GLUCOSE-ATP TRANSFERASES IN ADIPOSE TISSUE OF FASTED AND REFED RATS[1]

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Enzymes catalyzing the phosphorylation of glucose with the aid of adenosine triphosphate have been shown to exist in two molecularly distinct forms in liver (5,8,9,10). One of these (hexokinase) had a low K_m (0.01 mM) and was inhibited by glucose-6-phosphate, and the other (glucokinase) was a high (10 mM) K_m enzyme, not inhibited by glucose-6-phosphate (8). The glucose-adenosine triphosphate phosphotransferase of yeast has also been demonstrated to occur in multiple molecular forms (7). We have now demonstrated by starch-gel electrophoresis the presence of two glucose-adenosine triphosphate phosphotransferases in rat adipose tissue and shown the high K_m enzyme (glucokinase) to be susceptible to the nutritional manipulation of fasting and/or refeeding.

Sprague-Dawley rats weighing between 225 and 275 g when sacrificed were used. Prior to experimental use they had free access to Purina Laboratory Chow and water. "Fasted" rats were deprived of food for 48 hours but allowed water ad libitum; "refed" rats were fasted for 48 hours and then allowed

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free access for 24 hours to a purified high-carbohydrate low-fat ration consisting of a fat-free test diet supplied by Nutritional Biochemicals Corporation, to which 1% by weight of corn oil had been added. Rats were sacrificed by guillotine decapitation and the excised epididymal fat tissue was homogenized in one to two volumes cold 0.025 M Tris-HCl buffer pH 8.6 containing 5 x 10^{-3} M ethylenediamine tetraacetate. The homogenate was centrifuged for 30 minutes at 27,000 g, 0^{0} C, and the aqueous supernatant fraction retained.

The supernatant solution was assayed for glucokinase and hexokinase activity using essentially the method of Viñuela et al. (8). Soluble protein in the supernatant fraction was estimated according to the method of Lowry et al. (2).

Aliquots of the supernatant fluid were absorbed on 3 x 50 mm filter paper strips and subjected to starch-gel electrophoresis according to the method of Smithies (6), using a 0.3 M Tris buffer pH 8.6 with a constant current of 40 mA across the block for 12 hours at 0^{0}C. Two-centimeter strips were cut the length of the block and stained for the enzymes using a system containing in final concentration: 0.2 m molar NADP, 10 m molar $MgCl_2$, 10 m molar MgATP, 0.002 molar KCN, 0.16 mg/ml phenazine methosulfate, 0.1 mg/ml nitro blue tetrazolium, 0.4 i.u./ml crystalline glucose-6-phosphate dehydrogenase and glucose at either 0.1 molar (glucokinase and hexokinase) or 0.5 millimolar (hexokinase) in 0.05 molar Tris buffer pH 8.0 at 37^{0}C.

In liver from normal fed rats the glucokinase activity is three- to six-fold greater than the hexokinase (5,8). Contrastingly, adipose tissue from normal fed rats had hexokinase activity approximately twice that of glucokinase (Table 1). Fasting of rats for 48 hours reduced the adipose

tissue glucokinase activity to a very low level, but the effects of fasting on hexokinase are quantitatively much less marked, if indeed they are real. Refeeding of the fasted rats for 24 hours restored the glucokinase activity to about one-half of the normal level, and increased the slightly depressed hexokinase activity to normal.

TABLE I

EFFECT OF FASTING AND REFEEDING ON THE GLUCOSE-ATP PHOSPHOTRANSFERASES OF ADIPOSE TISSUE FROM RATS

Number of rats	Condition of rats	Glucokinase (units/mg protein)	Hexokinase (units/mg protein)
12	Fed	3.01 $\pm$ 0.21	5.91 $\pm$ 0.26
24	Fasted	0.76 $\pm$ 0.05	5.12 $\pm$ 0.17
15	Refed	1.41 $\pm$ 0.16	5.78 $\pm$ 0.24

Rats were fasted for 48 hours and refed for 24 hours. Methods for assay of the enzymes are in text. Enzyme units are millimicromoles NADPH formed per minute. Numbers following the values are standard errors.

Staining of the electrophoretic starch-gels for enzyme activity revealed two distinct bands for glucose-ATP-phosphotransferases with the higher concentration of glucose which detects both the glucokinase and hexokinase. When the gel strips were stained using 0.5 mM glucose as substrate, only one band (the slower migrating one) was visible (Fig. 1 and 2). Starch-gel strips obtained using the adipose tissue from 48 hour fasted rats revealed only hexokinase activity -- in some cases a very faint glucokinase band was detected (Fig. 1). Refeeding of the fasted rats for 24 hours provided adipose tissue which

gave both hexokinase and glucokinase activity when the enzymes were separated electrophoretically and stained (Fig. 2).

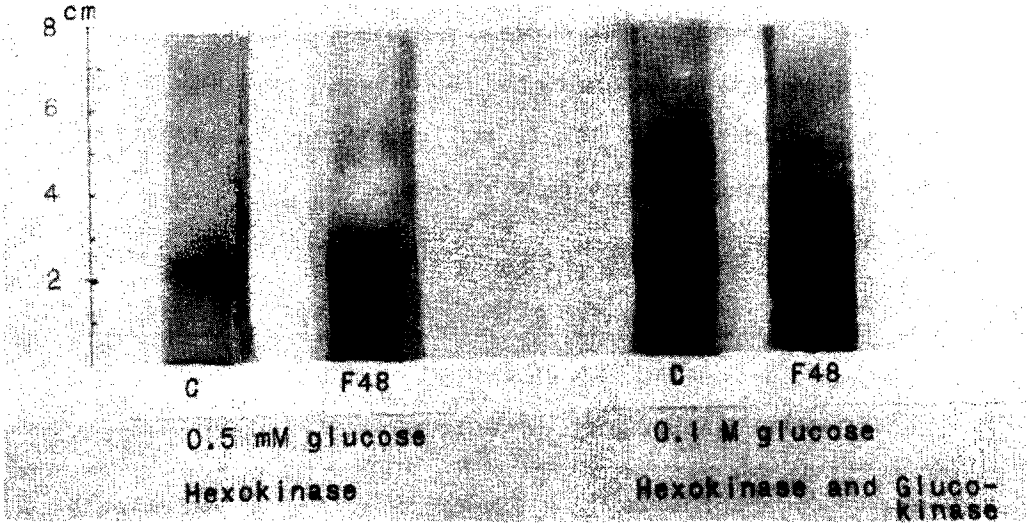

Figure 1 Bands of glucose-ATP phosphotransferase activity from adipose tissue of control and fasted rats, separated by starch-gel electrophoresis and stained using nitro blue tetrazolium as the final electron acceptor. Methods for electrophoresis and enzyme detection are in text. C refers to control, F48 refers to rats fasted for 48 hours.

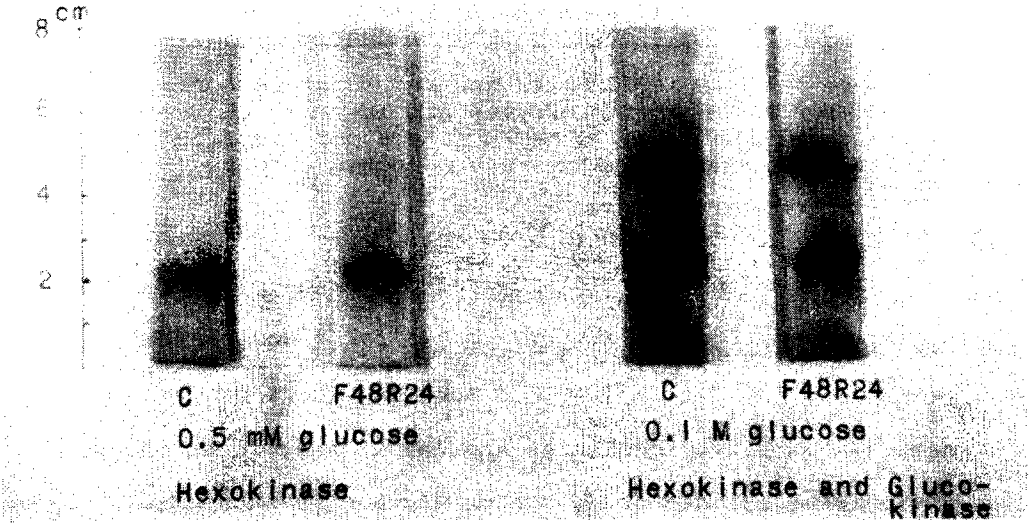

Figure 2 Bands of glucose-ATP phosphotransferase activity from adipose tissue of control and refed rats. Techniques were the same as for Figure 1. C refers to control, F48R24 refers to rats fasted for 48 hours and refed for 24 hours.

These results demonstrate that two enzymes which phosphorylate glucose occur in normal rat adipose tissue and they differ

markedly in K_m values. These two enzymes are unlike in charge since they can be separated by starch-gel electrophoresis. The observation that glucokinase, the high K_m enzyme, is reduced to very low levels in adipose tissue after a 48 hour fast, but returns toward normal values during a 24 hour refeeding period, suggests that the enzyme is readily inducible by its substrate in this tissue but that the elevated glucose levels associated with refeeding are required for this induction. On the other hand the hexokinase is not significantly altered during fasting, or fasting and refeeding.

The metabolic alterations in adipose tissue known to accompany fasting or fasting and refeeding are suggestive of concommitant alterations in enzyme activity. Earlier work (1,4) had demonstrated that adipose tissue from refed rats showed a marked increase over normal in lipid synthesis from glucose, implying a need for increased uptake and subsequent phosphorylation of glucose by the tissue. The enhancement of glucose uptake by adipose tissue during refeeding (3) may promote the induction of glucokinase which would in turn facilitate increased glucose phosphorylation at the higher intracellular glucose concentrations.

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