ACTIVITIES OF ENZYMES INVOLVED IN GLYCOLYSIS, GLUCOGENESIS AND HEXOSEMONOPHOSPHATE SHUNT IN RAT ADIPOSE TISSUE

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Studies during the past few years have emphasized that adipose tissue is capable of considerable metabolic activity, and is not simply a storehouse for fat (Ball and Cooper, 1960; Eichel, 1959; Hausberg, 1958; and Renold et al, 1959). Glucose metabolism by this tissue is markedly influenced by various endocrine hormones; e.g., insulin, corticoids and pituitary hormones, and such effects are readily demonstrated in vitro as well as in vivo. Recent investigations on the alternate pathways of glucose metabolism in adipose tissue indicated the operation of an active shunt in addition to the presence of glycolysis (Ball et al, 1959; Krahl, 1951-52; Martin et al, 1958; White and Engel, 1958; Winegrad and Renold, 1958; and Winegrad et al, 1959). These studies utilized differentially labeled glucose, and conclusions were reached on the behavior of metabolic pathways by determination of metabolites and end products of the various pathways. It has become of interest to obtain information regarding the activities and behavior of enzyme systems concerned with glucose metabolism in this tissue.

This preliminary communication reports the activity levels of enzymes responsible for channeling glucose-6-phosphate into four alternate pathways: glucose-6-phosphatase

(glucose release); phosphoglucomutase (glycogen synthesis); phosphohexoseisomerase (glycolysis); and glucose-6-phosphate dehydrogenase (hexosemonophosphate shunt). The activities of 6-phosphogluconate dehydrogenase, fructose-1,6-diphosphatase, and lactic dehydrogenase are also given. These carbohydrate-metabolizing enzymes were studied in the epididymal fat pad of rats and values were compared with activities found in the liver.

Male Wistar rats weighing 180-220 g were maintained on Purina Fox Chow and water ad libitum. Animals were killed by decapitation, bled, and livers and fat pads were rapidly excised. Tissues were minced with scissors, and 2.5 % homogenates were prepared in isotonic KCl. Supernatant fluid was obtained by centrifuging homogenates at 100,000 g for 30 minutes at 0°C. Nitrogen content was determined by the micro-Kjeldahl procedure. Glucose-6-phosphatase activity was measured in the homogenate (Cori and Cori, 1952), and the other enzymes were assayed in the supernatant fluid. Phosphoglucomutase activity was assayed according to Najjar (1948); phosphohexoseisomerase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase activities were followed by the methods of Glock and McLean (1953, 1956). Fructose-1,6-diphosphatase and lactic dehydrogenase activities were measured according to Weber and Cantero (1959). All enzyme assays were carried out at 37°C under linear kinetic conditions. Activities are expressed per mg nitrogen.

A comparison of nitrogen content and activities of carbohydrate-metabolizing enzymes in rat adipose tissue and liver is presented in Table I. There is only about one-seventh as much nitrogen in the adipose tissue as in the liver. However, adipose tissue exhibits more than five times as much glucose-6-phosphate dehydrogenase activity as the liver. It is of interest

that in the liver glucose-6-phosphate dehydrogenase activity is much lower than 6-phosphogluconate dehydrogenase activity, whereas in the adipose tissue both enzymes appear to be in the same range of activity. The high activities of the shunt enzymes in adipose tissue are in line with findings of Cohn and Joseph (1959) and with indications obtained by isotope studies of this tissue (Winegrad and Renold, 1958).

With the exception of glucose-6-phosphate dehydrogenase all enzyme activities measured in adipose tissue were less than those found in liver. The difference between the metabolic pathways of adipose tissue and liver is reflected in the virtual absence of two specific phosphatases (glucose-6-phosphatase and fructose-1,6-diphosphatase) in this tissue, whereas these enzymes play an important role in the gluconeogenetic processes in the liver. The ratios of glucose-6-phosphate dehydrogenase to 6-phosphogluconate dehydrogenase, phosphohexoseisomerase, and phosphoglucomutase are ten to twenty fold higher in adipose tissue than in liver (Table I). These findings show that the relative activities of the enzymes involved in the metabolism of glucose-6-phosphate are markedly different in adipose tissue from those found in liver.

These results indicate that the two major pathways of glucose metabolism in adipose tissue are the hexosemonophosphate shunt and glycolysis. The high activities of the two dehydrogenases of the shunt pathway emphasize the capacity of this tissue for TPNH production which is presumably a contributing factor to the marked capacity of adipose tissue for the synthesis of fatty acids. The behavior of enzyme systems of the adipose tissue under various dietary and hormonal conditions is under investigation.

TABLE I

Activities of enzymes involved in glycolysis, glucogenesis and hexosemonophosphate shunt in rat adipose tissue and liver*

	No. observed	Liver	No. observed	Adipose Tissue
Supernatant nitrogen	3	14.9 ± 0.6	5	2.4 ± 0.24
Lactic dehydrogenase	5	1149.0 ± 59	4	112.0 ± 24
Phosphohexoseisomerase	5	660.0 ± 17	5	187.0 ± 22
Phosphoglucomutase	5	37.8 ± 2.1	5	10.7 ± 3.8
Fructose-1,6-diphosphatase	5	31.1 ± 2.1	5	traces
Glucose-6-phosphatase	3	38.2 ± 3.7	5	traces
6-Phosphogluconate dehydrogenase	5	39.0 ± 3.1	5	20.2 ± 2.3
Glucose-6-phosphate dehydrogenase	3	5.5 ± 2.7	5	31.0 ± 10.5
		Ratios**		
Glucose-6-phosphate dehydrogenase/ 6-Phosphogluconate dehydrogenase		141		1535
Glucose-6-phosphate dehydrogenase/ Phosphohexoseisomerase		8		166
Glucose-6-phosphate dehydrogenase/ Phosphoglucomutase		146		2897

* The mean values and standard errors are tabulated. Enzyme activity is expressed in uM of substrate metabolized/hour/mg nitrogen at 37°C. Nitrogen content is given as mg/g wet weight of tissue.

** x 10^3

References

Ball, E. G., and Cooper, O., J. Biol. Chem., 235, 584 (1960).

Ball, E. G., Martin, D. B., and Cooper, O., J. Biol. Chem., 234, 774 (1959).

Cohn, G., and Joseph, D., Am. J. Physiol., 197, 1347 (1959).

Cori, G. T., and Cori, C. F., J. Biol. Chem., 199, 661 (1952).

Eichel, H. J., Biochem. and Biophys. Res. Comm., 1, 293 (1959).

Glock, G. E., and McLean, P., Biochem. J., 55, 400 (1953).

Glock, G. E., and McLean, P., Biochem. J., 63, 520 (1956).

Hausberg, F. X., Diabetes, 7, 211 (1958).

Krahl, M. E., Ann. N. Y. Acad. Sci., 54, 649 (1951-52).

Martin, D. B., Renold, A. E., and Dagenais, Y. M., Lancet, 2, 76 (1958).

Najjar, V. A., J. Biol. Chem., 175, 281 (1948).

Renold, A. E., Jeanrenaud, B., Winegrad, A. I., and Martin, D. B., Modern Problems in Pediatrics, 4, 121 (1959).

Weber, G., and Cantero, A., Cancer Research, 19, 763 (1959).

White, J. E., and Engel, F. L., Proc. Soc. Exptl. Biol. and Med., 99, 375 (1958).

Winegrad, A. I., and Renold, A. E., J. Biol. Chem., 233, 273 (1958).

Winegrad, A. I., Shaw, W. N., Lukens, F. D. W., Stadie, W. C., and Renold, A. E., J. Biol. Chem., 234, 1922 (1959).