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Interrelationships of Certain Metabolic Responses to Threonine Deficiency and to Various Dietary Carbohydrates in the White Rat

Elizabeth Bright

University of Arkansas, Fayetteville

THE INTERRELATIONSHIPS OF CERTAIN METABOLIC RESPONSES TO THREONINE DEFICIENCY AND TO VARIOUS DIETARY CARBOHYDRATES IN THE WHITE RAT

Elizabeth Bright[1]
University of Arkansas

The importance of the relative amounts of amino acids in a protein is a well known and accepted fact. An amino acid imbalance results from any change in the proportions of the amino acids in the diet that results in an adverse effect which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid(s) (1).

Threonine deficiency has been one of the most widely studied imbalances, partly because its manifestations in the white rat are similar to those of the calorie-protein deficiency disease, kwashiorkor, in humans.

As with all nutritional factors, amino acid deficiency does not function completely independently. One of the most pronounced symptoms of threonine deficiency is the deposition of excess lipid in the liver. However, the amount of liver lipid appears to depend partly on the type and the amount of carbohydrate in the diet (2).

It is not known just how the dietary carbohydrate influences the development of the threonine deficiency. This particular study was designed to observe the effect of various carbohydrates on threonine deficiency, and at the same time to gain some information about the influence of the amino acid imbalance on the handling of different carbohydrates.

Male weanling rats were used, six rats in each of six groups. All of the rations contained 9% casein as the sole protein source. At this level, tryptophan, methionine and threonine are the limiting amino acids. Three of the rations were supplemented with all three of these amino acids. The other three were supplemented with only tryptophan and methionine and, therefore, were deficient in threonine.

The dietary carbohydrate was either glucose, fructose, or a 1:1 combination of the two.

After the animals had been fed the diets for two weeks, they were sacrificed and the livers removed for analysis.

The total amount of liver lipid was determined as well as liver cholesterol and phospholipid levels. Triglyceride values were calculated by substracting the sum of the phospholipids and cholesterol from the amount of total lipid. Livers were also assayed for concen-

[1]Graduate Research Assistant, Department of Home Economics.

tration of glycogen and for activities of the glucose-6-phosphatase (G-6-Pase) and fructose-1,6-diphosphatase (FDPase)enzyme systems.

Threonine deficient diets containing each of the different carbohydrates were as readily consumed as the corresponding threonine supplemented diets. However, within each subgroup, that is, threonine supplemented or threonine deficient, diets containing fructose were not as readily accepted as those containing glucose (Table 1).

The threonine deficiency resulted in a decrease of weight gain with each of the carbohydrates. However this decrease was only slight when the dietary carbohydrate was glucose.

Generally, decreased food intakes accounted for decreased weight gain. However, there was a significant interaction of the fructose and of the threonine deficiency in depressing the food efficiency ratio of the group receiving this diet (Table 2).

Figure 1 illustrates the relative liver weights and the major components of the liver (g/100 g of body weight).

The substitution of fructose or the glucose/fructose combination for glucose in either the threonine supplemented or the threonine deficient diets, resulted in increased liver sizes. However, only with the threonine deficient diets did this substitution result in an increase in the milligrams of liver lipid per 100 g of body weight.

Although the liver sizes did not vary significantly between the group receiving threonine and the groups not receiving it with each carbohydrate, the livers of the animals fed the threonine deficient diets contained more lipid than those of their supplemented control group.

Levels of glycogen, protein and water were largely reflective of the total liver size.

Some differences in the composition of the total lipid were also found (Fig. 2). The level of cholesterol increased with the omission of threonine from the glucose and the glucose/fructose diets, and the phospholipid level increased slightly with the substitution of fructose for glucose in the threonine deficient diets. However, the total accumulation of lipid in the liver appears to be chiefly due to the net deposition of triglycerides.

Two types of enzyme adaptation have been proposed: an increase in specific activity and an increase in relative liver size (3). The total activity (i.e., units per 100 g of body weight) would represent the combination of the two methods, while specific activity (i.e., units per 100 mg of liver nitrogen) would represent the part of the adaptation accomplished by preferential synthesis of the enzymes. The graph (Fig. 3) shows both total and specific activity.

With both threonine supplemented and threonine deficient diets, the activities of the two enzyme systems follow similar patterns. Dietary

Table 1. Food intake and weight gain of rats on low protein diets, with or without supplemental threonine, and with various dietary carbohydrates.

	Diet	Food Intake		Weight Gain	
		Total	/wk	Total	/wk
		g	g	g	g
I	[1]G + Th	114 ± 5[2]	61 ± 3	43 ± 3	23 ± 1
II	F + Th	102 ± 6	55 ± 3	37 ± 3	20 ± 1
III	G/F + Th	121 ± 3	65 ± 1	47 ± 2	25 ± 1
IV	G – Th	113 ± 3	61 ± 2	38 ± 2	20 ± 1
V	F – Th	93 ± 6	50 ± 3	27 ± 3	14 ± 1
VI	G/F – Th	118 ± 6	63 ± 3	40 ± 2	22 ± 1

[1]G — glucose, F — fructose, G/F — glucose/fructose (1:1), Th — threonine

[2]Standard error of the mean

Table 2. Utilization of low protein diets, with or without supplemental threonine, and with various dietary carbohydrates. Gram weight gain per gram food intake.

	Diet	
I	[1]G + Th	.37 ± .02[2]
II	F + Th	.36 ± .01
III	G/F + Th	.39 ± .01
IV	G – Th	.34 ± .01
V	F – Th	.28 ± .01
VI	G/F – Th	.34 ± .01

[1]G — glucose, F — fructose, G/F — glucose/fructose (1:1), Th — threonine

[2]Standard error of the mean

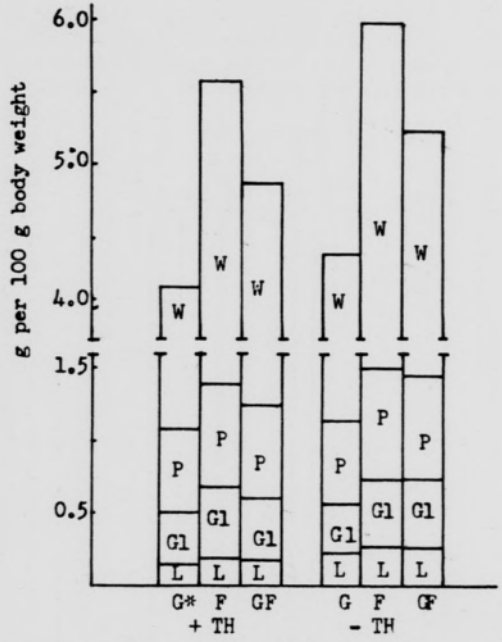

Figure 1 Composition of the liver.

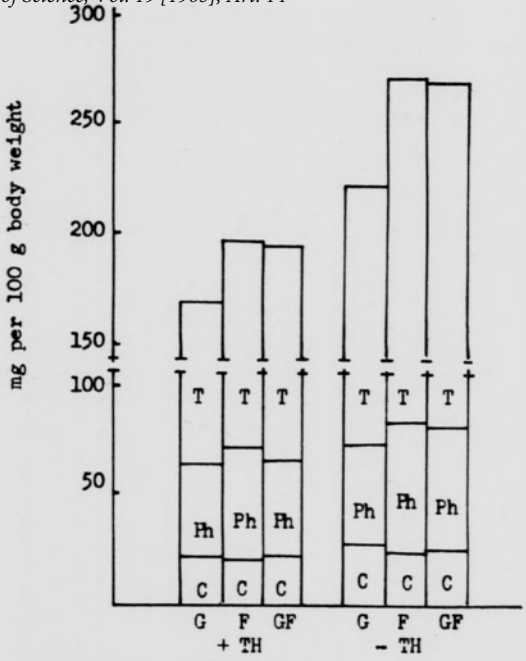

Figure 2 Composition of the liver lipid.

* G = glucose, F = fructose, GF = glucose/fructose (1:1), TH = threonine, W = water, P = protein, Gl = glycogen, L = lipid; T = triglycerides, etc. Ph = phospholipids, C = cholesterol.

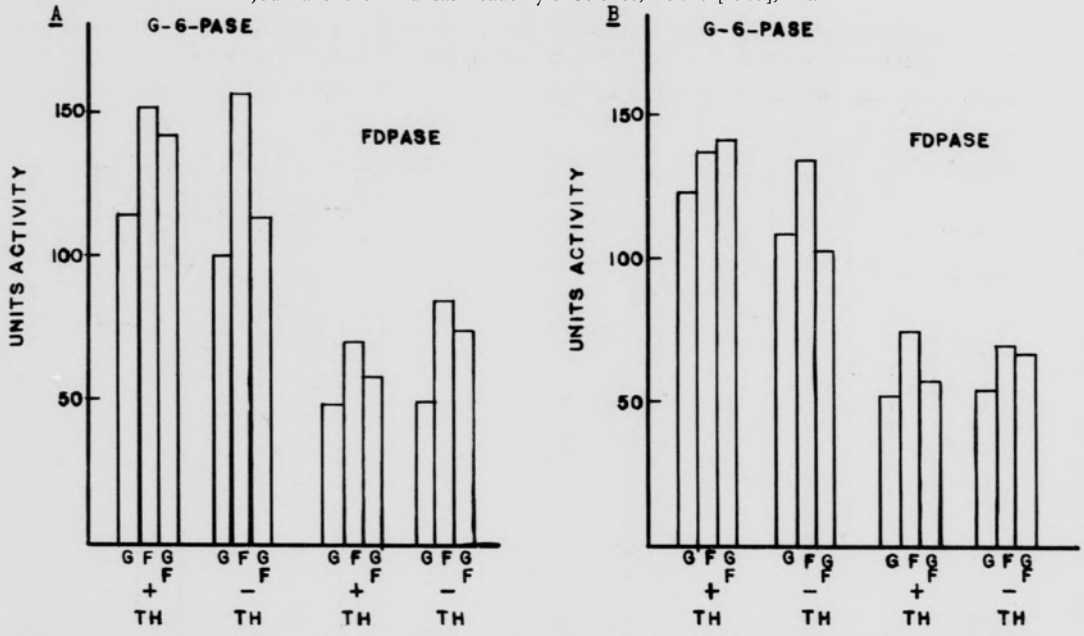

Figure 1 Response of the activities of glucose-6-phosphatase (G-6-Pase) and fructose-1,6-diphosphatase(FDPase) to low protein diets, with or without supplemental threonine, and with various carbohydrates. A units per 100 g body weight, B units per 100 mg liver nitrogen (specific activity). G = glucose, F = fructose, GF = glucose/fructose (1:1), Th = threonine, One unit of activity = the amount of enzyme which will release 1 µg Pi/minute.

fructose stimulated the activities of both systems. The response to the glucose/fructose combination was generally intermediate to that to glucose and to fructose.

The omission of threonine from the glucose diet resulted in a slight decrease in the G-6-Pase activity. This depression was due entirely to a decrease in the specific activity since the nitrogen contents of the livers of the two glucose-fed groups were the same. In one extensive study of G-6-Pase activity (4), the only treatment found to decrease the activity of this enzyme system was the feeding of a protein-free diet. This suggests that the effect of an amino acid imbalance is similar to that of a protein-free diet, and not merely an indication of a decrease in the level of utilizable protein.

The reaction catalyzed by G-6-Pase represents the final step in net glucose formation. The activity of FDPase could be indicative of the participation of the Embden-Meyerhof pathway (EMP) in glucose formation.

The relative changes in activities of these two enzyme systems in response to fructose feeding could be suggestive of the proportion of glucose formation due to the EMP. This is, a 1:1 ratio of the stimulation of the G-6-Pase and FDPase activities in response to fructose feeding would be consistent with a direct conversion of fructose to glucose via the EMP. A stimulation of the activity of FDPase less than that of G-6-Pase would indicate that some amount of fructose was being converted to glucose via a route not involving fructose-1,6-phosphate. Difference in degree of stimulation also might indicate a change in the synthesis or breakdown of glycogen.

Total G-6-Pase activity increased with the substitution of either fructose or the glucose/fructose combination for glucose in the threonine supplemented diets. This was accompanied by a proportionately smaller increase in the FDPase activity (figure 4), resulting in a greater difference between the activities of the two systems as compared with the difference for the glucose control group. Omission of the threonine supplement from the diet resulted in a much greater stimulation of FDPase activity by both fructose and the glucose/fructose combination. Differences between the activities were again greater with fructose than with glucose, but were less with the glucose/fructose combination.

Presumably, the greater the differences between the activities, the more precursors must be reaching glucose-6-phosphate by a route other the reversed glycolysis (i.e., EMP). One source of the glucose-6-phosphate would be the breakdown of glycogen. However, this was probably not the case, since those groups having the greater differences between the enzymes' activities, also showed a net increase in liver glycogen. Glycogen deposition would represent a drain on the glucose-6-phosphate and would indicate an even greater utilization of a pathway other than the EMP than is suggested by the relative enzyme activities.

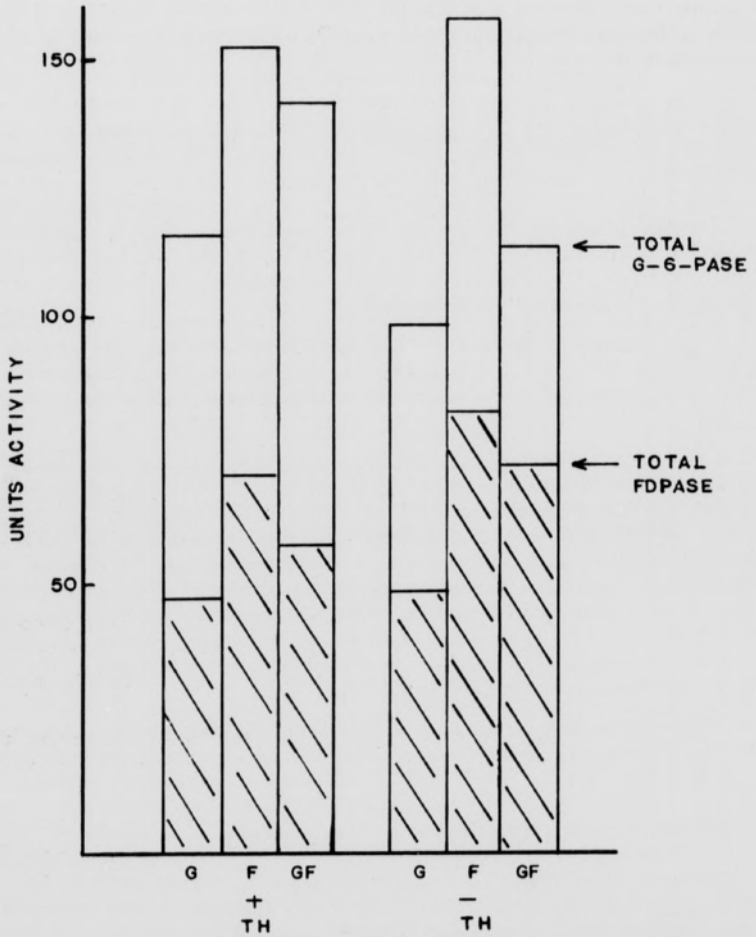

Figure 4 Difference in the total activities of glucose-6-phosphatase (G-6-Pase) and fructose-1,6-diphosphatase (FDPase) enzyme systems in response to low protein diets, with or without supplemental threonine, and with various carbohydrates. G = glucose, F = fructose, GF = glucose/fructose (1:1), Th-= threonine.

The data from the study suggest that the degree of participation of the pathways bypassing fructose-diphosphate may be modified by a deficiency of threonine. The mechanism(s) by which an amino acid imbalance can influence relative activities of alternate pathways of carbohydrate metabolism is not clear.

The possibility that the major pathway of the conversion of fructose to glucose may not be the EMP has been proposed by several authors (5-7). Although most of the previous studies were with purified enzyme systems, the fact that the substitution of fructose for glucose in the diet has been shown to stimulate at least two enzyme systems of the hexose monophosphate shunt (HMS) (8,9) suggests that the shunt may play an important role in fructose metabolism.

There has been much recent discussion about the relationship of lipogenesis to the shunt as well as to other NADPH producing systems. The majority of the reports indicate that the degree of lipogenesis influences the activity of the HMS rather than **visa versa.** In any case, the levels of liver lipid found under the conditions of this study do not correlate with the proposed pathways of carbohydrate utilization, as suggested by the relative activities of the two enzyme systems. Other factors, such as increased fatty acid oxidation, may possibly operate to decrease the net amount of lipid in livers in which the HMS is quite active.

LITERATURE CITED

1. Harper, A. E. 1959. Amino acid balance and imbalance. I. Dietary level of protein and amino acid imbalance. J. Nutrition 68: 405.
2. Macdonald, I. 1960. Some effects of carbohydrate in experimental low-protein diets. J. Physiol. 160: 306.
3. Friedland, R. A., and A. E. Harper. 1957. Metabolic adaptation of higher animals. I. Dietary effect of glucose-6-phosphatase. J. Biol. Chem. 228: 743.
4. Friedland, R. A., and A. E. Harper. 1958. Metabolic adaptation of higher animals. III. Quantitative study of dietary factors causing response in liver glucose-6-phosphatase. J. Biol. Chem. 233: 1.
5. Muntz, J. A., and M. Vanka. 1962. Studies on the utilization of fructose in rat liver **in vivo.** Fed. Proc. 21: 84.
6. _____. 1962. The metabolism of intraportally injected fructose in rat liver **in vivo.** J. Biol. Chem. 237: 3583.
7. Landau, B. R., and W. Merlevede. 1963. Initial reaction in the metabolism of D- and L-glyceraldehyde by rat liver. J. Biol. Chem. 238: 261.
8. Fitch, W. M., R. Hill and I. L. Chaikoff. 1960. The effect of fructose feeding on glycolytic enzyme activities of the normal rat liver. J. Biol. Chem. 234: 1048.
9. Fitch, W. M., and I. L. Chaikoff. 1960. Extent and patterns of adaptations of enzyme activities in liver of normal rats fed diets high in glucose or fructose. J. Biol. Chem. 235: 534.