Note

Effect of Carbohydrates and Starvation on Nitrogen Sparing Action of Methionine and Threonine in Rats

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It was previously reported¹⁻³⁾ that the dietary supplementation of methionine and threonine to a protein free diet had markedly reduced body weight loss and urinary nitrogen excretion. In these experiments, sucrose was used as a source of carbohydrate. Because, when the accumulation of liver lipid of rats fed a protein free diet supplemented with methionine was preliminary investigated at first, this fatty liver was derived from the feeding of sucrose more clearly than that of starch as a source of carbohydrate. While, the kind of dietary carbohydrate may affect the body protein metabolism through osmotic pressure and absorption of supplemented amino acids. In this paper, we have investigated the effects of sucrose or starch as a source of carbohydrate and starvation on nitrogen sparing action of methionine and threonine.

I. Effects of dietary supplementation of methionine and threonine on nitrogen sparing action of rats fed starch or sucrose as a source of carbohydrate were investigated

(Tables I and II). Young female rats of the Wistar strain weighing about 120 g were used with six rats per groups. The control diet was a protein free diet containing appropriate amounts of salts and vitamins⁴⁾ and 5% of corn oil. Sucrose or starch was used as a source of carbohydrate. Sucrose-diet was given as a powder or was

Fig. 1. Effect of the Dietary Form and Carbohydrates on the Body Weight Changes of Rats Fed a Protein Free Diet (PF) and the Protein Free Diet Supplemented with Methionine and Threonine (PMT) for 10 Days.

Vertical bars associated with 10 day data indicate standard deviations.

TABLE I. EFFECT OF THE DIETARY FORM AND CARBOHYDRATE ON THE URINARY UREA AND NITROGEN OF RATS FED A PROTEIN FREE DIET (PF) AND THE PROTEIN FREE DIET SUPPLEMENTED WITH METHIONINE AND THREONINE

Group diet	Initial weight (g)	Food intake (g/10 days)	Urinary urea (mg/3 days)	Urinary nitrogen (mg/3 days)
<i>Agar (sucrose)</i>				
1. PF	120.6 ± 6.7 ¹	74.1 ± 8.0	111.2 ± 24.5	82.2 ± 10.3
2. PF + Met + Thr	119.8 ± 4.5	71.8 ± 9.2	26.4 ± 6.1 ²	41.4 ± 5.9 ²
<i>Powder (sucrose)</i>				
3. PF	117.7 ± 6.0	74.3 ± 9.6	72.7 ± 7.7	75.8 ± 7.2
4. PF + Met + Thr	117.8 ± 5.3	77.9 ± 9.8	19.7 ± 5.9 ²	47.3 ± 3.8 ²
<i>Paster (starch)</i>				
5. PF	119.6 ± 5.6	106.0 ± 7.2	49.3 ± 9.3	52.6 ± 3.2
6. PF + Met + Thr	119.5 ± 5.0	102.4 ± 7.0	25.0 ± 2.7 ²	42.7 ± 1.9 ²

¹ Means ± S.D. of six rats.

² Significantly different from protein free diet group ($p < 0.05$).

TABLE II. EFFECT OF THE DIETARY FORM AND CARBOHYDRATE ON PLASMA UREA, URINARY NITROGEN AND NITROGEN-BALANCE OF RATS FED A PROTEIN FREE DIET (PF) AND THE PROTEIN FREE DIET SUPPLEMENTED WITH METHIONINE AND THREONINE

Group diet	Initial body weight (g)	Changes in body weight (g/10 days)	Liver weight (g)	Plasma urea (μ g/ml)	Urinary-nitrogen		Nitrogen- balance (mgN/day 7~10)
					Day 3~5 (mg/3 days)	Day 8~10 (mg/3 days)	
<i>Powder (sucrose), ad libitum</i>							
1. PF	125.8 $\pm$ 5.1 ¹	-20.2 $\pm$ 2.6	3.95 $\pm$ 0.30	159.9 $\pm$ 43.2	93.8 $\pm$ 6.8	81.7 $\pm$ 6.6	-90.2 $\pm$ 6.7
2. PF + Met + Thr	125.3 $\pm$ 3.8	-13.7 $\pm$ 4.5	4.71 $\pm$ 0.28	65.9 $\pm$ 13.6 ²	63.4 $\pm$ 4.3 ²	39.9 $\pm$ 2.3 ²	-49.9 $\pm$ 7.6 ²
<i>Paste (starch), ad libitum</i>							
3. PF	125.6 $\pm$ 3.5	-5.3 $\pm$ 3.6	3.77 $\pm$ 0.20	79.2 $\pm$ 23.5	70.1 $\pm$ 5.1	50.1 $\pm$ 6.0	-62.4 $\pm$ 3.8
4. PF + Met + Thr	125.8 $\pm$ 4.0	0.7 $\pm$ 2.9	5.07 $\pm$ 0.19	29.8 $\pm$ 7.1 ²	54.2 $\pm$ 3.2 ²	35.1 $\pm$ 1.4 ²	-29.3 $\pm$ 9.3 ²
<i>Paste (starch), pair-fed³</i>							
5. PF	124.7 $\pm$ 3.1	-15.3 $\pm$ 2.7	3.18 $\pm$ 0.09	158.9 $\pm$ 21.8	68.7 $\pm$ 7.8	63.2 $\pm$ 7.0	-71.9 $\pm$ 6.4
6. PF + Met + Thr	123.8 $\pm$ 3.7	-12.3 $\pm$ 1.6	3.76 $\pm$ 0.25	55.0 $\pm$ 11.5 ²	55.4 $\pm$ 5.3 ²	37.1 $\pm$ 3.7 ²	40.9 $\pm$ 7.4 ²

¹ Means $\pm$ S.D. of six rats.

² Significantly different from protein free diet group ($p<0.05$).

³ Paired-feeding to sucrose groups 1 and 2, respectively.

mixed with the same weight of 3% agar-solution to make agar-gel diet. Starch-diet was given as a paste with appropriate amounts of water. All rats were provided feed and water *ad libitum* for 10 days. Urine was collected for the periods of day 3~5 and 8~10 and urinary urea and nitrogen were determined by the method of Archibald⁵⁾ and by semimicro-Kjeldahl method.⁶⁾ In the feeding of the sucrose-diets, the body weight loss and the loss of urinary nitrogen and urea of rats fed the protein free diet supplemented with methionine and threonine were significantly smaller than those of rats fed the protein free diet (Fig. 1). The decrease of the urinary urea excretion of rats fed the amino acid supplemented diet was particularly remarkable. On the other hand, the losses of body weight of rats fed both starch-diets were almost the same. However, the output of urinary urea of rats fed the protein free diet was significantly greater than that of rats fed the amino acid supplemented diet. In spite of the kinds of sucrose or starch, the excretion of urinary urea of rats fed the protein free diet supplemented with methionine and threonine was markedly reduced as compared with that of rats fed the protein free diet (Table I). When amino acids were not supplemented to the control diet, the output of urinary urea of rats fed starch-diet was smaller than that of rats fed sucrose-diet. Then, the food intakes of rats fed the sucrose- or starch-diets were about 7 or 10 g per day, respectively. So, similar experiment was repeated but the food consumptions of rats fed starch-diet were equal to that of rats fed sucrose-diet (Table II). The changes in body weight of rats fed sucrose-diets (*ad libitum*) or starch-diets (paired feeding to sucrose groups) were the similar manner. However, the output of urinary nitrogen, plasma urea and nitrogen-balance of rats fed the protein free diet supplemented with methionine and threonine were significantly reduced as compared with those of rats fed the protein free diet. Therefore, the other factor except food intake might be concerned in this nitrogen sparing action of methionine and threonine. While, the small loss of body weight of rats fed starch-diets *ad libitum* might be considered to be due to the greater amounts of food consumption.

II. Effects of methionine and threonine in drinking water on changes in body weight, liver fat and urea in urine of starved rats were investigated (Table III). Young female rats of the Wistar strain weighing about 150 g were used with six rats per group. Methionine and threonine were dissolved in drinking water. In preliminary experiment, the food intake was less than 15 g per day and water intake was 12~25 ml. Then we planned that rats might ingest methionine and threonine corresponding to 15 g of protein free diet supplemented with 0.3% of methionine and 0.3% of threonine. In the control group, glutamic acid was dissolved in drinking water in order to equalize the nitrogen intake for all groups. Namely, 1.13% of glutamic acid (group 1), 0.5% of methionine and 0.63% of glutamic acid (group 2) or 0.5% of methionine and 0.5% of threonine (group 3) were provided to each groups as drinking water, respectively. All rats were provided no feed but water *ad libitum* for 4 days. Urine was collected for the periods of day 1~2 and 3~4 and urinary urea was determined. The losses of body weight in all groups were almost equal for 4 days of starvation. The accumulation of liver fat of rats drunk water supplemented methionine was partially prevented by additional supplementation with threonine (Table III). The output of urinary urea for the period of day 1~2 was the same among three groups and was higher than that of day 3~4. However, the urinary urea excretion of rats drunk water supplemented with methionine or methionine and threonine for the period of day 3~4 was significantly reduced as compared with that of rats drunk control water (1.13% of glutamic acid). The effect of additional threonine on urinary excretion of urea was not observed. The present results may show that, even in the condition of starvation, the output of urinary urea of rats is reduced by ingestion of methionine and threonine as the feeding test diets, fundamentally. There might be two reasons why the expected differences between the ingestion of methionine and that of methionine and threonine were not observed. The first reason may be considered that the catabolism of body protein in starved rats was remarkable, the output of urinary urea was extremely enhanced and the effects of supplementary methionine and threonine was

TABLE III. EFFECT OF METHIONINE AND THREONINE IN DRINKING WATER ON THE CHANGES IN BODY WEIGHT, LIVER FAT AND UREA EXCRETION OF STARVED RAT

Group	Drinking water	Changes in body weight (g/4 days)	Liver fat (%)	Urea-excretion	
				Day 1 ~ 2	Day 3 ~ 4
				(mg/day)	
1.	1.13% Glu (control)	-27.3 ± 1.8 ¹	10.2 ± 1.17	208.6 ± 11.4	146.4 ± 10.9
2.	0.5% Met + 0.63% Glu	-27.0 ± 0.6	14.0 ± 1.05 ²	204.4 ± 8.7	124.8 ± 2.7 ³
3.	0.5% Met + 0.5% Thr	-28.1 ± 0.7	12.6 ± 0.77	199.7 ± 7.4	122.9 ± 2.3 ³

¹ Means ± S.D. of six rats.

² Significantly different from control group ($p < 0.05$).

³ Significantly different from control group ($0.05 < p < 0.1$).

not clear. The second one could also be considered that only methionine might be a limiting amino acid endogenously in this starved condition in which much amounts of methionine and threonine were produced by the degradation of body proteins.

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