Human fat cell sizing—a quick, simple method

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Summary Two methods were used to determine the mean cell diameters of 37 samples of human adipose tissue, obtained by open or needle biopsy. Method I was the sizing of cells in cell suspensions and Method II was a quick, simple method of sizing cells from fixed sections. The agreement between the two methods was good ($r = 0.93$, $P = < 0.001$). The results using Method II were slightly lower than those using Method I, and a correction factor is suggested. Method II has several advantages over Method I and we propose that it is a suitable method for sizing cells when a quick method with a permanent record is required.

Supplementary key words Collagenase • histology.

Several methods for measuring the size of adipose cells have been described, all of which have some advantages and some disadvantages. Calculations of cell size from DNA and triglyceride content will underestimate fat cell size because of the large proportion of nonfat cells present in adipose tissue and the difficulty of completely separating these from fat cells. The osmium fixation method of Hirsch and Gallian (1) is a quick, automated method but it requires expensive apparatus and is probably biased against small cells (less than 25 μm diameter). Fat cell diameter measurements on frozen sections of adipose tissue agree well with other methods (2). However, they must be performed in the microscope and so this technique is slow and tedious and produces no permanent record. Fat cell diameter measurements can be made from collagenase suspensions of cells and this method has proved acceptable to several groups of workers (3, 4). We have also found it suitable except when studying the fat cells of extremely obese patients. These cells seem to be particularly fragile to collagenase and similar problems have been reported for fat cells of pigs[1] and obese mice.[2] The fat cells can be distinguished from fat droplets if the nuclei of the isolated cells are stained with acridine orange and viewed in a fluorescence microscope (5). However, this makes it necessary to view and size the cells in the microscope rather than from photographs and the technique again becomes slow and tedious.

Measurements of fat cell diameter from conventional thick or thin histological sections have been criticized (6) because the fixation procedure may cause shrinkage and mathematical assumptions have to be applied. Lemmonier (7) has devised a quick method of cell sizing from histological sections of adipose tissue but this relies on a correction factor derived solely from mathematical assumptions and we do not believe that it is possible to do this.

We wanted a method to use routinely and which could be used to provide a value for mean cell size quickly and simply. Therefore we used a technique similar to that of Lemonnier but compared the results using this method (Method II) to our results using isolated cells as a reference standard (Method I). In this way we were able to derive an empirical relationship between the values for mean cell diameters obtained by the two methods.

Subjects. Open biopsies of subcutaneous adipose tissue from the abdominal region were obtained from 11 subjects undergoing elective surgery (mean age 60.9 ± 4.35 years, mean relative weight[3] 1.09 ± 0.046).

Needle biopsies of subcutaneous adipose tissue were obtained from two different sites (usually from the arm and buttocks) in 13 overweight women (mean age 45.5 ± 2.95 years, mean relative weight 1.4 ± 0.085).

Method I. Cell sizing from isolated cell suspensions. Fat cell suspensions were made using the method of Smith, Sjöström, and Björntorp (2) and photomicrographs were taken at a known magnification. The diameters of isolated

[1] M. I. Gurr, personal communication.
[2] M. A. Cawthorne, personal communication.
[3] Relative body weight was calculated by dividing the subject's actual weight by their ideal weight for height as given by the Metropolitan Life Insurance Tables (using the middle of the medium frame range of weights).

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cells were measured on the photographs. Mean cell diameters and standard error of cell populations were calculated.

Method II. Cell sizing from fixed sections. From the same biopsies that were used for Method I, a small tissue sample was put immediately into Bouin's fixative and left for 1–2 days. The tissue was dehydrated, embedded in wax, sectioned (7 μm thick), then dewaxed, rehydrated and stained with aqueous aniline blue (0.5% w/v). Fields of representatively sized cells were photographed at a known magnification. Care was taken to choose fields containing mainly fat cells and minimal stromal-vascular areas. The number of cells occupying a defined area containing at least 200 cells was counted. Incomplete cells around the perimeter of the area were counted only if more than half their area fell within the defined area.

Calculation of apparent mean cell diameter (d'). The apparent mean cell diameter (d') can be calculated from these photographs using simple mathematics only if the following assumptions are made: (*1*) The cells are assumed to have retained their original size and not suffered from shrinkage. (*2*) The cells are assumed to cover the total area and intercellular space is assumed to be negligible. (*3*) The cells are assumed to be perfect spheres. (*4*) The cells are assumed to have all been cut through their equator thus revealing their maximum cross-sectional area.

In this way, the apparent mean cross-sectional area (s') can be derived as follows.

$$s' = \frac{A}{m^2 \times n}$$

where A = defined area (sq. cm)
m = final magnification of photograph
n = number of cells counted

and since $d' = \sqrt{\frac{4s'}{\pi}}$

$$\text{then } d' = \frac{2\sqrt{A} \times 10^4}{m\sqrt{n\pi}} \text{ (in } \mu\text{m)}$$

It is obvious that this formula will not give true values for the real mean cell diameter (d) because none of the assumptions used in its derivation is completely true. However, whereas assumptions *1*, *3*, and *4* would produce a value for d' which is smaller than d, assumption *2* would produce a value for d' which is larger than d.

It was our intention to use this mathematically derived formula to calculate values for d' which could then be compared to the values obtained for d using the reference method, Method I. In this way, an empirical relationship could be obtained between d' and d.

Results. **Table 1** shows the mean cell diameter and standard error of the mean obtained for the 37 samples using Method I and compares the results for the apparent mean cell diameter for these same samples using Method II. Figs. 1 and 2 show a typical sample of adipose tissue prepared for cell sizing by Method I (**Fig. 1**) and Method II (**Fig. 2**). The results are in good agreement ($r = 0.93$, $P(t_b') = <0.001$) although the average cell diameter determined by Method II is slightly

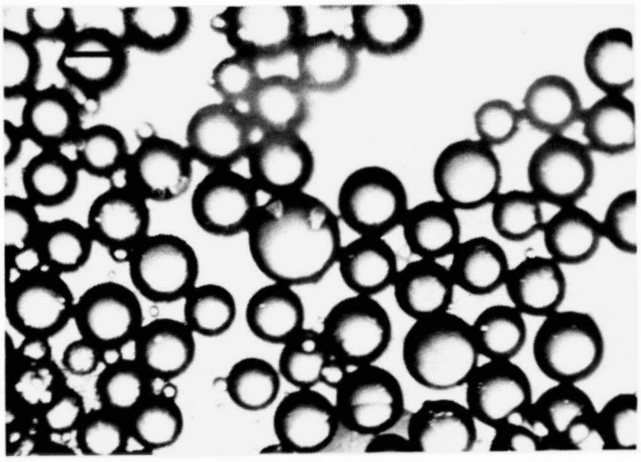

Fig. 1. Isolated fat cells (from sample No. 29) prepared by Method I. Scale marker = 100 μm.

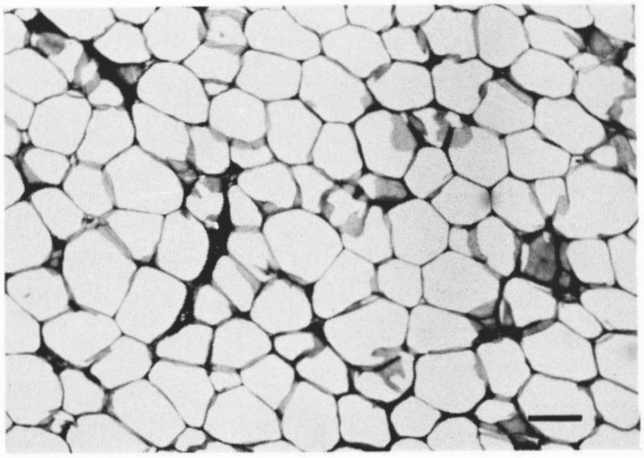

Fig. 2. A fixed section of adipose tissue (from sample No. 29) prepared by Method II. Scale marker = 100 μm.

smaller (83.3 μm) than that obtained by Method I (91.6 μm). A correction factor of 1.1 is proposed when sizing cells using Method II so that: d (real cell diameter) = 1.1 $\times$ d' (apparent cell diameter). This correction factor is constant over the whole range of cell sizes.

Discussion. We have derived an empirical relationship between the apparent mean cell diameter (d') calculated by Method II and the real mean cell diameter (d) measured by Method I.

In spite of all the assumptions made in deriving d' from conventional mathematical formulas, the values obtained for d' are closely related to the reference values d and the correction factor needed to convert d' to d is the same over a wide range of cell sizes.

The advantages of using Method II can be summarized as follows. (*a*) The apparatus required to produce fixed sections of adipose tissue is common to most laboratories and hospitals and the immediate fixation of tissue samples makes this a convenient method to use if samples are obtained away from the laboratory. The duration of fixation does not affect the

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

Biopsy	Sample	Method I(x)[a]	Method II(y)[a]
		(μm)	(μm)
Open	1	65.0 ± 0.95	57.8
	2	64.6 ± 1.52	59.7
	3	60.6 ± 2.48	62.2
	4	74.3 ± 3.65	70.6
	5	69.6 ± 1.20	61.8
	6	88.8 ± 2.86	80.0
	7	57.6 ± 0.76	59.7
	8	64.4 ± 1.51	58.4
	9	87.5 ± 1.74	76.4
	10	51.4 ± 1.17	40.1
	11	88.2 ± 1.87	80.8
Mean ± S.E.M. open biopsies		70.2 ± 3.9	64.3 ± 3.6
Needle	12	95.6 ± 1.60	92.2
	13	83.5 ± 1.81	85.9
	14	118.1 ± 1.95	94.1
	15	104.1 ± 2.24	103.5
	16	83.6 ± 1.26	82.9
	17	91.9 ± 1.79	82.9
	18	95.6 ± 2.27	88.5
	19	84.9 ± 1.58	79.1
	20	95.1 ± 1.73	88.5
	21	87.3 ± 1.70	85.4
	22	99.3 ± 2.07	88.1
	23	113.6 ± 1.76	85.1
	24	114.9 ± 2.02	100.1
	25	110.4 ± 1.99	97.2
	26	92.4 ± 1.42	74.7
	27	93.5 ± 1.50	83.2
	28	105.3 ± 2.62	98.7
	29	102.9 ± 2.01	100.1
	30	103.6 ± 1.55	93.7
	31	100.4 ± 1.67	92.6
	32	111.8 ± 2.05	101.5
	33	112.9 ± 2.54	103.5
	34	103.2 ± 1.44	95.3
	35	107.7 ± 1.93	98.7
	36	102.3 ± 1.83	93.0
	37	101.9 ± 2.09	87.2
Mean needle biopsies		100.6 ± 1.9	91.4 ± 1.5
Mean all biopsies		91.6 ± 2.9	83.3 ± 2.5

[a] The mean cell diameters of specimens of human adipose tissue (obtained from open or needle biopsies) were determined after isolation with collagenase (Method I) or from fixed sections (Method II) as described in the text. Results shown are means ± S.E.M. for Method I and means only for Method II. The average mean cell sizes of the 11 open biopsy specimens and the 26 needle biopsy specimens are shown separately and together. Regression equation for all samples:

$$y = 8.7 + 0.82x,\ r = 0.93,\ P(t_b) = <0.001.$$

final result. (*b*) Counting the number of cells in a particular sample is much quicker than actually measuring the diameters of cells (3 min cf. 20 min for 200 cells). Human errors are minimized particularly if the counting is done from photographs, which can be easily checked. Cell diameters determined by different investigators in our laboratory show a coefficient of variation of approximately 1%. (*c*) Cells of all sizes are included in the calculation.[4] The lower limit of apparent mean cell diameter is approximately 5 μm and there is no upper limit. (*d*) A fixed section is a permanent record of tissue sample.

It is important to point out that Method II can be used only if the fixed section shows a reasonably even distribution of fat cell sizes. Although this has been the case with most of the samples of human adipose tissue that we have sampled so far, we have noticed that in some samples of adipose tissue from young, developing animals or from genetically obese animals, there are pockets of small cells between regions of larger cells (8).

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[4] An estimate of the variance of cell diameter (s^2) can be obtained from knowledge of the variance of estimates of mean cell diameter (d_i) from several samples of the same adipose tissues. It is known from sampling theory that the standard deviation of several means (SEM) is equal to the standard deviation of the population divided by the square root of the number of observations in the samples. Accordingly

$$s^2 = \frac{\Sigma\, n_i}{j}\left(\frac{\Sigma\, d_i^2 - (\Sigma\, d_i)^2/j}{j-1}\right)$$

where j is the number of samples taken, d_i is the i'th estimate of mean cell size, and n_i is the number of cells counted; i 1 . . . . . j. The standard deviations (s) so derived accord well with those calculated by individual cell sizing as in Method I.

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