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Effects of Diet on the Activities of Enzymes Related to Lipogenesis in Rat Liver and Adipose Tissue[1] (38366)

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(Introduced by Richard A. Freedland)

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Alterations in pyruvate kinase (ATP: pyruvate phosphotransferase; E.C. 2.7.1.40) have been correlated with gluconeogenesis and lipogenesis in the liver (1); diets high in carbohydrate increase pyruvate kinase activity. The type of carbohydrate used in the diet would also appear to be a significant factor in determining the final activity of the enzyme (2). Diets high in sucrose stimulate pyruvate kinase (2) and fatty acid synthetase (3) to higher activity levels than diets containing starch. The present study was designed to extend the earlier findings with pyruvate kinase to three other enzymes in the liver which are closely related to lipogenesis (4); glucose-6-phosphate dehydrogenase (D-Glucose-6-phosphate: NADP oxidoreductase; E.C. 1.1.1.49), phosphogluconate dehydrogenase (6-Phospho-D-gluconate: NADP oxidoreductase; E.C. 1.1.1.43), and malic enzyme (L-Malate:NADP oxidoreductase [decarboxylating]). Since the majority of the *de novo* fatty acid synthesis in the rat occurs in the adipose tissue, the effect of diet on the four enzymes mentioned above also was investigated in epididymal adipose tissue.

Materials and Methods. Male Sprague–Dawley rats (Holtzmann Laboratories, Madison, WI) averaging 208 (165–235 g range) grams were allowed food and water *ad libitum*. In each experiment the rats were divided into four groups. The control group was fed finely ground Purina Lab Chow. In experiment 1, the other three groups were fed the following purified diet containing either glucose, sucrose, or glucose + fructose (1:1) as the carbohydrate: 65% carbohydrate, 25% casein, 5% corn oil, 4% Phillips-Hart salt mix, and 1% vitamins. In experiment 1 the control group was maintained on the ground Chow for 13 days, whereas the three groups eating the purified diets were all placed on the glucose diet for 3 days to accustom the rats to eating a purified diet. One group was then switched to a diet containing sucrose, another to a diet containing glucose plus fructose (invert sugar), and the third maintained on the glucose diet. Rats were then kept on the respective purified diets for 10 days. In experiment 2, rats were fed the purified diet described above containing either glucose, starch, or sucrose as the carbohydrate. In this experiment the rats were switched from a lab chow to one of the purified diets rather than prefeeding the glucose diet for three days and then switching. The rats were then maintained on the purified diets for 10 days as in experiment 1. Except for 6-phosphogluconate dehydrogenase (6PGDH), nearly identical enzyme activities were obtained in each experiment, suggesting that in this case prefeeding the glucose diets to adjust to the new type of diet was unnecessary. Following decapitation and exsanguination, the livers and epididymal fat pads were removed. Each liver was weighed and a 3 g portion of the median lobe homogenized in 12 ml ice-cold Tris–KCl buffer (0.01 *M* Tris [hydroxymethyl] aminomethane, 0.15 *M* KCl, 0.005 *M* ethylenediamine tetraacetic acid, and 0.01 *M* 2-mercaptoethanol; pH adjusted to 7.4 with HCl). The two epididymal fat pads were weighed, then homogenized in an equal volume of Tris–KCl buffer kept at room temperature. The homogenates were centrifuged in a refrigerated centrifuge for 70 min at 0–4°, and 34,000*g*.

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The resultant clear infranatants were used as the enzyme sources. Glucose-6-phosphate dehydrogenase (G6PDH) and 6PGDH were assayed by the method of Kornberg and Horecker (5) as modified by Kagawa *et al.* (6); pyruvate kinase (PK) by the method of Bucher and Pfleiderer (7) as modified by Szepesi and Freedland (8); and malic enzyme (ME) as described by Wise and Ball (9). Protein was determined by the Biuret method (10). Enzymatic activity is expressed as units/g wet weight of tissue.

Results. The average weight gained by rats on the various diets were: Experiment 1. Lab chow, 5.5 ± 0.7 g/day[4]; glucose, 7.5 ± 0.3 g/day; sucrose, 7.5 ± 0.3 g/day; and invert, 7.6 ± 0.5 g/day. Experiment 2. Lab chow, 7.0 ± 0.5 g/day; starch, 5.9 ± 0.3 g/day; glucose, 6.7 ± 0.7 g/day; and sucrose, 6.8 ± 0.5 g/day. The relative liver sizes (grams liver/100 grams body weight) of the rats fed diets containing sucrose and invert sugars were significantly larger than those fed the diets containing glucose, starch, or lab chow.

The activities of the four enzymes studies in the liver and in adipose tissue were significantly higher in rats fed any of the purified diets containing the four different carbohydrates (glucose, starch, sucrose or invert sugar) than in rats fed ground lab chow (Tables I and II). In adipose tissue, there were no significant differences in enzymatic activities among rats fed the three purified diets (Table II).

In contrast to adipose tissue, the enzymatic activities of these enzymes in liver extracts were significantly altered by the type of carbohydrate in the diet. The results of two separate experiments designed to test the effects of the type of carbohydrate on the enzymatic activities of the four enzymes are presented in Table I. The presence in the diet of either sucrose or invert sugar resulted in significantly higher activities of G6PDH, ME, and PK than observed in livers from rats fed either glucose or starch as the dietary carbohydrate. In no case was invert sugar more effective than sucrose in increasing the activities of these enzymes (Table I; Experiment 1). Livers of rats fed diets containing glucose had higher activities of malic enzyme than those fed diets containing starch (Table I; Experiment 2); however, in these same livers the activities of G6PDH, 6PDGDH and PK were identical.

Discussion. Yudkin and Krauss (2) reported that replacing starch with sucrose in their purified diet resulted in a fivefold increase in PK in the liver. In the present study, significant increases in PK in the liver were also observed when sucrose replaced starch or glucose in the diet (Table I), but the values were only about 30% greater. It is not readily apparent what caused the large discrepancy between the data presented here (Table I) and those of Yudkin and Krauss (2). A comparison of the two sets of data

[4] Weight gained per day ± SEM.

TABLE I. Effect of Diet on Glucose-6-phosphate Dehydrogenase, 6-phosphogluconate Dehydrogenase, Malic Enzyme, and Pyruvate Kinase in Liver of the Rat.

Diet	G6PDH units/g†	6PGDH units/g†	Malic enzyme units/g†	Pyruvate kinase units/g†
Experiment 1**				
Lab Chow(5)	$0.6 \pm 0.03^{*a}$	0.9 ± 0.44^{a}	0.7 ± 0.08^{a}	41 ± 1.8^{a}
Glucose (6)	2.2 ± 0.17^{b}	1.0 ± 0.11^{a}	1.8 ± 0.15^{b}	81 ± 3.5^{b}
Sucrose (5)	3.3 ± 0.44^{c}	$1.4 \pm 0.10^{a,b}$	3.5 ± 0.15^{c}	106 ± 6.5^{c}
Invert (6)	4.2 ± 0.27^{c}	1.4 ± 0.06^{b}	3.5 ± 0.63^{c}	100 ± 2.4^{c}
Experiment 2**				
Lab Chow (4)	$1.0 \pm 0.12^{*a}$	2.0 ± 0.08^{a}	1.6 ± 0.18^{a}	42 ± 0.8^{a}
Corn starch (4)	2.3 ± 0.40^{b}	$2.1 \pm 0.17^{a,b}$	2.2 ± 0.03^{b}	69 ± 3.3^{b}
Glucose (4)	2.6 ± 0.42^{b}	$2.5 \pm 0.18^{b,c}$	3.7 ± 0.27^{c}	66 ± 1.7^{b}
Sucrose (4)	3.8 ± 0.03^{c}	2.8 ± 0.23^{c}	4.9 ± 0.08^{d}	90 ± 1.5^{c}

* SEM.

** Numbers in parentheses indicate the number of animals in each group.

$^{a, b, c, d}$ Enzymatic activities with different superscript letters are significantly different from one another ($P < 0.05$).

† Units/g refers to units per gram wet weight of liver tissue.

TABLE II. Effect of Diet on Glucose-6-phosphate Dehydrogenase, 6-phosphogluconate Dehydrogenase, Malic Enzyme, and Pyruvate Kinase in Epididymal Adipose Tissue of the Rat.

Diet**	G6PDH units/g†	6PGDH units/g†	Malic Enzyme units/g†	Pyruvate kinase units/g†
Lab Chow (5)	0.5 ± 0.03*[a]	0.15 ± 0.01[a]	0.7 ± 0.08[a]	11.2 ± 0.17[a]
Glucose (6)	1.4 ± 0.14[b]	0.27 ± 0.03[b]	3.3 ± 0.35[b]	14.8 ± 0.53[b]
Sucrose (5)	1.2 ± 0.17[b]	0.24 ± 0.03[b]	3.0 ± 0.67[b]	14.3 ± 0.77[b]
Invert (6)	1.3 ± 0.13[b]	0.26 ± 0.02[b]	2.5 ± 0.31[b]	14.2 ± 0.43[b]

* SEM.

** Numbers in parentheses indicate the number of animals in each group.

[a, b, c, d] Enzymatic activities with different superscript letters are significantly different from one another (*PM* x 0.05).

† Units/g refers to units per gram wet weight of adipose tissue.

reveals that pyruvate kinase activities from the livers of rats fed diets containing sucrose or invert sugar were identical in the two studies and that the main difference is in values obtained from animals fed diets containing starch as the sole carbohydrate. It is possible that the discrepancy was a result of the use of different types of starches to prepare the diets in the two studies (corn starch vs. potato starch, etc.). The source of starch used by Yudkin and Krauss (2) was not reported, so this question must remain unanswered. Further study seems warranted to settle this discrepancy.

Since glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and malic enzyme produce the majority of the NADPH used for *de novo* synthesis of fatty acids in the cytoplasm (9), increases in the activities of these enzymes would indicate a greater maximum capacity of the tissue to produce NADPH. This, coupled with the fact that fatty acid synthetase in the liver is also increased by feeding diets containing sucrose (3), should mean that more lipids can be made by the liver for its own use or for transport to other tissues when the rat is fed diets containing sucrose. Michaelis and Szepesi (11) reported that rats fed diets containing sucrose had greater amounts of total lipid in the liver compared to those fed glucose. Bruckdorfer *et al.* (3) reported that rats fed diets containing sucrose had higher levels of plasma triglycerides and cholesterol compared to those fed starch. It therefore seems likely that, in the case of these enzymes, increases in enzymatic activity accurately reflect increased lipid content in the liver and plasma.

Of the four enzymes studied, only malic enzyme was significantly increased by feeding glucose rather than starch. Since the production of lipid in the liver was not measured in this study, it is not possible to determine if this difference was significant enough to have led to an increased production of lipid in the livers of rats fed glucose rather than starch.

That sucrose or invert sugar increases the activities of the enzymes related to lipogenesis studied in the liver (Table I) relative to activities in animals fed Lab Chow, glucose, or starch is very clear. The effect does not appear to be due to sucrose *per se*, but more likely to the fructose present in the disaccharide. The data do not agree with the hypothesis that diets containing sucrose stimulate glucose-6-phosphate dehydrogenase and malic enzyme more than diets containing invert sugar (12).

Summary. The activities of glucose-6-phosphate dehydrogenase, malic enzyme, and pyruvate kinase were significantly higher in the livers and adipose tissues from rats fed purified diets than in these tissues from rats fed commercial lab chow. The type of carbohydrate fed in the purified diet did not have a significant effect on the final levels of the three enzymes in epididymal adipose tissue, but did affect the levels of these enzymes in the liver: rats fed sucrose or invert sugar had higher activities than those fed glucose or starch. The activity of malic enzyme was higher in the livers of rats fed diets containing glucose than in those fed starch. No significant differences in enzyme activities were observed between rats fed diets containing sucrose or invert sugar.

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