Lipoprotein Lipase Activity in Adipose Tissue of Streptozotocin-induced Diabetic Rats

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Abstract

Lipoprotein lipase activity of epididymal adipose tissue was measured in streptozotocin-induced diabetic rats. Diabetic rats of 3, 10 and 34 days duration were examined. The enzyme activity in adipose tissue of diabetic rats was similar to that of control rats of the same ages, compared on the tissue weight basis. However, since adipose tissue weight was markedly reduced in rats with both acute and chronic diabetes, total enzyme activity in the whole tissue was very low in such animals regardless of the duration of diabetes. We wish to point out that contradictory results on the adipose tissue lipoprotein lipase activity in diabetic rats in the previous reports have arisen depending on differences in the methods chosen to express enzyme activity.

Different results on the adipose tissue lipoprotein lipase (LPL) activity in diabetic rats have been reported. This was low (Kessler, 1963; Schnatz and Williams, 1963; Elkeles and Hambly, 1977), only moderately decreased (Chen, *et al.*, 1979) or even normal (Redgrave and Snibson, 1977). This led us to re-examine the adipose tissue LPL activity in streptozotocin-induced diabetic rats. We have also examined whether the duration of diabetes has any effect on the tissue LPL activity in rats.

and control rats were fasted for 12 hours to avoid the nutritional effect on LPL. Animals were killed by decapitation and the epididymal adipose tissue was quickly excised.

Incubation of adipose tissue: The incubation method is the same as described previously (Murase *et al.*, 1981). A portion of the adipose tissue, weighing 30-100 mg, was minced into small pieces and placed in 1 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 40 mg of serum albumin (Fraction V, Sigma Chem. Co., St. Louis, USA) and 1.8 mg of glucose. Incubation was started with the addition of 2 units per ml of heparin (Novo Indust., Denmark) and was carried out for 60 min in a shaking water bath at 37°C with the gas phase of 95% O₂-5% CO₂. The incubation medium was then assayed for its LPL activity.

Materials and Methods

Animals: Male rats of Wistar strain were used in this study. Diabetes was induced by the intravenous administration of Streptozotocin (Upjohn Co., Kalazoo, Michigan), 50 mg per kg of body weight. Diabetic rats had blood glucose levels of more than 300 mg/dl. These rats were subjected to the experiments 3, 10 and 34 days after the induction of diabetes. Both diabetic

Measurement of enzyme activity: The enzyme activity was determined by the radioisotope method described previously (Yamada *et al.*, 1979). The activity was expressed as units; 1 unit represents 1 μ mole of free fatty acids liberated by hydrolysis per hour. All results were calculated per mg of incubated adipose tissue and then corrected for whole tissue, i.e., total weight of both left and right epididymal adipose tissue.

Statistical analysis: The results were expressed as mean $\pm$ SE. The statistical significance of the data was analyzed by Student's *t*-test.

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Table 1. Laboratory data for diabetic and control rats

Conditions	Duration (days)	Body weight (gm)	Adipose tissue weight (gm)	Enzyme activity	
				(U/gm tissue)	(U/whole tissue)
diabetes (n=5)	3	154± 9*	0.32±0.07**	16.7±3.1	5.0±0.7*
controls (n=5)		174± 5	0.67±0.08	18.6±4.3	12.1±2.6
diabetes (n=6)	10	188±14**	0.32±0.05***	13.6±5.3	3.0±0.2**
controls (n=5)		248± 6	1.68±0.22	11.6±2.1	20.1±5.0
diabetes (n=4)	34	181± 7***	0.19±0.08***	12.2±5.3	5.4±0.3**
controls (n=4)		279± 7	2.45±0.29	8.9±1.7	21.2±3.8

Mean±SE. *p<0.03, **p<0.01, ***p<0.001

Results

Properties of adipose tissue LPL: The addition of heparin to the incubation medium increased by four times the amount of LPL released from adipose tissue. This heparin-releasable enzyme had the characteristics of LPL in that it required the serum for activation (4.8 times activation by serum) and was inhibited by protamine sulfate (97% inhibition by 3 mg/ml of protamine sulfate) and had an alkaline pH optimum (pH 8.2).

Body weight and epididymal adipose tissue weight in diabetic rats: As shown in Table 1, diabetic rats had lower body weight than control rats. Epididymal adipose tissue weight was markedly reduced in rats with diabetes of 3, 10 and 34 days duration, compared with that of corresponding control rats.

Adipose tissue LPL activity: Adipose tissue LPL activity of diabetic rats was similar to that of control rats, when it was compared on the tissue weight basis (Table 1). However, when total enzyme activity in the whole adipose tissue of diabetic rats was compared with that of corresponding control rats, it was markedly low. Figure 1 shows the relationship between adipose tissue weight and the LPL activity. A high correlation was found between them ($r=0.81$, $p<0.001$).

Fig. 1. Relationship between epididymal adipose tissue weight and heparin-releasable lipoprotein lipase activity.

Discussion

Adipose tissue LPL is an enzyme which regulates the uptake of plasma triglyceride by the tissue (Robinson, 1970). Although most

studies show that rat adipose tissue LPL is an insulin-dependent enzyme (Borensztajn *et al.*, 1972; Garfinkel *et al.*, 1976), contradictory results have been published on the adipose tissue LPL activity in diabetic rats (Kessler, 1963; Schnatz and Williams, 1963; Elkeles and Hambly, 1977; Chen *et al.*, 1977 Redgrave and Snibson, 1977). The present study shows that this arises depending on the methods chosen to express enzyme activity. The enzyme activity of diabetic rats was similar to that of control rats of the same ages, when it was compared on the tissue weight basis. Redgrave and Snibson (1977) reported similar results. Chen *et al.* (1979) have emphasized that insulin deficiency caused only a moderate reduction in adipose tissue LPL activity. They also expressed the enzyme activity per gm of adipose tissue. However, we feel that it is inappropriate to express the enzyme activity on the tissue weight, because, as we observed in this study, diabetic rats exhibit a marked loss of adipose tissue weight. The present study shows that epididymal adipose tissue weight is closely related to its LPL activity. This indicates that the enzyme activity in whole adipose tissue may reflect adequately the functional state of the tissue. Probably it is more appropriate to express the enzyme activity per total tissue than per gm tissue weight. The present study demonstrates that diabetes causes a marked reduction in the total activity of LPL in the whole epididymal adipose tissue in rats. Such observations were made in rats with both acute and chronic diabetes.

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