Z. Ernährungswiss. 17, 206–209 (1978)

ISSN 0044-264 X

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Some aspects on liver enzymes in protein-energy malnutrition

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With 2 tables

(Received April 3, 1978)

Relation between nutrition and enzyme level patterns in tissues and body fluids became of prime importance in making a precise diagnosis of the different stages and various forms of malnutrition. Far from many factors such as inheritance, age, sex and environment, a change in the diet affects the activity of several enzymes.

Variations in the activities of tissue enzymes in response to diet composition and pattern of feeding has long been established. In this respect, *Vaughan* (1) had reported some variations in tissue enzymes in response to different dietary carbohydrates. Variations in the activities of various tissue enzymes in response to protein intake have been presented by *Knox* (2) and *Metwalli* (3). The role of feeding schedule on the levels of various enzymes in rat liver has been studied in details by *Potter* (4).

These enzyme changes in response to dietary variations have been considered to present either changes in the enzyme protein, apoenzyme, levels or the coenzymes or both. The present study was designated to investigate the response of liver alkaline phosphatase glutamic-pyruvic (GPT) transaminase and glutamic-oxalacetic (GOT) transaminase to protein depletion in comparison with a control group receiving an adequate amount of protein. These enzymes were selected since alkaline phosphatase contains Mg as an essential metal or coenzyme and transaminases contain pyridoxal phosphate as coenzyme.

The liver alkaline phosphatase activities were determined before and after Mg addition to the incubation media during its assay, while transaminase activities were determined before and after pyridoxine chloride injections.

Material and Methods

Animals

The present study was carried out on rats of Sprague Dawley strain of our own colony. Rats of three weeks old, weighing 35 ± 3 g were housed individually in wire- bottomed cages at room temperature of 23–25 °C and relative humidity of 45–55%. Animals were assembled into three groups, each of 32 animals. The first group was fed diets containing adequate quantity of protein (20%). The second was maintained on a low-protein diet (5%). These two groups were fed ad libitum. The third was a pair fed group receiving the high-protein diet in amounts equivalent to that actually consumed by the low-protein group. This is in order to avoid any

variations in food intake since it was very markedly low in the low-protein group compared with the high-protein group. The feeding experiment lasted for three weeks.

Diets

The diets prepared have the following formulae and were chemically analysed for crude protein and fiber content after its preparation

	High protein g/100 g (cooked	Low protein broad beans)
Protein (Casein)	20.00	5.00
Fat (Cotton seed oil)	10.00	10.00
Carbohydrate (Sugar cane)	64.55	79.55
Salt Mixtures (Spector 5)	3.50	3.50
Vitamin mixture (*Metta* and *Mitchell* [6])	1.00	1.00
Vitamin A and D	0.40	0.40
Vitamin E and K	0.05	0.05
Choline chloride	0.50	0.50

Enzyme assay procedures

At the end of the feeding experiment, animals were killed by decapitation, livers were rapidly removed, freed from blood with blotting or filter paper and washed with deionised water. Livers were homogenised with the fivefold of its volume of cold deionised water in a glass homogenizer surrounded by ice. Homogenates were filtered and aliquots were used for enzyme assays. The method described for alkaline phosphatase assay by *King* was followed (7). This was the technique for alkaline phosphatase. The effect of Mg ions on the enzyme activity was studied at concentration of 0.01 M. Enzyme units were expressed as mg P liberated per fifteen minutes per g tissue protein.

For transaminase activities, seven animals of each group were killed; another seven animals were injected by 1 mg pyridoxine hydrochloride per g body weight and killed after six hours. Livers were homogenised in 0.1 M phosphate buffer pH 7.4 and filtered. Enzyme activities were determined in the aliquots using the method of *Reitman* and *Frankel* (8). Activity units were expressed per g tissue proteins. Tissue proteins were determined according to the method of *Lowry* (9).

Table 1. Liver alkaline phosphatase activities before and after Mg addition to the incubation medium (units/g tissue protein).

Group	Alkaline phosphatase activity without Mg addition	Alkaline phosphatase activity after 0.01 M Mg addition	Percent activation
High protein* (9)	1.16 ± 0.14	2.72 ± 0.13	68.94
Low protein (9)	10.19 ± 0.68	10.20 ± 0.63	00.09
Pair fed (9)	1.46 ± 0.08	2.32 ± 0.42	58.90

* Number of animals studied
± Standard error

Table 2. Liver transaminase activities before and after pyridoxine injection (units/g tissue protein).

Group	GPT Before	After	Percent activation	GOT Before	After	Percent activation
High protein (7)	9,117 ± 915	152,250 ± 16,390	1569	6,510 ± 1,605	121,750 ± 5,593	1770
Low protein (7)	22,215 ± 1,480	54,417 ± 5,078	145	18,492 ± 3,871	42,945 ± 1,079	132
Pair fed (7)	7,520 ± 331	29,710 ± 1,020	295	5,020 ± 202	24,196 ± 1,650	318

± Standard error

Results and Discussion

The results obtained were presented in the accompanying tables.

The increased activities of liver alkaline phosphatase in the protein-depleted group in comparison with both the high protein and the pair fed groups agreed with that reported by *Ross* and *Batt* (10). These increased activities agreed also with the claim of *Vaughan* (1) that high carbohydrate content of the diet stimulates the synthesis of the protein apoenzymes responsible for carbohydrate metabolism. This a manifestation of the adaptive response mechanism reported by *Vaughan* (1).

The relatively marked activation of liver alkaline phosphatase in the high protein and the pair fed groups suggested that the apoenzyme protein synthesis is much active in protein-depleted animals. This suggestion is based on the observation that Mg addition to in the incubation media failed to activate the enzyme in the protein-depleted group. Also, the relatively low values of alkaline phosphatase activities in both high protein and the pair fed groups cannot be related Mg inavailability since both groups received the salt mixture. Mg intake of the low-protein group is much lower than the high protein fed group since the food intake of former group is markedly diminished. Differences in the nature of alkaline phosphatases can't be ignored since *Rosenberg* (11) reported such variations by using electrophoretic techniques.

Both liver GPT and GOT showed a similar response to protein depletion. Both enzymes were highly increased in the low protein fed group in comparison with both the high protein and the pair fed groups. Also, food restriction seems to have an inhibitory effect on both transaminases.

Pyridoxine injection activates significantly liver transaminases in all groups studied but this activation was comparatively higher in the group receiving the high protein diet. The percentage of activation reached about ten times that of the low protein fed group. Contrary to alkaline phosphatase, transaminases activities after pyridoxine injection exceeded that activity before and even after pyridoxine injection its corresponding values in the low protein and the pair fed groups. From the above finding, one can suggest that the increased activities of liver transaminases in the low protein fed group before pyridoxine injection was not due to increased enzyme protein synthesis. It can also be suggested that transaminase

apoenzyme synthesis is much higher in the cases receiving the high protein diet. The main role of pyridoxine and its metabolites was well established in the metabolism of carbohydrates (12) as well as in amino acids' synthesis (13). Thus in the cases of low protein diets, the animal adapts himself by increasing transaminases activities in order to metabolise the excess carbohydrates of the diet. A regulation mechanism between the apoenzyme and its coenzyme has been suggested and the relatively low response to pyridoxine injection in the protein-depleted group can be suggested as a result of decreased apoenzyme synthesis rather than coenzyme deficiency.

Finally, it can be suggested that increased activities of some liver enzymes in protein depletion vary in nature since alkaline phosphatases increased activities seem to be an increased enzyme protein synthesis; while increased transaminase activities was suggested to be a result of a regulation mechanism between the apoenzyme and its coenzyme.

Summary

Liver enzymes alkaline phosphatase and transaminases (GPT & GOT) were studied in cases of protein-depleted rats. Alkaline phosphatase activities were determined with and without Mg addition to the incumedia, since it is the essential metal for this enzyme. The liver transaminases were also determined before and after pyridoxine injection, which is the coenzyme for this group. Both liver alkaline phosphatase and transaminases activities were increased on protein depletion. The study indicates that the increased activities of liver alkaline phosphatase in protein-depleted animals is suggestive of increased enzyme protein synthesis. On the contrary, high activities of liver transaminases are suggestive to be a result of some regulation mechanisms between the enzyme protein and its coenzyme.

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