Absence of lipemia clearing factor lipase in human adipose tissue*

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SUMMARY
Clearing factor lipase activity was present in abundance in chicken adipose tissue extracts. No such activity was demonstrable in seven of nine samples of human adipose tissue removed at surgery. Slight lipolytic activity was found in the other two instances.

Lipoprotein lipase, or lipemia clearing factor lipase (which is more accurate terminology [1]), is a heparin-activated enzyme which has been found in rat, beef, and pig heart (2). The adipose tissues of rats, rabbits, and especially chickens contain greater quantities of the enzyme (3). This latter observation led to the suggestion that the major site of lipoprotein lipase activity was in the adipose tissue, where it might function in chylomeron dissolution, and also in the lipolysis of depot fat with resultant free fatty acid release. Clearing factor lipase has also been demonstrated in rat plasma after fat feeding (4), and in the plasma of some humans (5, 6) without the prior injection of heparin, indicating a possible intravascular lipolytic role. The evidence supporting this point of view has recently been summarized (7). In view of these facts, the presence of clearing factor lipase activity in human adipose tissue was investigated.

METHODS
Chicken adipose tissue was obtained as frozen pooled fat from commercial sources. Human material was removed at surgery and immediately frozen. When larger amounts of the human fat were available, an acetone powder extract was prepared within 1 to 2 days. Smaller pieces of tissue were kept frozen for as long as several weeks until a sufficient amount had accumulated. The yield of acetone powder was approximately 200 mg/100 g adipose tissue. A total of 1.5 to 2 kg of human adipose tissue was obtained and extracted. Ammonia extracts of the tissue acetone powders were prepared as described by Korn and Quigley (3) in a concentration of 50 mg/ml. The mixtures and substrates in the different experiments varied as described in the tables. All tubes (Tables 1 and 2) contained 1.4 ml 0.25 M NH₄–NH₄Cl pH 8.5, 0.25 ml 1 M CaCl₂, 0.5 ml heparin 0.1 µg/ml. In the experiments using human tissue (Table 2), 2 ml of ammonia extract and 0.5 ml of activated coconut oil were present in all incubation vessels. “Activated” (preincubated with human serum) coconut oil (Ediol®, Schenley) was prepared as outlined by Korn (8). In this study, free fatty acid release was measured in most experiments rather than glycerol, as the former is a more sensitive indicator of partial lipolysis of triglyceride. Free fatty acids were determined by the method of Grossman et al. (9) using 1 ml aliquots of the incubation mixtures removed at times indicated in the tables.

RESULTS
The data obtained in experiments with chicken adipose tissue enzyme are shown in Table 1. The values are expressed in microequivalents per milliliter. It is evident that splitting of a simple triglyceride occurred, but lipolysis was markedly enhanced when a lipoprotein substrate, “activated” coconut oil, was used. This activity was partially inhibited by protamine and 0.9 M NaCl (column 7), which inhibit the post-heparin enzyme but have no effect upon pancreatic lipase. The extract was quite active, 100 mg of acetone powder extract resulting in free fatty acid release at a rate of 13 µeq/ml per hour.
Table 2 presents the results of studies using nine different batches of human adipose tissue extract, and the same experimental design that gave maximal lipolysis with chicken fat enzyme. No activity is demonstrable in seven experiments. There is very slight release of free fatty acids in two instances (columns 4 and 5). The maximal rate of lipolysis with 100 mg human acetone powder extract was 0.8 μeq per ml per hour. Unfortunately, an insufficient amount of this sample was available and inhibitor studies could not be done. Experiments were also performed using human adipose tissue extracts in which the pH of the buffer and the heparin content were varied, but no increase in hydrolysis occurred. Phosphate buffer (pH 7.6) and physiological saline extracts of acetone powders did not show lipolytic activity when ammonia extracts had previously been inactive. The use of human low density lipoproteins, rich in triglyceride, as fat substrate did not result in any enhancement of lipolytic activity.

**DISCUSSION**

While this study was in progress, Angervall (10) reported similar experiments in which three of four samples of human adipose tissue extract did not induce lipolysis of an "activated" cream substrate. In one instance he found lipolytic activity, but at a markedly lower rate than that present in chicken fat extracts. Our results with seven individual samples and two pooled batches of human adipose tissue, and using "activated" coconut oil and human low density lipoprotein substrate, are in essential agreement with those of Angervall. Clearing factor lipase activity is not demonstrable in human adipose tissue using an extraction technique which shows abundant enzymatic activity in chicken fat and rat heart. The trace activity we found in a few instances in human extracts has not been identified by inhibitor studies. Furthermore, it may well have come from the capillaries of the adipose tissue rather than from adipose cells themselves. Robinson (11) has presented good evidence that the tissue factor of clearing factor lipase in rabbits is present in capillary walls.

Angervall (10) suggested that the absence of the enzyme in human adipose tissue might be due to the presence of inhibitors in the activating plasma. This explanation would not appear to be valid. In the present study aliquots of the same "activated" coconut oil substrate were used in both human and chicken experiments and excellent activity was present in the latter. With three samples of human extracts no lipolysis of low density lipoproteins occurred, and in these experiments serum was not present. These lipoproteins are an excellent substrate for human clearing factor lipase found in plasma, either endogenously or after the injection of heparin. Finally, Angervall (10) stated that only 22% inhibition of chicken adipose enzyme activity was found when plasma was added.

Using a histochemical technique, Gomori (12) found evidence of lipolytic activity in human adipose tissue. Renold and Marble (13) reported lipolytic activity in extracts of human abdominal and subcutaneous fat using an unphysiologic water soluble substrate, Tween 20. However, just as much of this activity was present in rat liver and in rat pancreas. These observations suggest that the substrate they used was also split by esterases, and therefore the nature of the enzyme involved is uncertain.

A legitimate question arises since our source material...
TABLE 2. FREE FATTY ACID RELEASE* USING ACETONE POWDER EXTRACT (APE) OF HUMAN ADIPOSE TISSUE. INCUBATION AT 37°C

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* µeq/ml
† P = Pooled from several subjects.
‡ I = Fat from one individual.

was obtained from patients who had not eaten for approximately 12 hours. In rats an overnight fast resulted in a 35:1 reduction in the lipase activity of adipose tissue as compared to that of fed rats who also were given sucrose (14). Other investigators (15) have not reported so marked a change due to fasting. They found a 75% reduction in adipose tissue enzymatic activity in rats fasted for 48 hours. The quantitative difference in results may well be the result of the added sucrose. It is unlikely that the absence of clearing factor lipase activity in most of our tissue samples was due to the 12 hours' prior abstinence from food. All the patients except one individual received an intravenous infusion of 5% glucose in physiological saline throughout the operative period. This was started prior to the induction of anesthesia, usually one-half to two hours before the sample of adipose tissue was removed. Furthermore, patients in the post-absorptive state release adequate quantities of clearing factor after the injection of small doses of heparin. Similarly, the endogenous plasma enzyme has been found in some fasting subjects (16). However, in view of our findings, and those of Angervall (10), it is probable that adipose tissue makes little or no contribution to the plasma content of clearing factor lipase in man.

It may not be amiss to draw attention once again to the marked species difference that our data show. The results of experiments with rat or chicken tissues or organs are not automatically transferable to man. It does not appear likely from the studies thus far that adipose tissue clearing factor lipase plays an important role in man. These human adipose tissue findings, however, are not evidence against the probable major role of this enzyme, derived from other tissues, in lipemia clearing.

REFERENCES