# Effect of Dextran Sulfate on Plasma Lipoprotein Lipase Activity in Obese Subjects and Diabetic Patients

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SUMMARY

**The changes in plasma lipoprotein lipase activity after intravenous injection of dextran sulfate, which stimulates release of lipoprotein lipase, have been studied in forty-two diabetic patients, eight obese subjects and eleven normal subjects.**

**In normal subjects, the response of lipoprotein lipase activity to dextran sulfate averaged 5.7±0.6 (S.E.) μEq. FFA/ml. at ten minutes and 6.5±1.2 (S.E.) μEq. FFA/ml. at twenty minutes.**

**A significantly decreased response of lipoprotein lipase activity to dextran sulfate was found in obese subjects and in diabetic patients, especially in those with hypertriglyceridemia. No correlation was observed between the cholesterol level and lipoprotein lipase activity. No significant relation between changes of lipoprotein lipase activity and the incidence of diabetic complications was found.** DIABETES *16*:238-41, April, 1967.

The recent report of Schnatz and Williams[1] on lipoprotein lipase in diabetic rat adipose tissue has suggested that a decreased activity of lipoprotein lipase may contribute significantly to the elevation of plasma triglycerides in diabetic rats. A significant increase in the activity of this enzyme was observed following the administration of insulin.[2]

It is well known that diabetic patients often show high concentration of triglycerides in plasma[3-5] and diabetics with vascular complications tend to show the greatest degree of hypertriglyceridemia.[5,6]

Hollenberg[7] found that lipoprotein lipase activity of rat adipose tissue was lower in the fasting state than in the fed state. He also noted that addition of heparin accelerated lipolysis by tissue from fasted rats only if glucose and insulin also were added to the medium.[8] Bragdon and Gordon[9] observed that carbohydrate feeding of rats shifted the distribution of injected labeled chylomicrons to adipose tissue instead of liver as in fasted rats.

Findings such as the foregoing suggest that the heparin-activated enzyme, lipoprotein lipase, is concerned with hypertriglyceridemia in diabetes mellitus. Presented here are the results of a study in obese and diabetic subjects of the change in lipoprotein lipase in the plasma following the intravenous injection of dextran sulfate which, like heparin, stimulates release of lipoprotein lipase.[10]

## METHODS

These studies included forty-two diabetic patients and eight obese subjects who were seen at intervals not exceeding three weeks at the diabetic clinic of Osaka University Hospital. Urine glucose, ketone bodies, albumin and blood pressure were determined at each visit. Fasting blood glucose, serum cholesterol and plasma triglyceride were analyzed once a month. All patients received a chest X ray, electrocardiogram, funduscopic examination and PSP test.

Obese subjects were those whose index of body weight* was more than 1.20 calculated as follows:

$$\frac{\text{body weight (kg.)}}{[\text{height (cm.)} - 100] \times 0.9}$$

and in whom oral glucose tolerance tests remained within normal range. Diabetic patients treated with insulin were omitted from the study.

All patients were fasted for fourteen hours prior to intravenous injection of 10 mg. per kg. of dextran sulfate. A blood sample was taken for determination of lipoprotein lipase activity immediately before and ten,

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*The index of body weight: according to the formula of the Ministry of Welfare (Japan).

twenty and thirty minutes after injection. The same samples were analyzed for blood sugar (Somogyi-Nelson), serum cholesterol (Zak-Henley) and plasma triglyceride concentration (Van Handel and Zilversmit). The lipoprotein lipase activity in each blood sample was determined by the modified method of Korn.[11] After adding 1 milliliter of plasma to standard fat emulsion (Fatgen: obtained from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) samples were incubated at *p*H 8.5 for thirty minutes at 37° C. Released free fatty acids served as an index of lipoprotein lipase activity and were expressed as μEq. FFA/ml. plasma.

Plasma lipoprotein lipase activity before administration of dextran sulfate was negligible (less than 0.4 μEq. FFA/ml.). These values are not indicated in the tables since no significant differences in activity were observed among normal, obese and diabetic groups.

## RESULTS

In eleven normal subjects tested, the lipoprotein lipase activity before administration of dextran sulfate was found to be negligible (0.3 ± 0.1 S.E. μEq. FFA/ml.). Intravenous injection of dextran sulfate increased the activity to an average of 5.7 ± 0.6 μEq. FFA/ml. at ten minutes and 6.5 ± 1.2 μEq. FFA/ml. at twenty minutes. The activity then decreased to 2.3 ± 0.2 μEq. FFA/ml. at thirty minutes.

Table 1 summarizes the values for plasma lipoprotein lipase activity after dextran sulfate injections in eleven normal subjects, eight obese subjects and forty-two diabetic patients. The lipoprotein lipase activity at both ten and twenty minutes after dextran sulfate injections was significantly lower in obese subjects than in normal subjects. In forty-two diabetic patients, lipoprotein lipase activity was found to be lower at twenty minutes than that in normal subjects. No difference was found at ten minutes.

A comparison of the degree of obesity and serum

TABLE 1

Plasma lipoprotein lipase activity after intravenous injection of dextran sulfate in normal, obese and diabetic subjects

| Subjects | Cases | Time after intravenous injection of dextran sulfate | | | |
|---|---|---|---|---|---|
| | | 10 minutes | | 20 minutes | |
| Normal | 11 | 5.7±0.6* | P | 6.5±1.2 | P |
| Obesity | 8 | 3.5±1.2 | <0.01 | 3.7±0.7 | <0.01 |
| Diabetes | 42 | 5.0±0.6 | >0.1 | 3.6±0.6 | <0.01 |

Mean ± standard error; values are expressed as μEq. FFA/ml. serum/30 min.

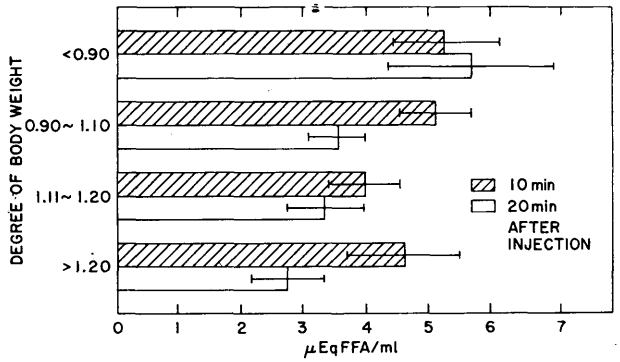


FIG. 1. Plasma lipoprotein lipase activity after dextran sulfate injection and degree of body weight in diabetics. Mean values and standard errors.

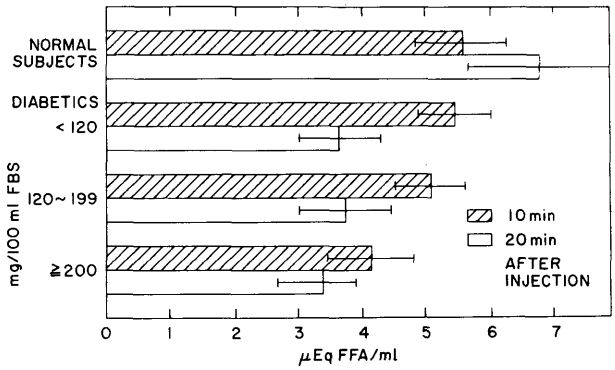


FIG. 2. Plasma lipoprotein lipase activity after dextran sulfate injection and fasting blood sugar in diabetics.

lipoprotein lipase activity in diabetics is shown in figure 1. Obese diabetics who had an index of body weight more than 1.20 showed lower plasma lipoprotein lipase activity at twenty minutes after dextran sulfate injection than diabetics without obesity.

Figure 2 indicates the relation between the plasma lipoprotein lipase activity and fasting blood glucose level in diabetic patients. Although the lipoprotein lipase activity at twenty minutes after dextran sulfate injection in all groups of diabetics was lower than in normal subjects, those with high blood glucose did not show significantly lower activity. No marked change at ten minutes after dextran sulfate was found, but the most hyperglycemic subjects exhibited slightly lower activity levels than those with less hyperglycemia.

Serum cholesterol level showed no correlation with lipoprotein lipase activity (figure 3). As indicated in figure 4, diabetics with a relatively high plasma triglyceride level (higher than 80 mg./100 ml.) showed a slightly decreased lipoprotein lipase activity at twenty

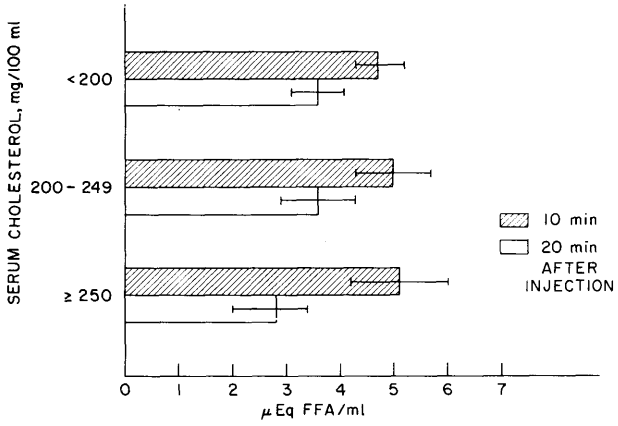


FIG. 3. Plasma lipoprotein lipase activity after dextran sulfate injection and serum cholesterol in diabetics.

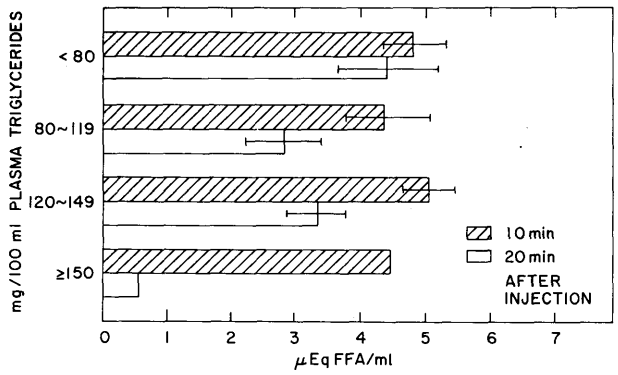


FIG. 4. Plasma lipoprotein lipase activity after dextran sulfate injection and plasma triglycerides in diabetics. Mean values and standard errors.

minutes after dextran injection. Furthermore, a marked decrease in lipoprotein lipase activity was found at twenty minutes after dextran sulfate injections in four diabetics with marked hypertriglyceridemia (higher than 150 mg./100 ml.).

The diabetic group was subdivided on the basis of presence or absence of clinical complications (hypertension, retinopathy, myocardial change and nephropathy). As shown in table 2, the present data do not clearly establish a relationship between a decreased plasma lipoprotein lipase activity and clinically apparent complications in most diabetics.

## DISCUSSION

The recent study of Rodbell,[12] demonstrating lipoprotein lipase activity in the fat cell but not in the stromal-vascular cells, has suggested that fat cells in the adipose tissue will release this enzyme into the blood with the aid of heparin or dextran sulfate. It is

TABLE 2

Plasma lipoprotein lipase activity after intravenous injection of dextran sulfate in diabetics with cardiovascular complications

| Subjects | Cases | Time after intravenous injection of dextran sulfate: 10 minutes | 20 minutes |
|---|---|---|---|
| No complication | 21 | 5.2±0.4* | 4.0±0.6 |
| Hypertension | 10 | 4.0±0.6 | 2.5±1.2 |
| Retinopathy | 15 | 4.5±0.6 | 3.6±0.8 |
| Myocardial change | 13 | 5.0±0.4 | 3.1±0.6 |
| Nephropathy | 2 | 6.9 | 2.2 |

Mean ± standard error; values are expressed as μEq. FFA/ml. serum/30 min.

Hypertension: over 150/90 mm. Hg; Myocardial change: EKG; Retinopathy: Wagener II, III, IV; Nephropathy: less than 20 per cent in PSP test.

known that lipoprotein lipase appears in the blood after the administration of heparin to rats in vivo.[13] In contrast to the negative findings by Angervall,[14] Nestel and Havel[15] and Stern et al.[16] clearly demonstrated the presence of considerable lipoprotein lipase activity in human adipose tissue incubated with heparin in vitro. It is also clear from the work of Yamada et al.[10] that the intravenous injection of heparin or dextran sulfate into human subjects causes the release and/or the activation of lipoprotein lipase activity. The authors have found that the increased activity of lipoprotein lipase in response to dextran sulfate was rather sluggish and continued longer compared with the response to heparin.[17] In the present study the response of plasma lipoprotein lipase activity to dextran sulfate agrees with other studies which have used a heparin-like substance as a component of the enzyme system.[18]

Lipoprotein lipase plays an important role in assimilation of plasma triglyceride by adipose tissue and is related to the uptake of triglyceride fatty acids from the blood by the extrahepatic tissues. Fasting induces a decrease in the activity released from adipose tissue by heparin, while refeeding is associated with an increase in this activity.[8,18] Lipoprotein lipase activity released from adipose tissue by heparin has been found to be decreased in alloxan diabetic rats.[1,10] Decreased activity in alloxan diabetic rats was improved by insulin treatment.[2] These findings suggest that lipoprotein lipase activity may be regulated by the blood glucose level or by insulin. Hollenberg has reported that glucose and insulin increased the heparin response of adipose tissue from fasting animals.[8]

In the present study of human subjects the response of lipoprotein lipase activity to dextran sulfate injec-

tion was decreased in obese subjects and in diabetics, especially in diabetic patients with high plasma triglyceride levels (over 150 mg. per 100 ml.). However, high serum cholesterol levels did not correlate with lipoprotein lipase activity.

Diabetics are known to develop vascular disease prematurely and frequently, as compared with nondiabetics. Hyperlipemia observed in nonketotic diabetics may be related to the increased incidence of vascular disease. Diabetic subjects with vascular complications have been reported to have higher cholesterol and triglyceride concentration than diabetics without complications.[5,6] However, we could not find a clear relationship between a decreased lipoprotein lipase activity and diabetic complications contrary to the results of Slack et al.[19]

The finding of decreased response of lipoprotein lipase activity to dextran sulfate suggests that there may be a decrease in this enzyme activity in adipose tissues of diabetic patients as well as in diabetic rats and that this decreased activity may contribute significantly to the elevated plasma triglyceride of diabetes mellitus. In view of the hypothesis that obesity predisposes to diabetes (a kind of prediabetic state), there may be, from the present study, some inference that decreased activity of lipoprotein lipase in such prediabetic states is associated with an imbalance between insulin and insulin antagonists.

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## REFERENCES

[1] Schnatz, J. D., and Williams, R. H.: The effect of acute insulin deficiency in the rat on adipose tissue lipolytic activity and plasma lipids. Diabetes *12*:174-77, 1963.

[2] Schnatz, J. D., and Williams, R. H.: Lipoprotein lipase and tributyrinase in rat adipose tissue. Metabolism *11*:349-56, 1962.

[3] New, M. I., Roberts, T. N., Bierman, E. I., and Reader, G. G.: The significance of blood lipid alterations in diabetes mellitus. Diabetes *12*:208-11, 1963.

[4] Wada, M.: Plasma lipids and diabetes mellitus. J. Jap. Internal Med. *53*:1381-85, 1964.

[5] Adlersberg, D., Wang, C. I., Rifkin, H., Berkman, J., Ross, G., and Weinstein, C.: Serum lipids and polysaccharides in diabetes mellitus. Diabetes *5*:116-20, 1956.

[6] Adlersberg, D., and Eisler, L.: Circulating lipids in diabetes mellitus. JAMA *170*:1261-65, 1959.

[7] Hollenberg, C. H.: Adipose tissue lipase II. Handbook of Physiology, Vol. 5, Adipose Tissue. Washington, D.C., p. 301-07, 1965.

[8] Hollenberg, C. H.: Effect of nutrition on activity and release of lipase from rat adipose tissue. Amer. J. Physiol. *197*:667-70, 1959.

[9] Bragdon, J. H., and Gordon, R. S., Jr.: Tissue distribution of C-14 after the intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat. J. Clin. Invest. *37*:574-78, 1958.

[10] Yamada, K., Kuzuya, H., and Noda, M.: Studies on some actions of sulfated polysaccharides on arteriosclerosis. I. Toxicity, lipolytic action and anticoagulant activity. Jap. Circ. J. *25*:497-502, 1961.

[11] Korn, E. D.: The assay of lipoprotein lipase in vivo and in vitro. Method of Biochemical Analysis, Vol. 7. New York, p. 145, 1959.

[12] Rodbell, M.: Localization of lipoprotein lipase in fat cells of rat adipose tissue. J. Biol. Chem. *239*:753-55, 1964.

[13] Korn, E. D.: Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. J. Biol. Chem. *215*:1-14, 1955.

[14] Angervall, G.: Lipoprotein lipase activity in adipose tissue from hen, chicken and adult human. Acta Physiol. Scand. *48*:71-77, 1960.

[15] Nestel, P. J., and Havel, R. J.: Lipoprotein lipase in human adipose tissue. Proc. Soc. Exper. Biol. Med. *109*:985-87, 1962.

[16] Stern, C. A., Iacono, J. M., and Muller, J. F.: Lipoprotein lipase in human adipose tissue. Proc. Soc. Exper. Biol. Med. *110*:366-68, 1962.

[17] Shigeta, Y., Nakamura, K., Hoshi, M., Kim, M., and Abe, H.: Unpublished data.

[18] Robinson, D. S.: The clearing factor lipase activity of adipose tissue. Handbook of Physiology, Vol. 5, Adipose Tissue. Washington, D.C., pp. 295-99, 1965.

[19] Slack, J., Seymour, J., McDonald, L., and Love, F.: Lipoprotein lipase levels and platelet stickiness in patients with ischemic heart disease and in controls, distinguishing those with an affected first-degree relative. Lancet *2*:1033-37, 1964.