ALTERATION IN SOME HEPATIC DEHYDROGENASES AND CERTAIN LIPID COMPONENTS IN THIAMIN DEFICIENCY

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Summary: An investigation was conducted to study the influence of thiamin deficiency on hepatic glucose-6-phosphate dehydrogenase, malic enzyme (NADP), lactate dehydrogenase and it's isoenzymes, total lipids, cholesterol and phospholipids in adult male albino rats. Typical thiamin deficiency symptoms developed in the 6th week. The specific activity of the said enzymes was significantly decreased in deficient rats as compared to pairfed controls. A significant drop in total lipid and phospholipid content was noted in deficient group while total cholesterol remained unchanged. Refeeding of control diet to deficient animals caused reversal of enzyme activities to normal.

Key words: Thiamin G-6-PDH ME (NADP) LDH cholesterol phospholipids

INTRODUCTION

Certain relationships of thiamin deficiency to lipid and carbohydrate metabolism have been studied by earlier workers. Boxer *et al.* (2) found decreased levels of liver lipids in thiamin deficiency. Rubina *et al.* (16) observed that when rats were starved and refed, there was greater deposition of body fat if thiamin was included in their diet at the time of refeeding. These studies reveal that, though the number of workers have studied the effect of thiamin deficiency on carbohydrate and lipid components hardly any systematic attempt has been made with respect to enzymatic changes, more so in hepatic dehydrogenases and lipid components. Therefore, we thought that it would be interesting to study the effect of thiamin deficiency on hepatic glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49), malic enzyme (NADP), (EC 1.1.1.40), lactate dehydrogenase (LDH, EC 1.1.1.27) and some lipid components.

MATERIALS AND METHODS

Male albino rats obtained from Hindustan Antibiotics Limited, Pune weighing 130-170 g were used in this experiment. All animals were given stock laboratory diet for one week prior to initiation of the experiment. The rats were housed in raised wire bottom cages at room temporature and their distribution was made as under :

Control group C — Rats fed adequate thiamin diet *ad lib*.

Deficient group D — Rats fed *ad lib* with the diet devoid of thiamin.

Pairfed group PFC — Rats pairfed with group D animals with adequate thiamin diet.

The composition of the diet is the same as reported earlier (9). After six weeks, some of the rats from both deficient and pairfed control groups were refed for 9 days with the control diet. At the end of six weeks, rats were killed under ether anaesthesia, livers were removed and processed for the assay of G-6-PDH by the modified method of Glock and Mclean (6) and ME and LDH by the methods of Ochoa (14) and Roman *et al.* (15) respectively. Protein was estimated by biuret method (7). Extraction of different lipids was carried out by the method of Folch *et al.* (4). Cholesterol was determined according to Sperry and Webb (17) and lipid phosphorous by the method of Fiske and Subba Row (3).

RESULTS AND DISCUSSION

Thiamin deficiency symptom such as reduced food intake, decrease in growth rate, muscular dystrophy and inability to stand independently were observed at the end of 6th week. In our series of investigations, thiamin deficiency resulted in decreased fat content of liver (Table II) and such decrease can be explained by the impaired fat synthesis from glucose in thiamin

TABLE I: Effect of thiamin deficiency on liver weight, body weight and RLS.

	Deficient	*Pairfed control*	*Ad lib fed control*	*Deficient refed*	*Pairfed control refed*
Liver wt. (g)	6.50±0.40	9.72±0.62	12.0±1.10	7.20±0.65	8.90±0.48
Final body wt. (g)	205.0±8.0	265.0±9.0	310.0±4.8	227.0±5.3	295.0±6.5
RLS	3.20±0.32	3.67±0.41	3.98±0.30	3.17±0.27	3.35±0.39

Mean ±SEM of 5 rats each.

TABLE II: Effect of thiamin deficiency on hepatic total lipid, total and free cholesterol phospholipids and total protein.

	Deficient	*Pairfed control*	*Ad lib fed control*	*Deficient refed*	*Pairfed control refed*
Total lipid	30.30±6.0*	48.0±3.5	50.00±5.6	48.8±4.7	49.20±3.5
Total cholesterol	2.82±0.60	2.75±0.31	2.65±0.22	2.92±0.38	2.84±0.34
Free/total cholesterol	0.74	0.49	0.46	0.48	0.44
Free cholesterol	1.86±0.45	1.35±0.58	1.22±0.37	1142±0080	1.29±0.40
Phospholipids	18.85±0.50	23.60±0.46	24.85±0.38	18.20±0.41	23.80±0.35
Total protein	150.0±5.1*	180.0±4.2	195.0±6.0	156.0±3.2	183.0±3.1

All values are expressed in *mg/g* fresh wet tissue.
Values are mean ±S.E.M. of 5 rats each.
*$P < 0.05$

TABLE III: Effect of thiamin deficiency on hepatic G-6-PDH and ME (NADP).

	Deficient	*Pairfed control*	*Ad lib fed control*	*Deficient refed*	*Pairfed control refed*
G-6-PDH	43.4 ± 2.5*	55.8 ± 2.3	58.5 ± 4.3	52.1 ± 3.3	56.3 ± 3.1
Malic enzyme	36.2 ± 2.2*	47.3 ± 3.0	50.4 ± 2.9	45.5 ± 3.0	49.0 ± 2.1

Mean ±SE of 5 rats each.
Specific activity is expressed as μmoles of NADPH formed/min/mg protein of enzyme extract.
*Significant when compared with both controls ($P<0.05$)

TABLE IV: Effect of thiamin deficiency on hepatic total LDH and its isoenzymes.

	Total LDH	*LDH_{4+5}*	*LDH_3*	*LDH_{1+2}*
Deficient	32.20 ± 0.92*	11.80 ± 1.21*	10.80 ± 1.00	9.20 ± 1.20
Pairfed control	35.85 ± 0.95	16.90 ± 1.12	10.10 ± 0.75	8.80 ± 1.00
Ad lib fed control	37.20 ± 1.10	17.80 ± 1.00	10.20 ± 0.83	8.20 ± 0.95
Deficient refed	35.10 ± 1.15	14.70 ± 0.95	11.90 ± 1.10	9.20 ± 0.93
Pairfed control refed	36.20 ± 0.85	16.10 ± 1.00	11.00 ± 0.68	9.10 ± 0.81

Mean ±SE of 5 rats each.
Specific activity is expressed as μg of pyruvate formed/15 min/mg protein of enzyme extract.
*Significant when comparad with both controls ($P<0.05$).

deficient group which might be due to the decreased activity of pyruvate and alpha keto glutarate dehydrogenase, diminished pyruvate carboxylase activity and decreased transketolase activity leading to decreased ability of the cells to form NADPH necessary in the reductive synthesis of fatty acids and cholesterol as reported by Veech *et al.* (18). Decrease in hepatic reported by Williams and Anderson (19). Our results are in concurrance with the findings of these workers. Conflicting results are available with respect to hepatic cholesterol concentration. Gubler *et al.* (8) reported decreased cholesterol concentration in liver of deficient animals even though the rate of cholesterol biosynthesis was higher. However, other workers (19) reported hardly any change in hepatic cholesterol concentration. In the present series of investigation also no change in cholesterol concentration was observed. It has been reported by many workers (5,11) that thiamin deficient animals decorboxylated at least as great percentage of pyruvate to acetyl CoA as did the well fed animals. Though the concentration of total

cholesterol in this study remains unaffected, there was found to be a tendency of increase in free cholesterol which might be due to the decreased biosynthesis of fatty acids required for esterification as suggested by Lynen *et al.* (12).

During repletion period, in deficient animals total liver lipid reached to a normal value as that of control animals which might be due to the increased appetite as revealed by increased food intake causing enhanced lipogenesis enforced by thiamin intake, However, dramatic increase in liver lipid content was not observed as was reported by Williams and Anderson (19) which might be due to the administration of thiamin, intraperitoneally adopted by these workers. A rapid fall in free cholesterol to total cholesterol ratio was noted in repletion period which could be due to more fatty acid biosynthesis and cholesterol esterification.

It is interesting to observe that the activities of G-6-PDH and malic enzyme (NADP) are significantly affected in thiamin deficiency. Benevenga *et al.* (1) reported that transketolase and G-6-PDH activities behave in a similar manner in various physiological conditions. Hence it could be that in thiamin deficient animals, the decreased action of G-6-PDH might be due to the decreased action of transketolase. Decreased activity of hepatic glycerophosphate and lactate dehydrogenase in thiamin deficient animals was reported by Jan Van Eys (10). In our study significant decrease in total LDH and LDH_{4+5} fraction was noted while LDH_3 and LDH_{1+2} remained unchanged. Such differential behaviour of isoenzyme pattern in rat adipose tissue was reported by Moore *et al.* (13).

Replenishing resulted in increased activities of these enzymes resulting towards normal metabolic pattern in animals.

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