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Human Adipose Tissue Lipoprotein Lipase: Comparison of Assay Methods and Expressions of Activity[1] (38526)

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Lipoprotein lipase (LPL), an important enzyme in the peripheral uptake of triglycerides (Tg) from plasma (1), has been measured in animal (2, 3), and more recently, in human (4, 5) adipose tissue. LPL from animal tissues usually has been assayed by one of two ways: as heparin releasable enzymatic activity from intact cells or as the activity in NH_4OH extracts of acetone–ether powder of adipose tissue. The former has been thought to correlate with postheparin lipolytic activity, the latter to represent the total enzymatic activity in adipose tissue (6). The purpose of the present communication is to compare these two techniques for assessment of enzymatic activity in adipose tissue of normal man. Since the degree of obesity varies considerably among humans and the LPL activity found may be dependent on the method of expression of activity, the activities were calculated per unit weight of adipose tissue and per fat cell for comparison.

Materials and Methods. Eight normal males, aged from 29–47 yr, were used in the study. Adipose tissue aspiration biopsies from subcutaneous buttock fat were performed by the technique of Hirsch *et al.* (7) after an overnight fast (12 hr). The tissue yield was usually 100–120 mg. After rinsing in approximately 100 ml of Krebs–Ringer phosphate (KRP) buffer (pH 7.4), tissue pieces were divided into three parts. One piece (5–10 mg) was used for measurement of fat cell diameter from a formaldehyde fixed, frozen section utilizing the method of Sjöström *et al.* (8). Fat cell size and the number of fat cells per gram of tissue were calculated from the fat cell diameter according to Goldrick (9). The two pieces for the LPL assays (40–50 mg each) were dried on Whatman filter paper and then weighed.

For the measurement of heparin releasable LPL, adipose tissue pieces were incubated for 45 min in a Dubnoff metabolic shaker at 37° in 2.5 ml of KRP buffer containing 2 unit/ml of heparin. Thereafter two 1.0 ml samples were taken for assay. The other tissue pieces were used to prepare an acetone–ether powder according to Nilsson-Ehle *et al.* (10). The acetone–ether powders were then homogenized in 0.4 ml of 0.05 *M* NH_4OH (pH 8.2) containing 0.15 *M* of NaCl, and the 0.4 ml samples used for assay. In separate experiments, acetone–ether powder was made also from tissue pieces, after they were first incubated with heparin and then rinsed in KRP buffer, to evaluate that amount of the total LPL activity of the tissue which is heparin releasable.

The substrate for LPL assay was prepared as follows: 100 μl of unlabeled triolein (25 mg/ml in benzene); 0.5 ml of 1-^{14}C-triolein (2 μCi/ml in benzene) and 10 μl of purified egg lecithin (12 mg/ml in chloroform–methanol, 1:1) were evaporated with nitrogen. This was then emulsified in 2 ml of a mixture of 10% fatty acid free bovine serum albumin (pH 8.0), normal human plasma, 2 *M* Tris–HCl buffer and distilled water (4:1.5:5:5:9.5) for 3 min using Branson 125 Sonifier (setting 3 and maximal tuning). The tube was kept in ice during and after sonification.

For lipoproprotein lipase assay 1.0 ml of incubation medium or 0.4 ml of NH_4OH homogenate of acetone–ether powder, and 0.2 ml of substrate were incubated for 30–60 min at 37° in Dubnoff metabolic shaker. The reaction was terminated by adding 10 ml of

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Dole's extraction mixture. One ml of KRP or 0.4 ml of NH_4OH, and 0.2 ml of substrate, to which Dole's mixture was added was used as a control blank in the assay. Free fatty acids (FFA) were extracted as previously described (11). The enzymatic activity was calculated as nanoEq FFA released/min/g of tissue or per 10^6 cells.

The coefficient of variation (replicate analysis) for the heparin releasable activity within assays was 5.9%. This calculation could not be done for the extract of the acetone–ether powders of adipose tissue since they were not done in duplicate. To account for variability between assays a postheparin pooled plasma sample was run in each assay: 10 μl of postheparin plasma and 0.2 ml of the substrate were incubated and free fatty acids extracted at the same time as the adipose tissue LPL. PHLA values usually differed less than 10%. On occasions when this was exceeded the results were corrected to the corresponding mean PHLA values.

The percent of ideal body weight was calculated from the mean ideal body weight for height obtained from the Metropolitan Life Insurance tables.

Results and Discussion. LPL activities from heparin incubated adipose tissue and acetone–ether powder of adipose tissue were expressed as activity per gram of adipose tissue or per 10^6 cells of adipose tissue (Table I). Heparin releasable enzymatic activity is about two times higher than the activity in acetone–ether powder, and there is a significant positive correlation between these activities ($r = 0.92$, $P < .01$; Fig. 1). This result would be unexpected, if LPL of acetone–ether powder represents the total enzymatic activity, and only a part of that activity can be released by heparin, as has been previously suggested (6). However, in studies with rat adipose tissue (11, 12), these two enzymatic activities were similar, and in the study of Stewart and Schotz (13), the "total" LPL activity of rat adipose tissue (measured from buffer homogenate) was only one fifth of that activity released into the medium with heparin. One explanation for this apparent discrepancy could be the activation of LPL during release process (13), so that the activity in the heparin extract may be higher even if only a part of the originally inactive enzyme is released. On the other hand, only a part of the total LPL activity of acetone–ether powder might be extractable with the conventional methods (14). That one of the above possibilities exists, is supported by the results of the present study: Of the original LPL activity 44–70% remained in acetone powder of adipose tissue after preincubation with heparin (Table II). These results fit quite well with studies in animals, in which adipose tissue LPL activity decreased 20–30% (6) or 70–80% (15), after *in vivo* heparin injection. However, such studies should be interpreted cautiously, because even after rinsing, some LPL activity released and activated by heparin may be left in the tissue.

The subjects used in this study were selected to provide a wide range of adult-

TABLE I. ADIPOSE TISSUE LPL IN EIGHT NORMAL SUBJECTS.[a]

Subject	Heparin releasable LPL		LPL of acetone–ether powder	
	g	10^6 cells	g	10^6 cells
E.B.	16.5	7.9	9.8	4.7
J.B.	11.2	4.7	3.9	1.6
R.C.	5.4	1.8	2.2	0.8
G.L.	5.1	2.8	2.1	1.2
O.P.	3.4	1.4	1.7	0.7
P.R.	8.6	3.2	4.2	1.6
P.S.	6.3	4.0	3.6	2.3
S.W.	5.2	1.6	1.3	0.4
Mean ± SD	7.7 ± 4.0	3.4 ± 2.0	3.6 ± 2.5	1.7 ± 1.3

[a] The activities are expressed as nanoeq FFA liberated/min/g of adipose tissue or 10^6 fat cells.

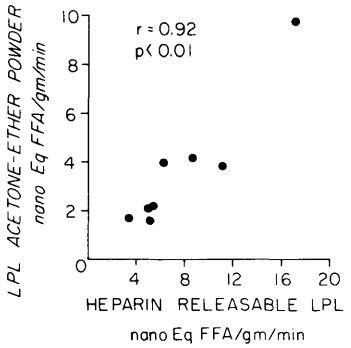

FIG. 1. Relationship between heparin releasable LPL and acetone–ether powder LPL in human adipose tissue.

TABLE II. THE LPL ACTIVITY IN ACETONE–ETHER POWDER BEFORE AND AFTER INCUBATION OF TISSUE PIECES WITH HEPARIN.[a]

	A	B	
Subject No.	LPL in acetone powder	LPL in acetone powder after heparin extraction	B/A × 100
1	2.12	1.37	64.6%
2	3.03	2.12	69.9%
3	2.32	1.01	43.5%
4	2.35	2.17	64.7%

[a] The activities are expressed as nanoEq FFA/min/gm of adipose tissue.

onset obesity. It has been suggested that in this type of obesity fat cell number approximates normal while fat cell size is increased (16, 17). The results of this study support this concept, since there is a positive correlation between obesity (expressed as percent ideal body weight) and fat cell diameter ($r = 0.91$, $P < .01$; Fig. 2A) and an inverse correlation between obesity and fat cell number per unit weight of adipose tissue ($r = -0.89$, $P < .01$; Fig. 2B). Thus, the LPL activity per unit weight and per fat cell would be quite different among these subjects; the more obese the person is, the lower the enzymatic activity per unit weight of tissue as compared to the activity per fat cell.

The results of this investigation demonstrate that the method of expression of the LPL activity may be important in comparative clinical studies. Many diseases, e.g., diabetes and endogenous hypertriglyceridemia, are frequently associated with obesity. If subjects are not weight-matched, low enzymatic activity per unit weight may then lead to an erroneous conclusion even though LPL activity per total body fat would be normal. Since fat cell size, but not fat cell number, increases in adult-onset obesity, the ratio between total body fat and fat cell size remains the same. Therefore, if LPL activity is expressed per fat cell, the influence of obesity can be avoided.

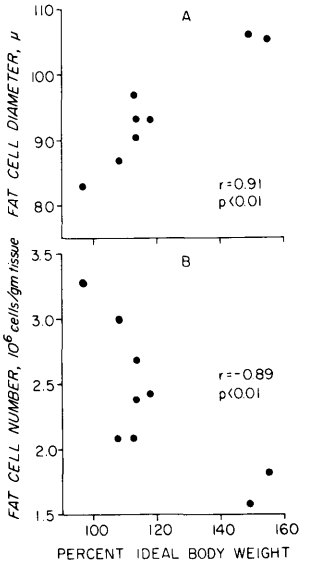

FIG. 2. Relationship between ideal body weight and fat cell diameter (A) and fat cell number per gram of adipose tissue (B) in normal subjects.

Summary. There was a positive correlation in normal man between heparin releasable lipoprotein lipase and lipoprotein lipase of ammonium hydroxide homogenate of acetone–ether powder in adipose tissue. Heparin releasable as lipoprotein lipase activity was about twice as high as the enzymatic activity in acetone powder, even though 40–70% of the original activity remained in the tissue after incubation with heparin. This might indicate that activation of the enzyme is associated with its release by heparin from tissue. The lipoprotein lipase activity per unit weight and per fat cell were affected differently by obesity: In obese subjects lipoprotein lipase per unit weight was proportionally lower than the activity per fat cell. The expression of

activity per fat cell appears to avoid the effect of obesity, and hence increased fat cell size, on values obtained.

1. Garfinkel, A. S., Baker, N., and Schotz, M. C., J. Lipid Res. **8,** 274 (1967).
2. Korn, E. D., and Quigley, T. W., Biochim. Biophys. Acta **18,** 143 (1955).
3. Cherkes, A., and Gordon, R. S., Jr., J. Lipid Res. **1,** 97 (1959).
4. Nestel, P., and Havel, R. J., Proc. Soc. Exp. Biol. Med. **109,** 985 (1962).
5. Persson, B., Björntorp, P., and Hood, B., Metabolism **15,** 730 (1966).
6. Robinson, D. S., Adv. Lipid Res. **1,** 133 (1963).
7. Hirsch, J., and Goldrick, R. B., J. Clin. Invest. **43,** 1776 (1964).
8. Sjöström, L., Björntorp, P., and Vra'na, J., J. Lipid Res. **12,** 521 (1971).
9. Goldrick, R. B., Amer. J. Physiol. **212,** 777 (1967).
10. Nilsson-Ehle, P., Tornquist, H., and Belfrage, P., Clin. Chim. Acta **42,** 383 (1972).
11. Pykälistö, O., Vogel, W. C., and Bierman, E. L., Biochim. Biophys. Acta, **369,** 254 (1974).
12. Brady, M., and Higgins, J. A., Biochim. Biophys. Acta **137,** 140 (1967).
13. Stewart, E. J., and Schotz, M. C., J. Biol. Chem. **249,** 904 (1974).
14. Bensedaun, A., Ehnholm, C., Steinberg, D., and Brown, W. V., J. Biol. Chem. **249,** 2220 (1974).
15. Payza, A. N., Eiber, H. B., and Walters, S., Proc. Soc. Exp. Biol. Med. **125,** 188 (1967).
16. Salans, L. B., Cushman, S. W., and Weisman, R. E., J. Clin. Invest. **52,** 929 (1973).
17. Sjöström, L., and Björntorp, P., Acta Med. Scand. **195,** 201 (1974).

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