Brief Notes and Comments

The Effects of Fasting and Streptozotocin Diabetes on Hepatic Triglyceride Lipase Activity in the Rat

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SUMMARY

The activity of hepatic triglyceride lipase in the rat was reduced by fasting. Withdrawal of insulin from insulin-treated streptozotocin-diabetic rats resulted in a decrease in hepatic triglyceride lipase activity. The behavior of the enzyme in both situations was similar to that of adipose tissue lipoprotein lipase. It is concluded that hepatic triglyceride lipase, like adipose tissue lipoprotein lipase, is under hormonal regulation by insulin. DIABETES *26*:58-60, January, 1977.

The clearance of dietary and endogenous triglyceride from the circulation is thought to be mediated by action of the enzyme lipoprotein lipase in extrahepatic tissues, mainly adipose tissue, heart, and muscle.[1] Although triglyceride lipase activity similar to that of lipoprotein lipase has been demonstrated in the liver,[2] it has not been thought to play an important part in the removal of triglyceride from the circulation.[2,3] Lipoprotein lipase can be released into the circulation by heparin, and Krauss et al. have demonstrated that hepatic triglyceride lipase is heparin-releasable and makes a significant contribution to plasma postheparin lipolytic activity.[4] The aim of this study was to ascertain whether hepatic triglyceride lipase activity is changed by alteration in the hormonal environment, e.g., by fasting and experimental diabetes.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (300 gm.) were used. In the fasting experiments the animals were deprived of food for 24 hours. Diabetes was induced by intraperitoneal injection of streptozotocin (Upjohn) 65 mg./kg. in citrate buffer. Diabetes was controlled by daily subcutaneous injections of soluble and protamine zinc insulin, four units of each at 4 p.m. for five to eight days. The animals, fed ad libitum, were killed in pairs at 10 a.m., insulin having been omitted from one the previous evening. Acetone-ether extracts of adipose tissue (epididymal fat pad), heart, and liver were prepared according to the method of Borensztajn et al.[5] Lipoprotein lipase activity was measured as the release of ^{14}C-labeled fatty acid from a triolein substrate by a method modified from Nilsson-Ehle et al.[6] To 5 ml. of 7 mmol/L. triolein (Sigma) in benzene was added 2.25 μCi. of glyceroltri[$1\text{-}^{14}C$]oleate (Radiochemical Centre, Amersham, 55 mCi./mmol). The benzene carrier was evaporated off with nitrogen. Triton X100 (50 μl. of 1 per cent solution in 0.2 mol/L. Tris HCl buffer, pH 8.0), fasting human serum (150 μl.) and bovine serum albumin (Armour fraction V) (225 μl. of 1 per cent solution in 0.2 mol/L. Tris-HCl buffer, pH 8.0) were added. The volume was made up to 3 ml. with 0.2 mol/L. Tris-HCl buffer, pH 8.0. The solution was sonicated (M.S.E. ultrasonic disintegrator) for two periods of 90 seconds at 8 μ peak to peak. Aliquots of the extracts of the three tissues were assayed in conditions of excess substrate. The reaction was carried out at 37° C. and continued for 45 minutes

over which time it was shown to be linear. The reaction was terminated by the addition of 5 ml. of Dole's solution.[7] Free fatty acids (FFA) were then separated with alkaline ethylene glycol.[8] One-milliliter samples of the lower phase were counted in 10 ml. Bray's solution in a Wallac beta counter (80,000 Series). Quenching was automatically corrected for by use of an external standard. DNA in tissue extracts was estimated by the method of Abraham et al.[9] Results were expressed as nmoles of ^{14}C fatty acid released/gm./min. or /μg. DNA/min.

RESULTS

Twenty-four-hour fasting produced a fall both in adipose tissue lipoprotein lipase and hepatic triglyceride activity, both on a weight and DNA basis (table 1). The activity of heart lipoprotein lipase rose on fasting, but not significantly.

In the diabetic rats, overnight withdrawal of insulin produced the expected rise in blood glucose (table 2). There was a significant fall in both adipose tissue lipoprotein lipase and hepatic triglyceride lipase activity, on a weight and DNA basis, in rats from which insulin had been withdrawn. In the insulin-withdrawal group, heart lipoprotein lipase activity rose, but not significantly.

DISCUSSION

The effects of fasting and experimental diabetes on adipose tissue and heart lipoprotein lipase activities previously described[5,10-12] are confirmed in this study. The role of insulin in the regulation of adipose tissue and heart lipoprotein lipase activity has also been well documented.[10,13,14] The findings that the activity of hepatic triglyceride lipase falls on fasting and when insulin is withdrawn from streptozotocin-diabetic rats suggests that it behaves like adipose tissue lipoprotein lipase under these conditions and that it too is under hormonal regulation by insulin. Other hormones, such as glucagon, may also be important in the regulation of lipoprotein lipase activity, especially in the heart.[15]

The function of hepatic triglyceride lipase is not yet clear. It is now believed that very-low-density lipoprotein is catabolized in the circulation to low-density lipoprotein through intermediate density lipoproteins.[16] It has been suggested that the function of hepatic triglyceride lipase is to remove lipoprotein remnants produced in this reaction.[3,4] It may be speculated that in conditions of insulin deficiency or ineffective insulin action, the hepatic removal of such remnants could be inefficient. This could lead to the accumulation of triglyceride-enriched low-density lipoprotein such as has been shown to occur in diabetes.[17] Such particles have been shown to readily enter the arterial wall.[18] However, the function of hepatic triglyceride lipase awaits clarification.

TABLE 1

Adipose tissue, heart lipoprotein lipase, and hepatic triglyceride lipase activity in fed and 24-hour-fasted rats
Mean ± S.E.M. n = 13. N. S. (not significant).

	Fed	Fasted	P
Lipase activity nmol/gm./min.			
Adipose tissue	51.3 ± 6.8	11.5 ± 1.3	< 0.001
Heart	15.2 ± 1.6	18.3 ± 2.2	N.S.
Liver	4.9 ± 0.6	2.8 ± 0.5	< 0.001
Lipase activity pmol/μg. DNA/min.			
Adipose tissue	488.5 ± 105.5	95.1 ± 16.9	< 0.001
Heart	13.7 ± 2.9	21.3 ± 7.0	N.S.
Liver	3.4 ± 0.8	1.4 ± 0.5	< 0.01

TABLE 2

Adipose tissue, heart lipoprotein lipase, and hepatic triglyceride lipase activity in streptozotocin-diabetic rats
Mean ± S.E.M. n = 9. N.S. (not significant).

	Insulin-treated	Insulin withdrawn overnight	P
Lipase activity nmol/gm./min.			
Adipose tissue	38.9 ± 8.5	5.4 ± 2.0	< 0.01
Heart	7.6 ± 1.3	10.0 ± 1.3	N.S.
Liver	2.9 ± 0.3	2.2 ± 0.2	< 0.01
Lipase activity pmol/μg. DNA/min.			
Adipose tissue	225.0 ± 55.8	20.3 ± 6.6	< 0.01
Heart	5.3 ± 0.9	7.2 ± 0.6	N.S.
Liver	1.5 ± 0.1	1.1 ± 0.1	< 0.001

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