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Lipoprotein-lipase Activity in Subcutaneous, Adipose Tissue in Healthy Subjects

Variation of Activity in a Population of 60-year-old Men

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ABSTRACT

The lipoprotein-lipase activity (LPLA) in the abdominal, subcutaneous, adipose tissue was studied in a random sample (n=69) of 60-year-old men. A new method for the quantification of LPLA was applied. The mean value was 67 mU/g when expressed per gram (wet weight) of adipose tissue. Several subjects within the lower part of the range of adipose-tissue LPLA values had low concentrations of serum-triglycerides (S-TG). There was no correlation between the LPLA and S-TG concentrations in the fasting state. Among the 69 subjects, four had newly detected diabetes mellitus and had significantly lower LPLA in the adipose tissue than the control group. The fat-cell size and the LPLA per gram of adipose tissue were not correlated. Thus, obesity without diabetes mellitus does not imply a low LPLA concentration in adipose tissue. The variation of the concentration of adipose-tissue LPLA in the fasting state in this population was explained only to a minor extent by the variation of S-insulin and blood-glucose parameters, when analysed statistically by a stepwise multiple-regression technique.

INTRODUCTION

Lipoprotein lipase (LPL) is an enzyme in extrahepatic tissues that hydrolyses the triglycerides (TG) of serum (S-) chylomicrons and very-low-density lipoproteins (VLDL) to partial glycerides, glycerol and free fatty acids. The fatty acids pass the cell membranes and are either oxidized or stored as resynthesized TG (22).

The main function of adipose tissue is the storage of TG in the adipocytes. Consistent over-consumption of food leads to the enlargement of the adipocytes (hypertrophic obesity (3)). Enlarged adipocytes contain more lipoprotein-lipase activity (LPLA) than small adipocytes (21). Furthermore, a positive correlation has been reported (12) between fat-cell size and LPLA per gram of adipose tissue.

Several factors associated with food consumption are known to influence the concentration of LPLA. Human-adipose-tissue LPLA increased significantly in healthy control subjects when they were put on a carbohydrate-rich, formula diet (21). This increase of LPLA through the diet was related to the changes in insulin concentration. The decreased, fasting concentration of adipose-tissue LPLA that has been reported in diabetes mellitus (12, 18, 21) indicates that insulin is involved in the maintenance of a "normal", fasting, LPLA concentration in adipose tissue. The S-insulin concentration thus seems to be an important factor in the regulation of adipose-tissue LPLA (21).

In evaluating the results of clinical studies, e.g. on adipose-tissue LPLA and its relationship to the S-TG concentration, it must be considered that alcohol intake may be associated with high LPLA of adipose tissue (2), sometimes in combination with hypertriglyceridemia (HTG) (17).

In this study, the LPLA of abdominal, subcutaneous, adipose tissue was determined by a method, recently described (14). Needle-biopsy specimens of adipose tissue were taken in a random subsample of apparently healthy, 60-year-old men. The influence of fat-cell size, previous alcohol intake, and S-insulin and blood-(B-) glucose concentrations on the concentration of LPLA and the relationship between the LPLA concentration in adipose tissue and the S-TG concentration were studied.

MATERIALS AND METHODS

Population

All the men born in 1915 and living in Uppsala, Sweden, were invited to a health-screening investigation (29) during the period August–November 1975. Three hundred and thirty-one individuals attended the investigation (par-

ticipation rate 78.4%). An extended survey, including adipose-tissue LPLA determination, was made of a randomly chosen subsample ($n=69$). All these individuals stated that they had no disease and were not receiving pharmaceutical treatment or keeping dietary prescriptions.

Experimental design

The examination took place in the morning after an overnight fast. Smoking had not been allowed since the night before. A needle-biopsy specimen (9) of subcutaneous, adipose tissue was taken after 15 min rest in a supine position. Venous-blood samples were drawn through an indwelling catheter for the determination of S-lipoprotein, S-TG and S-cholesterol (chol) concentrations, fasting (f) B-glucose and fS-insulin concentrations, and S-γ-glutamyltransferase (GT). An injection of glucose, 0.5 g/kg body weight, was then given for 2 1/2 min. Blood samples were taken 4 and 6 min after the injection started for the determination of S-insulin concentrations. Blood-glucose concentrations were determined in samples taken 20, 30, 40, 50 and 60 min after the injection.

Methods

The adipose-tissue-biopsy specimen was washed in physiological saline, blotted on filter paper and divided into four portions weighing 10–30 mg each. Three of these were stored in plastic ampoules in liquid nitrogen until analysed for LPLA. One portion was frozen at −20°C and the fat-cell size was determined (25). The method of determining adipose-tissue LPLA has previously been described in detail (14). The reaction medium consisted of a glycine buffer (2.1 mol/l, pH 8.3, [I]=0.08), albumin (0.11 mmol/l), heparin (1 g/l), serum diluted 1 : 120 and a triglyceride emulsion with a final TG concentration of 1.2 mmol/l. The final incubation volume was 0.6 ml. A stock emulsion had been produced by Vitrum AB, Stockholm, Sweden, in the same way as Intralipid®. Trace amounts of ^{3}H-trioleate (NEN, USA) had been added to the soybean oil before emulsification to a specific radio-activity of 13 Ci/mol TG, using egg lecithin as an emulsifier. The biopsy specimen was weighed in frozen condition, put into the reaction medium and after an equilibration the linear release of fatty acids was determined. The radio-active, fatty acids released were measured by liquid scintillation counting and the total release of fatty acids per minute was calculated and related to the wet weight of the tissue. One enzyme unit (U) was defined as the release of 1 μmol of fatty acid per minute. LPLA was expressed in milliunits (mU) per gram of adipose tissue or per 10^6 adipocytes. The activity per 10^6 cells was calculated by multiplying the activity per gram by the cell weight (21).

TG and chol concentrations were determined (24) in whole serum and in the top (corresponding to VLDL) and bottom fraction at density=1.006 after preparative ultracentrifugation. The low-density lipoproteins (LDL) were precipitated from the bottom fraction at density=1.006, using a heparin-manganese chloride solution and the lipid concentrations were determined in the supernatant corresponding to high-density lipoproteins. Hyperlipoproteinemias were typed according to the WHO recommendation (1). The cut-off point for the VLDL-TG concentration was 1.4 mmol/l and for the LDL-chol concentration 5.2 mmol/l. These cut-off points represent the upper 85th percentile in a local control material of healthy men (6). B-glucose concentrations were analysed by a glucose-oxidase method (10) and the elimination-rate constant (K-value) for B-glucose during the i.v., glucose-tolerance test (IVGTT) was calculated by the least-squares method (11). Serum-insulin concentrations were analysed by the Phadebas Insulin Test (Pharmacia, Uppsala, Sweden). The mean value of the S-insulin concentrations at 4 and 6 min was called "peak insulin". The ratio between the peak insulin and the fS-insulin concentrations was called the "insulin index" (4, 30).

Serum GT was analysed mainly according to Szasz, but the 3-carboxylated, L-γ-Glutamyl-4-nitroanilide was used as substrate (27). Concentrations above 0.5 μkat/l were regarded as elevated (13).

Statistics

All results were computerized. Statistical calculations were made according to Snedecor and Cochran (26). Mean values were calculated after logarithmic transformation of the data when the distribution of measurements was significantly skewed to the right. The coefficients of correlation were calculated on log transformed data and after pairwise elimination of missing data. In five individuals, the tissue sample was not large enough to permit cell-size determination. In two subjects, the K-value, and the lipoprotein concentrations and in three subjects, the S-insulin concentrations were not determined. A stepwise, multiple-regression analysis was performed, entering variables according to their degrees of partial correlation (7).

Comparison between groups of unequal size was made with the Wilcoxon-White, rank-sum test and the probability that the groups were of different populations has been indicated.

RESULTS

Characteristics of the population

The mean concentration of LPLA in adipose tissue was 67.4 mU/g, with a range of measurements from 14.7 to 195.9 mU/g (Table I). The distribution of the measurements of LPLA per cell was significantly skewed to the right and the mean value was 13.3 mU/10^6 cells (range 2.1–101.5 mU/10^6 cells) (Table I).

The mean value, SD and range for cell weight, body weight, fS-TG and VLDL-TG concentrations, fB-glucose concentration, K-value, fS-insulin and peak S-insulin concentrations, insulin index and S-GT concentration are presented in Table I.

The distribution of the S-VLDL-TG concentrations was significantly skewed to the right and the mean value was 0.76 mmol/l (range 0.17–3.93 mmol/l). Eight individuals had increased concentrations of S-VLDL-TG (=hypertriglyceridemia=HTG), two as type IV hyperlipoproteinemia (HLP),

Table I. *Characteristics of the population—60-year-old men*

Number of observations (n), mean value and S.D., t-value for test of skewness of the distribution with level of significance ($2p$) indicated, antilog value for the mean value calculated on logarithmically transformed data and range of distribution are given for the parameters in the table. HAT-LPLA=human-adipose-tissue lipoprotein-lipase activity, VLDL=very-low-density lipoprotein, GT=glutamyl-transferase

	n	Mean value	S.D.	Skewness	$2p$	Mean value antilog	Range
HAT-LPLA, mU/g	69	67.4	41.6	1.22	n.s.		14.7–195.9
HAT-LPLA, mU/10^6 cells	64	16.8	14.3	3.54	<0.001	13.3	2.1–101.5
Cell weight, μg	64	0.27	0.19	0.17	n.s.		0.06–0.56
Body weight, kg	69	77.7	11.7	0.62	n.s.		51.0–109.0
fS-triglycerides, mmol/l	69	1.51	0.89	2.30	<0.025	1.31	0.30–5.80
f-VLDL-triglycerides, mmol/l	67	0.91	0.60	2.25	<0.05	0.76	0.17–3.93
fB-glucose, mmol/l	69	5.5	1.0	2.41	<0.020	5.4	4.3–9.8
K-value	67	1.58	0.48	0.11	n.s.		0.61–2.65
fS-insulin, mU/l	66	13.0	7.3	1.39	n.s.		4.0–37.0
Peak insulin, mU/l	66	59.4	29.4	0.77	n.s.		8.5–145.0
Insulin index	66	5.5	3.0	0.90	n.s.		0.9–15.0
S-GT, μkat/l	69	0.33	0.33	3.99	<0.001	0.25	0.08–2.23

four as type IIB HLP and two as type III HLP. Increased LDL-chol concentrations were found in the four individuals with type IIB and in five patients with type II A HLP. The LDL-chol concentration ranged from 2.33 to 6.55 mmol/l with a mean value of 4.23 mmol/l.

The distribution of the fB-glucose measurements was significantly skewed to the right and the mean value was 5.4 mmol/l (range 4.3–9.8 mmol/l). Four individuals had B-glucose concentrations of 7.2, 7.5, 9.4 and 9.8 mmol/l in combination with K-values below 0.9. These individuals were regarded as patients with newly detected diabetes mellitus (DM).

The distribution of the S-GT concentrations was significantly skewed to the right and the mean value was 0.25 μkat/l (range 0.08–2.23 μkat/l). Six individuals had increased concentrations of S-GT.

Concentration of adipose-tissue LPLA in subjects with DM, HTG or increased S-GT concentration

The mean values of adipose-tissue LPLA per gram and per cell in the three groups with HTG, DM and increased S-GT concentration are presented in Table II. In addition, the mean value in the group of individuals who had "normal" concentrations of S-VLDL-TG, fB-glucose and S-GT is presented as the control group. It is evident from Table II that the DM group (with a mean value of 24 mU/g) was significantly different from the control group (mean value 71 mU/g). Neither the group with HTG (mean value 46 mU/g) nor the group with increased S-GT concentrations (mean value 103 mU/g) were significantly different from the control group. None of the subgroups (with HTG, DM or increased S-GT concentrations) was significantly different from the control group when tested with regard to the adipose-tissue LPLA concentration per cell (Table II).

Table II. *Concentrations of adipose-tissue LPLA in controls and patients with HTG, DM and high S-GT*

A. Human-adipose-tissue (HAT-) lipoprotein-lipase activity (LPLA), mU/g. Mean value ($\bar{x}$) and S.D. and mean value ($\bar{x}_{log}$) when calculated on logarithmically transformed data are given for the control group and three subgroups in the study: HTG=fS-VLDL-TG >1.4 mmol/l, DM=fB-glucose >7 mmol/l and K-value <0.9, high S-GT=S-GT >0.5 μkat/l. Number of subjects in the subgroup=n. The statistical significance that the subgroups were different from the control group was tested by the Wilcoxon-White, rank-sum test and the level of significance ($2p$) is indicated in the table. B. Human-adipose-tissue (HAT-) lipoprotein-lipase activity (LPLA), mU/10^6 cells. Data presented as under A

		Controls	HTG	DM	High S-GT
A. HAT-LPLA, mU/g	n	51	8	4	6
	$\bar{x}$	71.2	45.6	24.4	103.3
	S.D.	41.9	11.5	9.3	47.2
	$2p$	–	n.s.	<0.01	n.s.
B. HAT-LPLA, mU/10^6 cells	n	46	8	4	6
	$\bar{x}$	15.5	13.6	8.1	36.2
	$\bar{x}_{antilog}$	12.7	13.0	7.8	18.2
	S.D.	9.6	4.6	2.2	34.7
	$2p$	–	n.s.	n.s.	n.s.

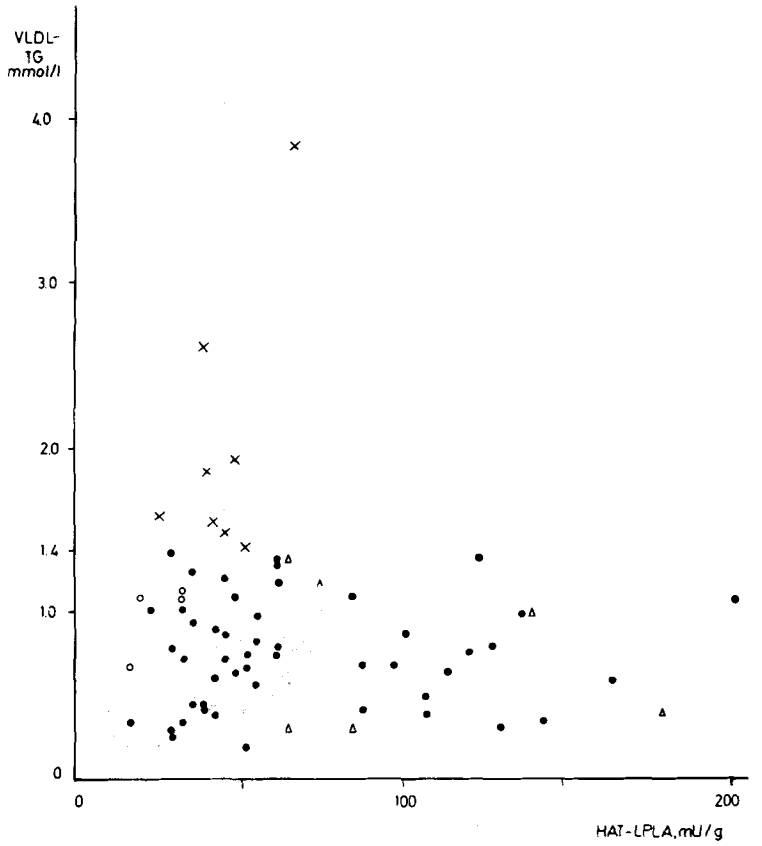

Fig. 1. Relationship between human-adipose-tissue (HAT-) lipoprotein-lipase activity (LPLA) (mU/g) and very-low-density-lipoprotein (VLDL), triglyceride (TG) concentration (mmol/l). Four subgroups have been indicated by the following symbols: S-VLDL-TG> 1.4 mmol/l (×), fB-glucose>7 mmol/l and K-value<0.9 (○), S-glutamyltransferase>0.5 μkat/l (△), controls (●).

Relationship between the fasting concentrations of adipose-tissue LPLA and S-VLDL-TG

There was no significant correlation between the adipose-tissue LPLA per gram and the S-VLDL-TG concentration ($r=-0.10$), nor between the adipose-tissue LPLA per cell and the S-VLDL-TG concentration ($r=-0.08$).

The relationship between the adipose-tissue LPLA per gram and the S-VLDL-TG concentration is shown in Fig. 1. As is evident from the figure, several subjects had low, fasting, VLDL-TG concentrations in combination with low adipose-tissue LPLAs per gram. On the other hand, high adipose-tissue LPLAs and high VLDL-TG concentrations did not co-exist in this random population.

Relationship between measurements of obesity and concentration of adipose-tissue LPLA

There was no significant correlation between body weight and adipose-tissue LPLA per gram ($r=-0.16$) or between cell weight and adipose-tissue LPLA per gram ($r=-0.15$). As is evident from Fig. 2, both subjects with diabetes mellitus and subjects with increased S-GT had large fat cells but low and high adipose-tissue LPLA concentrations respectively. There was still no significant correlation ($r=-0.23$) between cell size and adipose-tissue LPLA per gram when only the results of the control group were considered.

On the other hand, there was a significant correlation between cell weight and adipose-tissue LPLA per cell ($r=0.57$, $2p<0.001$). As is evident from Fig. 3, one individual had an extremely high adipose-tissue LPLA concentration per cell (102 mU/10^6 cells). This individual had both a large cell weight and a high adipose-tissue LPLA/g and belonged to the subgroup with increased S-GT concentrations. However, the correlation between cell weight and adipose-tissue LPLA per cell was significant also in the group of controls ($r=0.41$, $2p<0.01$).

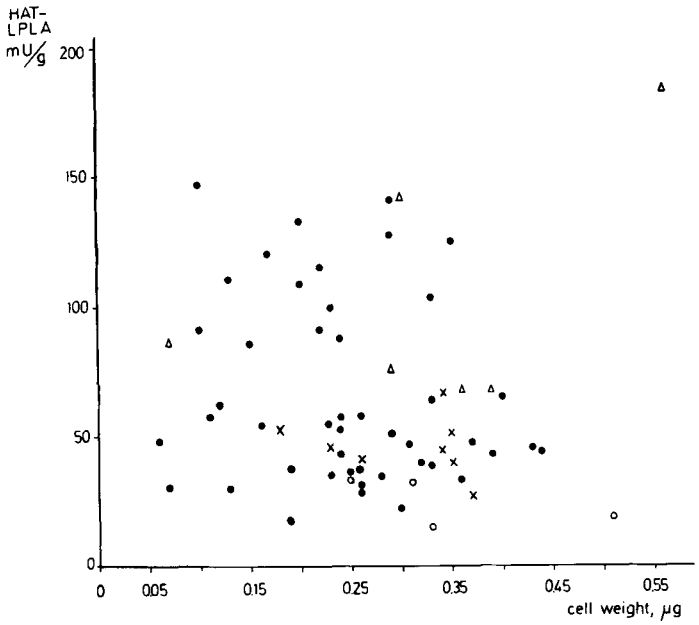

Fig. 2. Relationship between fat-cell weight (µg) and human-adipose-tissue (HAT) lipoprotein-lipase activity (LPLA), mU/g. Results for individuals belonging to the four subgroups indicated in Figure 1.

The variation of the fasting, adipose-tissue LPLA concentration and its regulation

There was a significant, inverse correlation between the fB-glucose concentration and the adipose-tissue LPLA concentration ($r=-0.27$, $2p<0.05$) mainly due to the four individuals with increased fB-glucose concentrations. No significant correlation existed after the exclusion of these four individuals. No significant correlations were found between the K-value of the IVGTT, fS-insulin concentration, peak S-insulin concentration, insulin index or S-GT concentration respectively, on the one hand, and the adipose-tissue LPLA per gram, on the other. The variations of fB-glucose, K-value, S-GT and insulin index were found to explain only about 11% ($R^2=11.1$) of the variation of adipose-tissue LPLA concentration per gram when analysed by the stepwise, multiple-regression technique.

The fS-insulin concentration was significantly correlated to the amount of adipose-tissue LPLA per cell ($r=0.25$, $2p<0.05$).

Serum GT and adipose-tissue LPLA per cell were significantly correlated ($r=0.39$, $2p<0.01$). The latter correlation disappeared if individuals with increased S-GT concentrations were excluded.

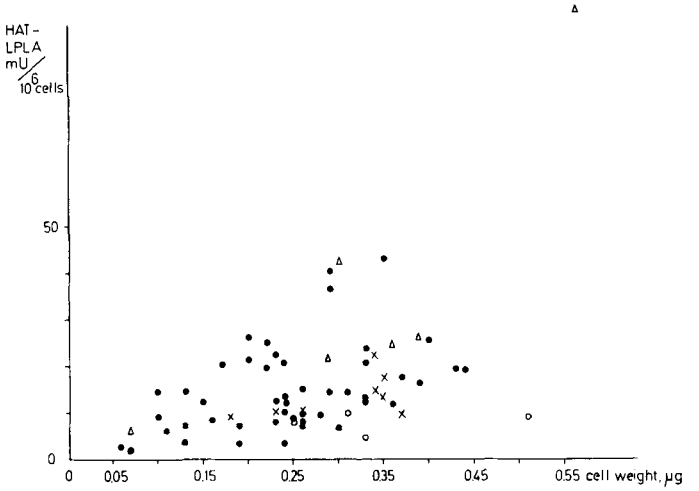

Fig. 3. Relationship between fat-cell weight (µg) and human-adipose-tissue (HAT) lipoprotein-lipase activity (LPLA), $mU/10^6$ cells. Results for individuals belonging to the four subgroups indicated in Figure 1.

DISCUSSION

Many authors have earlier presented "normal values" of adipose-tissue LPLA (12, 16, 18, 21). These values range from 6 to 100, expressed as mU/g (21, 18). Several factors may have contributed to this large variation, some of them probably methodological. In the present method, a reaction medium based on a glycine buffer and containing a high concentration of heparin was used. In this system, values of LPLA about six times higher were obtained than in a reaction medium based on a Tris buffer (14). The technique used for the preparation of the emulsion influences the results of the enzyme determinations. The same homogenisation technique was used for the preparation of our emulsion as for Intralipid® (Vitrum, Sweden). A comparison was made between an emulsion produced by this technique and an emulsion homogenized by ultrasonication in a standardized manner, using the same batch of egg phospholipid, soybean oil and ^{3}H-triolein. (This ultrasonicated emulsion was kindly made by Drs P. Belfrage and H. Tornqvist, of Lund, Sweden. The emulsion was transported by air and used about four hours after sonication.) The values of adipose-tissue LPLA obtained with the ultrasonicated emulsion were about 1.5 times higher than with the other emulsion (unpublished results). The composition of the emulsion, with regard to both the triglyceride fatty acids and the emulsifier, is a factor which influences the results of the LPLA measurements (20). Analysis of adipose-tissue LPLA yielded values 5–7 times higher with Ediol® as substrate than with ultrasonicated, triolein emulsion (20). Persson used Ediol as substrate, which may explain the high values of adipose-tissue LPLA reported by him (18). The conditions of incubation and the type of emulsion are probably the main reasons for the higher values of adipose-tissue LPLA (about 60 mU/g) in the present study than, for example, the values reported by Pykälistö et al. (about 6 mU/g (21)).

In earlier studies of adipose-tissue LPLA, biopsy specimens from the gluteal site have been used (12, 16, 17, 18, 19, 21) but not adipose tissue from the abdominal site as in the present study. The choice of site for tissue-sampling may also contribute to the different results, as regards the adipose-tissue LPLA concentration in control groups. In a study of obese women (who had been fasting overnight), we found that adipose tissue from the abdominal site had a lower LPLA concentration per gram than adipose tissue from the arm, the buttock or the leg (to be published). However, there was a good correlation between the activities of the different sites, so that, when the activity was low in tissue from the abdominal site, it was low also in tissue from the other sites in that individual. This means that in the fasting state any subcutaneous site may be used, but that the tissue specimens should always be taken from the same site.

In the present study, no correlation could be found between the fasting concentrations of adipose-tissue LPLA and S-VLDL-TG since a low LPLA per gram was found in subjects with low S-VLDL-TG concentrations (Fig. 2). These findings confirm previous findings in a group of normotriglyceridemic, 50-year-old men (15). They are in accordance with findings by Pykälistö et al. (21) but in contrast to findings by Persson (18). He found a significant, inverse correlation between the adipose-tissue LPLA per gram and the S-TG concentration in males with normal S-TG values. However, the partial-correlation coefficient between the adipose-tissue LPLA per gram and the S-TG concentration was not significant when other factors that influenced the relationship were kept constant (22).

The level of S-GT concentration is correlated to the previous alcohol intake (23). The discriminating capability of the S-GT concentration as an indicator of habitual alcohol consumption was evaluated in this health survey of 60-year-old men and a preliminary report has been given (28). Among the 69 individuals in the present study, six had increased S-GT. They all had adipose-tissue LPLA concentrations in the upper half of the distribution of adipose-tissue LPLA measurements. This is in accordance with earlier studies (2), in which a daily intake of alcohol was found to be accompanied by an increase of the adipose-tissue LPLA concentration.

Four patients with newly detected diabetes mellitus had a significantly lower, mean adipose tissue LPLA concentration than the control group, a finding which is in good agreement with earlier results by others (12, 19, 21). On the other hand, our study indicates that the variation of fB-glucose within normal ranges and the variations of the fS-insulin concentration and the insulin index do not explain more than a minor part of the variations of the fasting value of adipose-tissue LPLA per gram found in a random population sample. This may mean that the S-insulin variations in the fed state are impor-

tant for an adequate increase of adipose-tissue LPLA but that the fasting adipose-tissue LPLA measured per gram is not mainly determined by the S-insulin or B-glucose concentrations. The effect of S-insulin concentrations on the fasting adipose-tissue LPLA becomes evident only in the insulin-deprived state of diabetes mellitus.

The present study did not show any correlation between cell size and adipose-tissue LPLA per gram. This is in accordance with our findings in another study of obese women (to be published). Results different from ours have been reported earlier. Jaillard et al. (12) found a significant correlation between cell size and adipose-tissue LPLA per gram. That study, however, included patients with both juvenile and adult types of diabetes mellitus, which may explain the results. Persson (18) found a significant, inverse correlation between a body-weight index and the adipose-tissue LPLA per gram. That study was not carried out on a random population and the calculations were made on results obtained during a six-year period. With regard to B-glucose concentration, only patients with fB-glucose concentrations above 8.3 mmol/l were excluded in that study. These factors may explain the different results in the two studies. Our finding that there was no correlation between cell size and adipose-tissue LPLA per gram may be interpreted as showing a tendency of the tissue in healthy individuals to compensate for hypertrophy of the fat cells by increasing the amount of LPLA per cell so much that the concentration per gram is kept constant. An important factor in this compensatory mechanism may be the increased fS-insulin concentration in cases of obesity, as the fS-insulin concentration was significantly correlated to the amount of adipose-tissue LPLA per cell.

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