# Lipoprotein Lipase in Human Adipose Tissue.* (27519)

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The presence of lipoprotein lipase, or clearing factor enzyme, in the plasma of various animals and man has been demonstrated by many observers and its characteristics well defined(1,2). Heparin injection has been shown to increase lipoprotein lipase activity both *in vivo*(1) and *in vitro*(3).

Clearing factor enzyme has also been demonstrated in adipose tissue of various animals, including chickens and rats(2) and it too is heparin responsive(4). Using a histochemical technic, Gomori(5) found evidence of lipolytic activity in human adipose tissue. Renold and Marble(6) reported lipolytic activity in extracts of human abdominal and subcutaneous fat using Tween 20® as a substrate. Havel(7) has demonstrated lipoprotein lipase in human adipose tissue using 2 different technics. One method utilized intact adipose tissue slices and the other an acetone powder extract of adipose tissue. Other investigators have recently questioned the existence of lipoprotein lipase activity in human adipose tissue(8,9). In view of these discrepancies, we are reporting our observations on the presence of lipoprotein lipase in surgically-removed, human adipose tissue.

*Method.* Human adipose tissue was obtained from the subcutaneous fat layers of patients undergoing elective surgical procedures at Cincinnati General Hospital and Cincinnati Veteran's Administration Hospital. All patients appeared to be in good nutritional states prior to surgery and received 5% glucose in water immediately before and during surgery. In an effort to observe possible age effects, an elderly group (over 60 years of age) and a young group (under 15 years of age) were selected. In choosing the adipose tissue samples, care was taken to select fat free of all macroscopic blood, blood vessels or other tissue. In early experiments 100 mg samples of adipose tissue were used, but with simple quantitative adjustment of the components of the reaction mixture, it was possible to analyze 20 mg samples which are herein reported.

Upon removal, adipose tissue was immediately frozen and a 20 mg sample homogenized at 4°C in 1.1 ml of the following medium: 0.5 ml Krebs-Ringer phosphate buffer, 0.5 ml normal human serum, 0.05 ml Ediol-Schenlabs (50% coconut oil emulsion) and 0.05 ml heparin. Heparin (Hepathrom®) was diluted so that each ml of incubation medium contained 90 $\gamma$ of heparin. One and one-tenth ml of the homogenate was incubated at 37°C with gentle shaking. Duplicate 0.2 ml aliquots were removed at 0, 30 and 40 minutes and immediately placed in an ice water bath. The further enzymatic hydrolysis of the triglyceride (Ediol) in the aliquot was inhibited by addition of 0.2 ml of cold TCA and 1.6 ml of cold $H_2SO_4$. After cold centrifugation and filtration of the aliquot, 0.25 ml of the filtrate was removed and the amount of glycerol determined by Korn's(2) modification of Lambert and Neish's method (formaldehyde-chromotropic acid technic). A control flask containing the reaction mixture without adipose tissue was similarly incubated. On numerous occasions no change was found in the glycerol content of the control flask. Lipolysis was determined by the change in glycerol content of the mixtures after 0, 30, and 40 minutes of incubation and expressed as $\gamma$ glycerol/g wet tissue/hr.

Preliminary studies,‡ not reported here, on

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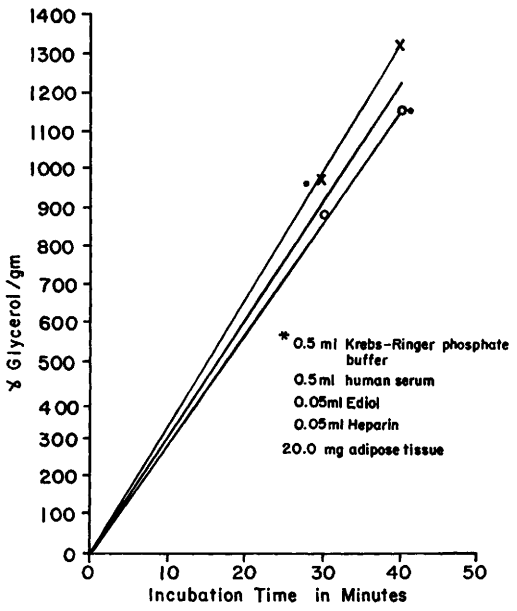


FIG. 1. The relationship of incubation time and glycerol production in a reactive mixture* containing 3 representative samples of human adipose tissue, indicating the "zero order" nature of the enzyme over the first forty minutes of incubation.

the kinetics of the enzyme reaction, indicated the optimal interval for incubation to be 40 minutes. Over this period the reaction proved to be "zero order" (Fig. 1) with the activity falling off randomly thereafter. These observations agree essentially with those of Kern, *et al.*(10) who studied the enzyme in blood. Therefore all values were arbitrarily calculated from the 40-minute incubation sample.

Addition of 10 $\mu$M/ml of sodium pyrophosphate to the reaction mixture completely inhibited hydrolysis of the substrate triglyceride which is evidence that the enzyme we are measuring in human adipose tissue is that described by others and is not some other variety of lipase(2).

*Results.* Using non-heparin stimulated human adipose tissue, we found average lipoprotein lipase activity to be approximately 600 $\gamma$ glycerol/g/hr. When heparin was added to the incubation medium the average of 8 different samples was 1418 $\gamma$ glycerol/g/hr (Table I). The range was from 1173 $\gamma$/g/hr to 1993 $\gamma$/g/hr. Average lipoprotein lipase activity for 3 patients under 15 years of age was 1310 $\gamma$ glycerol/g/hr as compared to the average of 1497 $\gamma$ glycerol/g/hr for 5 patients over 60.

*Discussion.* Using the method of direct incubation of homogenized adipose tissue, our results clearly indicate the presence of lipoprotein lipase in human subcutaneous adipose tissue. The adipose tissue samples were from patients in good nutritional state to whom carbohydrate was being given intravenously, obviating the possible depression of the enzymatic activity by carbohydrate deprivation (4).

The lipoprotein lipase activity of the 2 age groups varied only slightly, with perhaps greater activity in our older age group. The small number of subjects in each group precludes generalization regarding adipose tissue lipoprotein lipase activity and age.

It is of interest that data obtained in our early experiments with rabbit adipose tissue (using 100 mg samples) correlated well with Hollenberg's results(4). Using non-heparin stimulated, carbohydrate-fed rat adipose tissue, he obtained enzyme activities ranging from 10-20 $\mu$eq of non-esterified fatty acids (NEFA)/g/hr, with the majority between 15-20 $\mu$eq. In our experiments using non-heparin stimulated rabbit adipose tissue, we found 600 $\gamma$ glycerol/g/hr or approximately 19.5 $\mu$eq NEFA/g/hr. Furthermore, our average figure of 1418 $\gamma$ glycerol/g/hr or 46 $\mu$eq NEFA/g/hr for 8 samples of heparin stimulated human adipose tissue compares well with Hollenberg's range of 40-50 $\mu$eq NEFA/g/hr for his carbohydrate-fed, heparin stimulated rat adipose tissue samples.

Havel's finding of lipoprotein lipase activ-

TABLE I. Lipoprotein Lipase Activity in Eight Samples of Human Adipose Tissue.*

| Subjects | Surgical procedure | Age | Sex | γ glycerol per g wet tissue/hr |
|---|---|---|---|---|
| 1. R.D. | Inguinal hernia | 67 | ♂ | 1173 |
| 2. J.M. | " " | 65 | ♂ | 1993 |
| 3. S.L. | AK amputation | 63 | ♂ | 1737 |
| 4. J.K. | Epigastric hernia | 69 | ♂ | 1737 |
| 5. W.L. | Inguinal " | 73 | ♂ | 1480 |
| 6. E.B. | Umbilical " | 13 | ♀ | 1283 |
| 7. H.M. | Inguinal " | 1.3 | ♂ | 1469 |
| 8. B.W. | " " | 1 | ♂ | 1173 |

* 20 mg samples, incubated at 37°C for 40 min.

ity equivalent to 14 $\mu$M fatty acids/g/hr in a patient primed with glucose and insulin, using a direct incubation technic as well as an acetone-powder extraction method, lends support to the presence of lipoprotein lipase in human adipose tissue. Although insulin, long known to decrease the free fatty acid release from adipose tissue(11), was being given in large amounts, appreciable lipoprotein lipase activity was found in the adipose tissue of the patient.

Recently there have been two reports of virtual absence of lipoprotein lipase in human adipose tissue. Angervall(9) reported lack of activity in 3 of 4 samples and insignificant activity in the fourth. Macroscopic blood, which is known to contain inhibitors of lipoprotein lipase, was present on the tissue and might account for his findings. In the other report(8), in which acetone powder extracts from 7 human beings showed no lipoprotein lipase activity, serum was not present in 3 of the incubation mixtures. Two others showed only slight activity. The importance of serum albumin as a fatty acid acceptor in this type system was well demonstrated by Reshef, *et al.*(12). Furthermore, the apparent advantage of using whole tissue over acetone extracts, as suggested by Angervall(9), may also be responsible for his negative results.

If one accepts the importance of the role of adipose tissue as a potent repository source of energy *via* release of FFA, the demonstration in adipose tissue of an enzyme capable of hydrolyzing triglyceride and freeing fatty acid radicals is not surprising. Its exact physiological role, and especially the part played by heparin *in vivo,* are not completely clear. It seems clear, however, from our data that such an enzyme is present in human adipose tissue.

*Summary.* Subcutaneous adipose tissue was obtained from 8 patients undergoing various surgical procedures. All were in good nutritional balance. In each instance it was possible to demonstrate considerable lipolytic activity *in vitro* in the fatty tissue. It was further possible to identify this hydrolytic enzyme as lipoprotein lipase, similar in its characteristics to that previously reported in animal blood and tissue and human blood.

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## Studies on the Metabolism of 5-Hydroxytryptamine (Serotonin) II. Effect of Tryptophan Deficiency in Rats. (27520)

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During studies(1,2) on the effect of reserpine on 5-hydroxytryptamine (5-HT) metabolism in tryptophan deficient rats enough data accumulated to permit a separate presen-

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