Kaolin) which had no effect on jejunal thickness. Second, Hindmarsh *et al.* (8) could find no change in amino acid active transport by semistarved hamster small intestine despite considerable thinning of the intestine. Third, the mid-small intestine of the normal rat is thicker than the lower region (3), yet the mid-region absorbs L-histidine better (9).

Yasumoto *et al.* (30) reported that the activities of ouabain-sensitive ATPase were increased in the intestines of semistarved rats. Semistarvation and complete starvation (for short periods) are known to enhance the absorption of amino acids across the intestine (8, 12, 18, 22, 25, 30). Ouabain-sensitive ATPase which forms the enzymatic basis

of energy dependent active transport across the cell membrane was also reported to have two fold higher activity in children suffering from Kwashiorkor (11). Therefore, increased energy metabolism in the intestinal tissues of protein-deficient rats, might be related to enhanced rates of amino acid absorption.

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