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Effect of Thiamine Deficiency on Gluconeogenesis and Transketolase Activity in Isolated Hepatocytes (40907)

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Abstract. The effect of a 4-week thiamine deficiency on gluconeogenesis from xylitol, lactate, pyruvate, dihydroxyacetone, and fructose in isolated rat hepatocytes was investigated. Xylitol is metabolized via transketolase, a thiamine-requiring enzyme, whereas the remaining substrates are converted to glucose via enzymes which do not require thiamine. At 7 days, transketolase activity was 81% of the value obtained with hepatocytes isolated from pair-fed control rats and gluconeogenesis from all the above substrates was normal, indicating an excess of transketolase activity present. By 14 days, however, gluconeogenesis was depressed (65–88% of control). At 28 days, both transketolase activity and gluconeogenesis from xylitol were markedly depressed (32 and 73% of control, respectively), but moreover, those substrates not utilizing thiamine-requiring enzymes for gluconeogenesis were equally or more greatly depressed in their gluconeogenic rates (42–70% of control). These data indicate (i) transketolase activity is present in amounts greater than that needed for normal hexose monophosphate shunt operation, and (ii) thiamine deficiency markedly depresses hepatic gluconeogenesis from a variety of substrates, indicating a generalized effect of the absence of this vitamin on metabolism, and more specifically, that the depression in gluconeogenesis from xylitol could be due to a decrease in the activity of non-thiamine-requiring enzymes. The need for caution in interpreting cause-and-effect relationships between enzymes and pathway flux is stressed.

Early work in our laboratory (1) demonstrated that thiamine deficiency in rats depressed the activity of three hepatic enzymes: glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), and citrate cleavage enzyme (EC 4.1.3.8), each of these being non-thiamine requiring. As a result, we decided to investigate other nonspecific effects of thiamine deficiency upon liver metabolism, particularly with respect to gluconeogenesis. The objective of this study was to compare the effect of thiamine deficiency on gluconeogenesis from a substrate which must be metabolized via a thiamine-requiring enzyme, to substrates whose metabolism is independent of thiamine. Xylitol was chosen for the former case, as it must be metabolized via transketolase to enter the gluconeogenic scheme at the level of the triose phosphates or as fructose 6-phosphate. Glucose production from lactate, pyruvate, fructose, and dihydroxyacetone was measured as these compounds do not utilize thiamine-requiring enzymes for conversion to glucose.

Materials and methods. Animals and diet. Male, Sprague–Dawley rats (Hilltop Lab Animals, Scottsdale, Pa.) were used in all experiments. The rats were housed individually in suspended wire-mesh cages in a 23° room with a 12-hr light–dark cycle. Animals were divided into two groups, designated as thiamine-deficient and pair-fed controls.

The control diet (2) consisted of 65% glucose, 25% vitamin-free casein, 5% corn oil, 4% salt mix,[1] and 1% vitamins.[2] For the thiamine-deficient diet, an equal amount of dextrose was substituted for thiamine HCl in the vitamin mixture made from the purified vitamins. Food was removed 24 hr

[1] The salt mix contained: (%) $CaCO_3$, 29.29; $CaHPO_4 \cdot 2H_2O$, 0.43; KH_2PO_4, 34.31; NaCl, 25.06; $MgSO_4 \cdot 7H_2O$, 9.98; $Fe(C_6H_5O_7) \cdot 6H_2O$, 0.623; $CuSO_4$, 0.156; $MnSO_4 \cdot H_2O$, 0.121; $ZnCl_2$, 0.020, KI, 0.0005; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.0025; $Na_2SeO_3 \cdot 5H_2O$, 0.0015. Q. R. Rogers and A. E. Harper, J. Nutrition 87, 267 (1965).

[2] The vitamin mix contained: (mg/kg diet) vitamin A concentrate (500,000 units/g), 18; vitamin D concen-

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prior to killing. In the thiamine repletion study, deficient animals were injected subcutaneously with 60 μg thiamine hydrochloride/100 g body wt/day, and the controls were injected with an equal volume of saline. Hepatocytes were isolated by the method of Berry and Friend (3), with modifications, cell preparations, and incubations as described by Cornell *et al.* (4).

Metabolite assays. Glucose was determined by the method of Krebs *et al.* (5) and ketone bodies were measured by the method of Williamson *et al.* (6) in order to assess mitochondrial redox state. DNA content (7) and lactate concentration (8) were also determined. Pyruvate levels were measured on an Aminco–Bowman spectrophotofluorometer. The assay system for pyruvate contained 0.4 ml Tris buffer (0.1 *M* Tris, 2 m*M* EDTA, pH 7.6), 0.1 ml NADH (0.08 mg/ml), and 0.5 ml sample. The reaction was started with the addition of 1 μl lactate dehydrogenase and pyruvate concentration was quantitated by comparison to a standard curve. The cytosolic redox state was calculated from the ratio of lactate to pyruvate.

Transketolase assay. An aliquot of the final cell suspension was removed for measuring transketolase activity. The cell suspension was kept on ice and homogenized using four strokes of a Teflon pestle in a Potter–Elvehjem homogenizer, at medium speed. The homogenized cells were centrifuged at 20,000*g* for 30 min and the supernatant assayed for enzyme activity. The assay was a modification of that reported by Benevenga *et al.* (2). Rather than using ribose 5-phosphate as substrate (2), a pentose phosphate mix containing ribose 5-phosphate, ribulose 5-phosphate, and xylulose 5-phosphate was generated and used in the assay. This was accomplished by incubating ribose 5-phosphate in a 0.25 *M* glycylglycine buffer, pH 7.5, at 37° with ribose 5-phosphate epimerase and phosphoriboisomerase. After 10 min, the solution was acidified, neutralized, and stored frozen in small aliquots. One unit of transketolase is the amount of enzyme which produced 1 μmole NADH/min.

All chemicals were obtained from Sigma Chemical Company, St. Louis, Missouri.

Statistics. Data were analyzed using Student's *t* test (9).

Results. Food intake and weight gain of rats fed the control or thiamine-deficient diet indicated an acute appetite depression and subsequent weight loss in the thiamine-deficient rats beginning near Day 13. This pattern is in agreement with observations made by others (10, 11).

Gluconeogenesis from saturating concentrations of xylitol and transketolase activity was measured in cells isolated from thiamine-deficient and pair-fed control rats after 7, 14, and 28 days of the dietary treatment. The data graphed in Fig. 1 are expressed as a percentage of the pair-fed control value. After 7 days of dietary treatment, transketolase activity fell to 73% of the control value, whereas gluconeogenesis from xylitol was not changed. At 14 days, gluconeogenesis was depressed. By 28 days, transketolase activity was 32%, and gluconeogenesis was 73% of control values. The average value for glucose production from xylitol in hepatocytes isolated from control rats was 0.222 μmole/min/mg DNA, and that for transketolase activity was 0.525 units/mg DNA. This is equivalent to 1.24 μmole glucose/min/g liver and 2.93 units of transketolase activity/g liver, assuming 1 g liver from a 48-hr starved rat contains 5.6 mg DNA, as reported by D. L. Story *et al.* (12). The value for transketolase activity agrees with those reported by others (2, 13, 14). Addition of thiamine pyrophosphate to the transketolase assay system failed to stimulate enzyme activity, possibly indicating a lack of apoenzyme. Similar observations have been made by other investigators (13, 15, 16).

In order to examine restoration of both transketolase activity and capability to convert xylitol to glucose, rats receiving the

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trate (400,000 units/g), 2.5; α-tocopherol, 50; ascorbic acid, 450; inositol, 50.0; choline chloride, 750.00 (added as a 50% solution); menadione, 22.5; *p*-aminobenzoic acid, 50.0; niacin, 45.0; riboflavin, 10.0; pyridoxine·HCl, 10.0; thiamine HCl, 10.0; Ca pantothenate, 30.0; biotin, 0.2; folic acid, 0.9; vitamin B_{12}, 0.0135 (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp., Cleveland, Ohio).

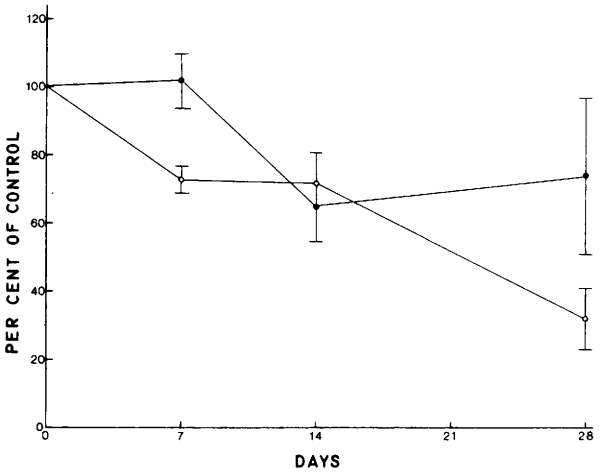

FIG. 1. Gluconeogenesis from 10 m*M* xylitol (●) and transketolase activity (○) ± SEM in hepatocytes isolated from thiamine-deficient rats throughout the 28 days of dietary treatment. Values are expressed as a percentage of the pair-fed control value. Average glucose production from hepatocytes isolated from pair-fed controls was 0.222 μmole/min/mg DNA and transketolase activity was 0.525 units/mg DNA. Endogenous glucose production has been substracted (n = 3–5).

dietary treatment for 28 days were repleted with thiamine. Gluconeogenesis from xylitol returned to the control value by Day 7, whereas transketolase activity was 81% of control. By 14 days, both parameters returned to 100% of control.

Glucose production for lactate, pyruvate, dihydroxyacetone and fructose through the 28 day period is shown in Fig. 2. At 7 days, gluconeogenesis from the four substrates was the same for both thiamine-deficient and pair-fed controls. By 14 days, however, a depression in gluconeogenesis was seen in hepatocytes isolated from the deficient animals, and by 28 days, gluconeogenesis was some 60% of control (average value for all substrates).

Mitochondrial and cytosolic NADH/

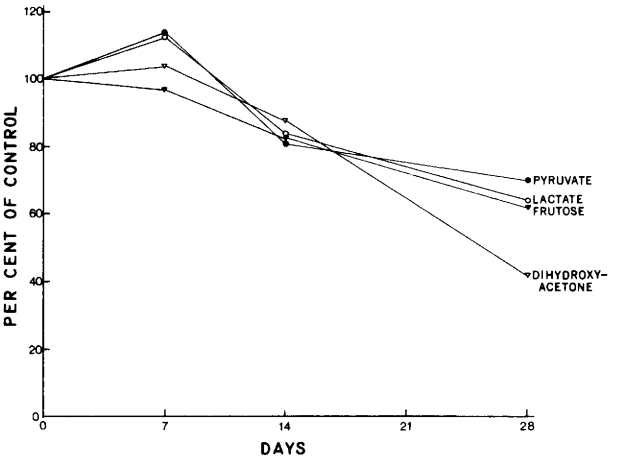

FIG. 2. Gluconeogenesis from pyruvate (●), fructose (▼), lactate (○), and dihydroxyacetone (▽) (10 m*M*) in hepatocytes isolated from thiamine-deficient rats during the 28 days of dietary treatment. Values are expressed as a percentage of the pair-fed control value. Control rates of gluconeogenesis from pyruvate, fructose, lactate, and dihydroxyacetone are 0.283, 0.710, 0.218, and 0.371 μmole/min/mg DNA, respectively. Endogenous glucose production has been substracted (n = 2–4).

NAD ratios were estimated by measuring the ratio of β-hydroxybutyrate to acetoacetate, and lactate to pyruvate, respectively (17). These data are shown in Table 1. In the presence of 10 m*M* xylitol, the cytosol became fourfold more reduced ($P < 0.01$), while the mitochondria became less reduced by 26% ($P < 0.001$), compared to no xylitol addition. There was no significant difference in redox state in either compartment between hepatocytes isolated from deficient or pair-fed control rats.

Discussion. Comparison of transketolase activity to xylitol conversion to glucose after 28 days of a thiamine-deficient diet, would suggest that the decrease in transketolase activity caused the depression in gluconeogenesis from xylitol. However, examination of these two parameters earlier in the dietary treatment is necessary to identify a causative agent. At Day 7, a depressed transketolase activity, with normal rates of xylitol conversion to glucose, demonstrates that transketolase activity is present in excess, and is not rate-limiting for gluconeogenesis from xylitol. By Day 14, both glucose production from xylitol and transketolase activity were depressed. However, gluconeogenesis from lactate, pyruvate, dihydroxyacetone, and fructose was also depressed. These data suggest that the decrease in xylitol conversion to glucose could be due to a generalized effect of thiamine deficiency on gluconeogenesis and not necessarily to changes in transketolase activity. It is interesting to note that this occurred at the same time that the acute weight loss and marked appetite depression was seen in the rats.

TABLE I. EFFECT OF 10 mM XYLITOL ON CYTOSOLIC AND MITOCHONDRIAL REDOX STATE IN HEPATOCYTES ISOLATED FROM THIAMINE-DEFICIENT AND PAIR-FED CONTROL RATS[a]

	Cytosol[b] (Lactate/Pyruvate)	
	−Xylitol	+Xylitol
Deficient	100	319 ± 76
Control	100	550 ± 216
	Mitochondria[c] (β-Hydroxybutyrate/Acetoacetate)	
	−Xylitol	+Xylitol
Deficient	100	71 ± 12
Control	100	78 ± 7

[a] Values are percentages, averaged throughout 28 days of dietary treatment.

[b] The absolute ratio for deficient and control without xylitol is 42.3 and 37.2, respectively. $n = 9$.

[c] The absolute ratio for deficient and control without xylitol is 1.6 and 1.8, respectively. $n = 10$.

Two major points are borne out by these results: (i) the nonspecific effect of thiamine deficiency on metabolism, especially with respect to non-thiamine enzymes, and (ii) caution must be observed when correlating flux through a pathway with enzyme activity. Indeed, as shown here, depressed transketolase activity after 28 days of a dietary thiamine deficiency does correspond to depressed glucose production from xylitol. However, this correlation in decreased transketolase activity and depressed gluconeogenesis from xylitol may not necessarily represent a causal relationship. This is clearly indicated by the fact that the rates of gluconeogenesis from many substrates, which do not require thiamine-dependent enzymes for this process, were as greatly depressed as that of xylitol.

In regard to point (ii), McCandless *et al.* (18) reported that after 35 to 40 days of thiamine deficiency, despite a 90% decrease in hepatic transketolase, flux through the hexose monophosphate pathway was unaltered. This study has at least two limitations. First, the use of tracer levels of [^{14}C]glucose as substrate will not cause maximum flux through the hexose monophosphate shunt, especially since levels of NADP and NADPH have been shown to be controlling factors in this pathway (19, 20). Levels of the oxidized form of this coenzyme will rise when lipogenesis is occurring, which would be close to nil in these animals. In the work reported here, by using saturating levels of xylitol as substrate, the flux through transketolase is rapid as indicated by the rapid rates of gluconeogenesis, and the levels of NADP or NADPH will not control this portion of the pathway. Second, the use of the ratio of $^{14}CO_2$ produced from [6-^{14}C]glucose to [1-^{14}C]glucose to estimate flux through the hexose monophosphate shunt is limited. The authors state that the

ratio was the same for normal versus thiamine-deficient animals, hence the flux was unchanged. However, a depression in the first two enzymes of the pathway (1), along with depressed pyruvate and α-ketoglutarate oxidation (21, 22) could decrease $^{14}CO_2$ from both [1-^{14}C]- and [6-^{14}C]glucose to similar extents, leaving the ratio unchanged. Other limitations with the use of this procedure for estimating hexose monophosphate flux have been reported (23).

Changes seen in compartment redox states upon xylitol addition are similar to observations made by Wood and Krebs (24), despite a difference in systems (perfused liver versus isolated cells). Xylitol dehydrogenase rapidly oxidizes xylitol, producing NADH which reduces the cytosol. This has caused concern over the use of xylitol as an energy source in parenteral nutrition.

Thiamine deficiency appears to cause a general decay in metabolism. Even by correcting for depressed food intake of the thiamine-deficient rats by using pair-fed control animals, the deficiency clearly caused aberrations in gluconeogenesis where thiamine-requiring enzymes were not involved. Whether the problem is specific in affecting other enzymes involved in gluconeogenesis (fructose diphosphatase, for example) or is a generalized effect, by affecting energy metabolism, remains to be further investigated.

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