Comparison of two methods for determining human adipose cell size

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Summary The mean cell sizes of specimens of human adipose tissue were determined on sectioned slices according to the method described by Sjöström et al. (*J. Lipid Res.* 1971. **12:** 521–530) and on adipocytes isolated after treatment of the tissue with collagenase. The average mean cell sizes from 11 biopsy specimens were 94.4 and 94.0 μm, respectively ($r = 0.964$; $P(t_b) < 0.001$; $y = 0.90x + 9.74$), for the two methods. There was no indication of an increased rupture of isolated large human adipose cells. Thus, with precautions (freshly siliconized glassware and omitting the centrifugation of the isolated cells), the collagenase method may be used for metabolic as well as morphologic studies of human adipose tissue.

Supplementary key words collagenase

DETERMINATION OF adipocyte size is of importance because cell size influences the rates of metabolism of cells as well as the sensitivity to insulin (1–7). Different preparatory methods for cell size determination have been used, such as isolation of the adipocytes after treatment of the tissue with collagenase (5, 8), fixation with osmium (9), or determinations on frozen-cut specimens (10). The collagenase method may lead to the rupture of human adipose cells (2) which may predominantly affect the larger cells. If such is the case, the collagenase method would be less suitable for metabolic and morphologic studies of human adipose tissue in vitro. We have used a collagenase method for several years (5), and it is the purpose of the present paper to compare the results obtained using this technique with those obtained by the direct method described by Sjöström, Björntorp, and Vrána (10). This latter method gives results (10) which in turn are well correlated with the osmium fixation technique described by Hirsch and Gallian (9).

Cell isolation with collagenase (method I). Biopsies of subcutaneous adipose tissue were obtained in connection with operations on patients with cholecystolithiasis. After excision of the tissue, 15–20 smaller specimens, weighing about 20–30 mg each, were dissected from different parts of each biopsy and incubated in freshly siliconized flasks containing 3 ml of Krebs-Ringer bicarbonate buffer with 4% albumin (bovine albumin, fraction V, Armour Pharmaceutical Co., Eastbourne, England) and collagenase (type I, lot no. 81C-0080, Sigma Chemical Co., St. Louis, Mo.) added at a concentration of 5 mg per 3 ml of medium. The flasks were incubated without shaking at 37°C and pH 7.4. After 30 min the specimens were gently stirred for about 5 sec with a plastic spatula to dislodge the fat cells. The flasks were then incubated for another 30 min without shaking. After this period of time the adipocytes were almost completely freed. The remaining stroma was carefully removed with the plastic spatula. No centrifugation of cells was performed. From our experience there may be some variability between different lots of collagenase, and concentrations two to three times greater than that used in the present study may be required for the liberation of the cells.

With a siliconized pipette, 0.5 ml of the cell suspension was removed and placed in a glass chamber. The

diameters of 100 consecutive cells were determined using a Zeiss photomicroscope with a calibrated ocular. The fat cells were recognized by their spherical shape, the light-refractile lipid droplet, and the presence of nuclei that can be stained with crystal violet (11). However, it has not been found necessary to stain the samples routinely with crystal violet. At a magnification of 200, the caliper scale was calibrated so that the unit marks had a constant interval of 3.80 μm. The error of the method, determined according to Dahlberg (12), was calculated from 20 duplicate determinations and was found to be 3.4%.

Determinations on sectioned specimens (method II). From the same biopsies that were used for cell isolation with collagenase, a small tissue sample (one lobule) was dissected prior to the isolation procedure described above. The mean cell sizes were determined as described in detail previously (10). Briefly, the tissue samples were fixed in 35% formaldehyde for 7 min and then frozen-cut to yield slices 200 μm thick. The slices were floated in a closed chamber containing an isotonic solution, and they were then examined with a microscope supplied with a calibrated ocular. The largest diameter of 100 cells was determined with a technique that avoids selection of large cells.

Results and discussion. The adipose cells isolated with collagenase are spherical and show no tendency to form conglomerates. The nuclei may be visualized by the addition of crystal violet. As shown in Table 1, the mean cell sizes determined after isolation with collagenase (method I) are in good agreement with the results obtained with the sectioned specimens (method II) (r = 0.964, $P(t_b) < 0.001$). The mean cell sizes of the biopsies used in the study were from about 70 to 120 μm, i.e.,

TABLE 1. Mean cell sizes of specimens of human adipose tissue determined by two methods

Sample	Method I (x)	Method II (y)
	μm	
1	84.2 ± 2.05	78.3 ± 1.76
2	106.9 ± 1.99	103.7 ± 1.94
3	91.8 ± 2.01	97.4 ± 1.80
4	76.5 ± 1.53	76.5 ± 1.42
5	84.7 ± 1.78	88.9 ± 1.82
6	110.2 ± 2.07	105.4 ± 1.92
7	80.1 ± 1.50	85.9 ± 1.38
8	88.4 ± 1.82	85.6 ± 1.65
9	120.9 ± 1.71	123.2 ± 1.92
10	115.9 ± 1.92	114.1 ± 2.54
11	74.0 ± 1.64	79.8 ± 1.47

The mean cell sizes of specimens of human adipose tissue were determined after isolation with collagenase (method I) and on sectioned slices as described by Sjöström et al. (10) (method II). Results shown are means ± SEM. The average mean cell sizes of the 11 specimens are 94.0 μm (method I) and 94.4 μm (method II) (r = 0.964; $P(t_b) < 0.001$; $y = 0.90x + 9.74$).

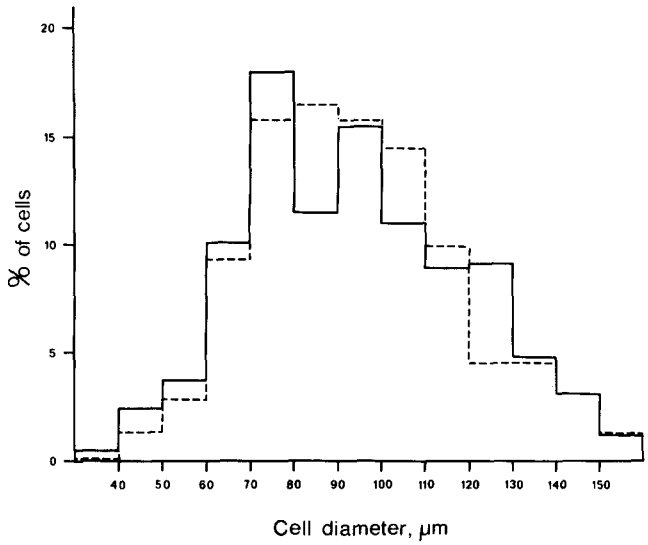

FIG. 1. Distribution of adipose cell sizes of 11 specimens determined after isolation with collagenase (——) or on sectioned slices (- - - -) as described by Sjöström et al. (10).

the mean cell sizes usually found in human adipose tissue. As shown in Fig. 1, the distributions of the cell sizes obtained with the two methods are also similar. With the precautions used there is no indication of an increased rupture of large adipose cells. In our experience this may easily occur unless freshly siliconized glassware is used. In addition, the centrifugation procedure of the isolated cells as originally suggested by Rodbell (13) leads to an increased rupture of human adipocytes. In the present study it was not found necessary to wash the samples prior to the sizing procedure. However, careful washing with warm buffer may be advantageous if the cells are used for subsequent metabolic studies (6).

The results of the present study show that with adequate precautions the collagenase method provides isolated cells that may be used for metabolic as well as morphologic studies of human adipose tissue. However, the microscopic method (10) is more convenient for determinations of adipose cell size in clinical studies since it is less time-consuming and requires a smaller tissue sample.

In a previous study (10) it was suggested that there is a limited variation in the mean fat cell size of a given region of the subcutaneous adipose tissue. This was confirmed in the present study, since in method II the mean cell size was determined from only one lobule while in method I fat cells from a large number of lobules were examined.

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