Effect of cell size on lipid synthesis by human adipose tissue in vitro

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ABSTRACT When adipose tissue cells were incubated with collagenase for different periods of time, cell populations with different mean cell sizes were obtained from the same tissue sample. Lipid synthesis from glucose was studied as a function of adipose cell size and number. The incubations were performed in Parker medium 199, which is suitable for tissue culture of human adipose tissue. The results show that the larger cells of a specimen have a greater rate of lipid synthesis than the smaller cells of the same specimen. This is mainly due to an increase in the synthesis of glyceride-glycerol. Addition of insulin stimulated lipid synthesis. However, the larger adipose cells were less sensitive to the stimulating effect of insulin than the smaller cells.

SUPPLEMENTARY KEY WORDS adipocyte size · adipocyte number · cell fractions · glucose incorporation · insulin

Several investigations (1, 2) have shown that increasing adiposity is associated with an increase in the adipose cell size. Recent studies indicate that there are metabolic differences between adipose cells of different sizes. Salans, Knittle, and Hirsch (3) found that large adipose cells were less sensitive than small cells to the stimulating effect of insulin on the oxidation of glucose to CO_2. Nestel, Austin, and Foxman (4) reported that large adipose cells were less responsive to the stimulating effect of insulin on the uptake of labeled triglyceride fatty acids.

The morphologic changes of human subcutaneous adipose tissue maintained in vitro for prolonged periods of time have been studied (5). During an observation period of 14 days it was found that large adipose cells had a greater tendency to decrease in size than small cells. Metabolic differences may be a reason for this finding.

This paper describes a method to obtain cell populations with different mean cell sizes from the same tissue sample. Incorporation of glucose into the lipids was studied as a function of adipose cell size and number.

MATERIALS AND METHODS

Source of Tissue

Biopsies of subcutaneous adipose tissue were obtained in connection with operations on patients with cholecystolithiasis or on patients undergoing exploratory laparotomy. Patients with diabetes mellitus, jaundice, or malignant disease were excluded. All patients were fasted overnight. Anesthesia was induced with a short-acting barbiturate and maintained with halothane, nitrous oxide, and oxygen. The biopsies were usually obtained at the beginning of the operations. The specimens used for metabolic studies were obtained from 11 patients. The age, sex, height, and weight of these patients are shown in Table 1. Immediately after excision the biopsy specimens were placed in a vessel containing 20 ml of Parker medium 199 modified to a glucose concentration of 1.0 mM (Statens Bakteriologiska Laboratorium, Stockholm, Sweden; for composition see Ref. 6).

Tissue Preparation and Cell Isolation Technique (Method I)

In order to determine the mean cell size of the biopsies the following procedure was used. Smaller specimens, weighing about 20–30 mg each, were dissected from different parts of the biopsies. The fat cells were isolated from the stroma by incubating the specimens with collagenase (Sigma type 1, Sigma Chemical Co., St. Louis, Mo.) for 60 min according to the method introduced by Rodbell (7), and using the modifications described by Martinsson (8). The diameters of 100 cells were mea-

TABLE 1 DATA OF PATIENTS EMPLOYED IN THE METABOLIC STUDIES

Patient	Age	Sex	Height	Weight
	yr		*cm*	*kg*
1	71	F	177	73.0
2	40	M	181	65.0
3	45	M	186	88.0
4	60	F	165	64.0
5	56	M	182	102.5
6	60	M	182	73.0
7	75	M	178	70.0
8	73	F	163	83.0
9	37	M	182	84.5
10	67	M	163	72.5
11	71	F	154	62.5

sured with a calibrated ocular in a Zeiss photomicroscope and the mean cell size was determined. The coefficient of variation calculated from ten consecutive determinations of the mean cell size was 3.0% (5).

Tissue Preparation and Cell Isolation Technique (Method II)

Another procedure was used to obtain cell populations with different mean cell sizes from the same tissue sample. About 4–5 lobules were excised as completely as possible from the biopsies. Smaller specimens, weighing about 20–30 mg each, were carefully dissected from the outer cell layers of the lobules. The specimens were then incubated at 37°C in a siliconized glass vial containing 5 mg collagenase (Sigma type 1, Sigma Chemical Co.) dissolved in 3 ml Krebs-Ringer bicarbonate buffer, pH 7.4, with albumin added at a concentration of 4% (w/v). After 5 min the specimens were carefully stirred with a plastic spatula in order to dislodge the fat cells. The specimens were then placed in other vials containing collagenase and buffer as described above and incubated for another 55 min, i.e., the total incubation time was 60 min. (The populations of adipose cells freed by incubating specimens with collagenase for 5 min and 60 min are henceforth referred to as 5-min fractions and 60-min fractions, respectively.) The adipose cells had then been almost completely dislodged. The remaining stroma was removed with a plastic spatula. The mean cell sizes of the cell fractions were determined with a calibrated ocular as described above. In four experiments cell fractions were collected after 5, 10, 15, 30, 45, and 60 min and the mean cell sizes were determined.

The cell diameters were determined on cells isolated in Krebs-Ringer bicarbonate buffer. As controls, the diameters were determined on cells isolated in the modified Parker medium 199. Since the same results were obtained, the less expensive bicarbonate buffer was routinely used.

Assay Procedures with Cells

In 11 sets of experiments, prior to the isolation procedure described above, specimens obtained from the outer cell layers of the lobules were incubated in 2 ml of the modified Parker medium 199 with the addition of 0.15 μCi of glucose-1-^{14}C (Radiochemical Centre, Amersham, England) per 2 ml medium. In each set of experiments insulin (recrystallized pork insulin, Vitrum AB, Stockholm, Sweden) was added to the medium in two vials at a concentration of 0.1 IU/ml and two vials were without insulin. Equivalent amounts of tissue, between 300–500 mg in weight, were added to each vial. The vials were incubated for 2 hr at 37°C and at pH 7.4 ± 0.2; the gas phase was air. After incubation with labeled glucose the specimens were washed with warm physiological saline and then incubated with collagenase to obtain the 5-min and 60-min fractions as described above. In order to maintain equal times of exposure to collagenase the cells of the 5-min fractions were left in the collagenase-containing buffer until the 60-min fractions had been collected. Control experiments have shown that the mean cell size was not significantly changed by this procedure.

Lipids were extracted with chloroform–methanol 2:1 (v/v) as described by Folch, Lees, and Sloane Stanley (9). Aliquots of the chloroform phase were evaporated to dryness and 10 ml of scintillation fluid (0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene dissolved in toluene) was added. The radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer. Quenching was corrected for by means of internal standardization. Aliquots of the chloroform phase were taken for determination of the glyceride-glycerol according to the method described by Carlson (10). After saponification and acidification the fatty acids were extracted with *n*-heptane and the radioactivity was determined as described above.

Determination of Fat Cell Volume and Number

The mean cellular volume was calculated on the assumption that the cells were spherical and the formula suggested by Goldrick (2) was used:

$$V = \frac{\pi}{6}(3\sigma^2 + \bar{X}^2)\bar{X}$$

in which V = volume
σ^2 = variance of the mean cell size
$\bar{X}$ = mean cell size

The mean cellular weight was calculated on the assumption that the density of fat cells is that of triolein (11). When the glyceride-glycerol content of the specimens is known the number of fat cells can be calculated.

RESULTS

Studies of Adipose Cells Isolated According to Method II

Morphologic Studies. By varying the times of incubation with collagenase it was possible to obtain cell fractions with different mean cell sizes from the same tissue sample. A representative experiment is shown in Table 2. The cell fractions obtained after 5 min and 10 min have significantly smaller mean cell sizes than the cell fraction obtained after 60 min. The cell fraction with the smallest mean cell size was obtained after 5 min ($P < 0.0005$ compared with 10 min fraction). At this time about 10–15% of the total number of cells had been freed. Very few cells were isolated if a shorter incubation length was used. For these reasons the only cell fractions routinely studied were those obtained after 5 min and 60 min.

The adipose cells of specimens from 33 patients were isolated according to methods I and II. Fig. 1 shows the distribution of the cell sizes obtained with method I. 10% of the cells were smaller than 65 μm, and the average mean cell size was 94.4 μm. Fig. 2 shows distribution curves of adipose cell sizes obtained from 92 determinations of the 5-min and 60-min fractions. In the 5-min fractions 49% of the cells were smaller than 65 μm as compared with 14% in the 60-min fractions. The average mean cell sizes were 70.9 μm and 92.9 μm, respectively ($P < 0.0005$), i.e., volume ratios of about 1:2.25. The coefficients of variation calculated from determinations of the mean cell sizes of eight consecutive 5-min and 60-min fractions were 5.0% and 3.2%, respectively.

Metabolic Studies. Incorporation of labeled glucose in the presence or absence of insulin was determined in 43 5-min and 60-min fractions. The average mean cell sizes were 67.8 μm and 87.1 μm, respectively. The distribution of the cell sizes was similar to that shown in Fig. 2.

Table 3 shows the cell sizes and the incorporation of ^{14}C from labeled glucose into the lipids of the 5-min and 60-min fractions. The cells of the 60-min fractions incorporated more glucose into the total lipids measured per 1000 cells than did the cells of the corresponding 5-min fractions. The incorporation of label into the

TABLE 2 MEAN CELL SIZES OF CELL FRACTIONS OBTAINED FROM THE SAME TISSUE SAMPLE BY INCUBATING WITH COLLAGENASE FOR DIFFERENT PERIODS OF TIME

Time of Incubation with Collagenase	Mean Cell Size	*P* Value, Compared with Cell Fraction Obtained after 60 Min
min	*μm*	
5	62.0	<0.0005
10	79.6	<0.025
15	83.4	NS*
30	86.5	NS
45	82.3	NS
60	86.3	—

Specimens of adipose tissue were prepared from the outer cell layers of the lobules and incubated with 5 mg of collagenase dissolved in 3 ml of Krebs-Ringer bicarbonate buffer with albumin added. The mean cell size of the cells freed after 5, 10, 15, 30, 45, and 60 min was calculated from the determination of the diameters of 100 cells.

* NS, not significant.

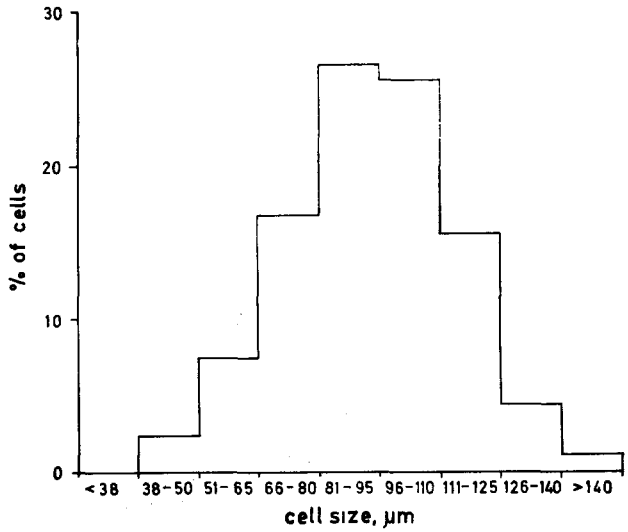

FIG. 1. Distribution of adipose cell sizes determined on biopsies from 33 patients. In each experiment specimens were incubated with collagenase for 60 min and the diameters of 100 cells were measured.

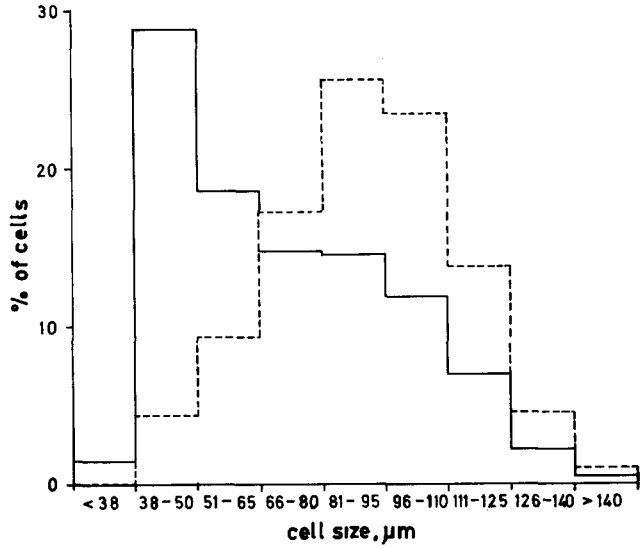

FIG. 2. Distribution of the cell sizes obtained from 92 determinations of the 5-min and 60-min fractions. The tissue samples were obtained from 33 patients. In each experiment specimens were incubated with collagenase, and the cells released after 5 min and 60 min were collected. In each cell fraction 100 cells were measured. ——, cells released after 5 min; - - - cells released after 60 min.

TABLE 3 CELL SIZE AND INCORPORATION OF LABELED GLUCOSE INTO THE LIPIDS OF THE 5-MIN AND 60-MIN FRACTIONS

	5-Min Fractions	60-Min Fractions	*P* Value
Average mean cell size (μm)	67.8 ± 1.8	87.1 ± 3.6	<0.0005
Basal incorporation rate			
Total lipids ($cpm/10^3$ *cells/ 2 hr*)	2.3 ± 0.4	9.1 ± 1.1	<0.0005
% in glyceride-glycerol	85 ± 6	94 ± 3	NS*
% in glyceride-fatty acids	15 ± 6	6 ± 3	NS
Incorporation rate with insulin			
Total lipids ($cpm/10^3$ *cells/ 2 hr*)	3.6 ± 0.4	12.9 ± 1.5	<0.0005
% in glyceride-glycerol	89 ± 7	90 ± 7	NS
% in glyceride-fatty acids	11 ± 7	10 ± 7	NS

Specimens of adipose tissue were incubated in duplicate with labeled glucose and with or without the addition of insulin for 2 hr in a modified Parker medium 199. The specimens were then incubated with collagenase, and cell fractions were collected after 5 min and 60 min. The values shown represent the mean ± SEM of 11 experiments.
* NS, not significant.

fatty acids of the 5-min and 60-min fractions accounted for about 15% and 6%, respectively, of the total radioactivity. However, due to the large individual variations the difference was not significant. Apart from this discrepancy the distribution of the radioactivity was similar. The relationship between mean cell size and incorporation of glucose into the total lipids measured per 1000 cells is illustrated in Fig. 3. It is noted that increasing cell size is accompanied by an increase in glucose incorporation.

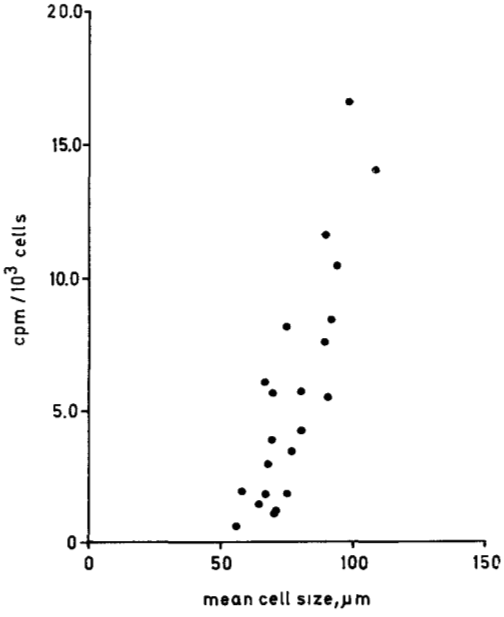

FIG. 3. Effect of cell size on incorporation of glucose-1-^{14}C into total lipids. Specimens from 11 patients were incubated with label for 2 hr in a modified Parker medium 199 in duplicate flasks. Mean cell size and incorporation of label into the cells of the 5-min and 60-min fractions were determined.

In the presence of insulin the average increases in synthesis of total lipids by the 5-min and 60-min fractions were about 57% ($P < 0.005$) and 43% ($P < 0.005$), respectively. Thus, the cells of the 60-min fractions seem to be less sensitive to the stimulating effect of insulin on lipid synthesis than the cells of the 5-min fractions ($P < 0.01$). Due to the large individual variations there was no significant increase in the synthesis of glyceride-fatty acids in the presence of insulin.

DISCUSSION

It was recently reported by Salans et al. (3) that biopsies with different mean cell sizes incorporate glucose at similar rates when calculated on a per cell basis. Björntorp (12), on the other hand, reported that on a per cell basis specimens from obese patients, which have enlarged cells, have a greater rate of lipid synthesis than specimens from nonobese patients. In these investigations adipose cells of different sizes were not obtained from the same tissue samples. It has been shown that age (13) as well as nutritional differences (14–16) between the donors of the tissue affect glucose metabolism and insulin sensitivity. For these reasons it seemed desirable to develop a method to obtain adipose cells of different sizes from the same tissue samples.

Morphologic studies of human subcutaneous adipose tissue in culture have shown that the cells along the borders of the lobules are significantly smaller than the cells within the lobules (5). The same observation was made by Bjurulf (1) in fixed, frozen-cut samples. By using specimens from the outer cell layers of the lobules, and by varying the incubation time with collagenase, cell fractions with different mean cell sizes were obtained. A probable reason for this is that the effect of

collagenase is initially exerted at the surface of the specimens. The difference in average mean cell size between the 60-min fractions and that determined with method I is presumably caused by the different dissection techniques used.

All incubations with labeled glucose were performed in Parker medium 199 because it is suitable for tissue culture of human adipose tissue (5). However, for the short-term incubations used in the present study, glucose concentration was modified to 1.0 mM because the stimulating effect of insulin on lipid synthesis is increased at low glucose concentrations (17, 18).

The results of the present investigation show that incorporation of glucose into the lipids of human adipose tissue is dependent upon cell size. The larger cells of a specimen have a greater rate of glucose incorporation than the smaller cells of the same specimen. Although not shown in Table 3, similar results were obtained when incorporation of glucose was expressed per unit of tissue lipid. Furthermore, the larger adipose cells were less sensitive than the smaller cells to the stimulating effect of insulin on glucose incorporation into the lipids. The large dose of insulin used (0.1 IU/ml) seems to exclude the possibility that this difference is due to the degradation of insulin by the enzyme system described by Rudman, Garcia, Di Girolamo, and Shank (19). The main contribution of glucose carbons was to the glycerol moiety, which is in accordance with other investigations (13, 20). All incubations with labeled substrate were performed prior to the isolation procedure since isolated human adipose cells may easily rupture.

In the present investigation, incorporation of glucose into the lipids is expressed in terms of the cellularity rather than in the customary manner, i.e., per unit of tissue lipid. This is in accordance with the suggestions of several investigators (3, 8, 21). The numbers of cells in the specimens were calculated from determinations of the cell diameters. As shown by Goldrick (2), measurement of the cell diameter is a satisfactory estimation of the lipid content of large cells, but it tends to overestimate the lipid content of small cells. The reason for this is the relative differences in cytoplasmic volumes. It was calculated by Goldrick (2) that the cytoplasm of small cells occupied about 8% of the cell volume, but the corresponding value for large cells was about 0.8%. This indicates that the calculated number of cells in a cell fraction with a small mean cell size is an underestimation of the true number. However, this does not affect the principal findings of the present investigation regarding the differences in glucose incorporation, but would rather tend to further accentuate them.

The differences in results from those obtained by Salans et al. (3) probably reflect the differences in methodology. In their study, specimens were obtained by needle aspiration from the subcutaneous tissue of the buttocks and incubated in Krebs-Ringer bicarbonate buffer. The method used to determine the cellularity of the specimens differed from that used in the present investigation.

At this time we can only speculate on the reason for the increased rate of glucose incorporation into the larger cells. However, the results indicate that the larger cells have an increased need of α-glycerophosphate, presumably for the esterification process. Possibly, the larger adipose cells have a greater lipolytic rate (4). Since no albumin was present the release of fatty acids to the medium is diminished but not inhibited (22). Increasing the intracellular free fatty acid pool increases the requirements for α-glycerophosphate for esterification of the fatty acids (23, 24).

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