

A technique to study the relationship between adipose cell size and lipogenesis in a heterogeneous population of adipose cells

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Summary A technique is described for the calculation of the incorporation of radioactive substrate into lipid in adipose cells that have been isolated and separated into groups according to their diameter from a single sample of adipose tissue containing a heterogeneous population of cells. After incorporation of radioactive substrate, a section of adipose tissue is fixed in osmium tetroxide and the fixed cells isolated and separated into specific diameter ranges using a series of nylon screens. The separated cells are weighed, decolorized with hydrogen peroxide, and the radioactivity is determined in the cells from each diameter range. With this method, true comparisons can be made between adipose cell size and lipogenic activity of isolated cells of known diameter which have been subjected to various nutritional or hormonal treatments. Results with sheep adipose tissue show that large adipose cells are considerably more active in the synthesis of lipid than small cells from the same sample of adipose tissue.—**Hood, R. L., and R. F. Thornton.** A technique to study the relationship between adipose cell size and lipogenesis in a heterogeneous population of adipose cells. *J. Lipid Res.* 1980. **21**:1132–1136.

Supplementary key words sheep · lipogenesis · osmium tetroxide

The number and volume of adipose cells in rats (1–3), pigs (4, 5), sheep (6), and cattle (7) increase until about the first 15 weeks, 5 months, 11 months, and 14 months of age, respectively. In the adult animal further accumulation of fat is due solely to filling of existing cells by lipid. Exceptions to this normal pattern are found in conditions of genetic obesity (e.g., the Zucker rat (8)).

The size and number of adipose cells in the adipose tissues of an animal are influenced by genotype, age, and nutritional and endocrine influences as well as experimental treatments. On this basis, several research groups have realized the importance of studying the relationship between the size of adipose cells and their lipogenic or lipolytic activity. Metabolic studies on adipose cells of known size and lipid con-

tent have enabled relationships to be drawn between cell size or cell number and several biochemical parameters, including lipolytic response (3, 9, 10), lipid synthesis (9, 11–13), NADPH-generation (14), insulin sensitivity (15, 16), and lipoprotein lipase activity (16). In most studies, animals of different age or weight have been used to prepare populations of adipose cells of different mean size. This has made the interpretation of data from these studies difficult and often conflicting (8, 17), as it has not been possible to distinguish between the effect of age and the effect of cell size or degree of obesity. To overcome this problem, Björntorp and Karlsson (18) attempted to isolate adipose cells of different sizes within a single population of cells using the differential flotation rates of large and small cells. This method was time consuming and rupture of large cells was frequently a problem. Other studies have described methods to measure the incorporation of radioactive substrate into either a single adipose cell (12) or about 1000 adipose cells (9). These two methods involve the isolation of adipose cells after fixation with osmium tetroxide and are not suitable where large numbers of cells are involved due to color quenching that occurs when osmium tetroxide is present in liquid scintillation counting systems. To overcome the quenching problem a method, utilizing hydrogen peroxide, has been described to decolorize the black osmium-lipid complex which forms when adipose cells have been treated with osmium tetroxide (19).

This communication describes a method to determine the relative incorporation of radioactive substrate into lipid in adipose cells of different sizes within a heterogeneous population of adipose cells which have been isolated from a slice of adipose tissue. Using this technique it is possible to study the effect of cell size on lipogenesis within the same tissue or animal and to determine if adipose cells of a particular size respond differently to hormonal or nutritional treatments.

METHODS

Three Dorset Horn × Merino wethers were fed ad libitum a diet of lucerne chaff (25%), crushed barley (60%), and cottonseed meal (15%). When the sheep had reached about 90 kg, they were slaughtered and subcutaneous adipose tissue was sampled from over the rump and intramuscular adipose tissue from the longissimus dorsi muscle.

Thin (<1 mm) slices of subcutaneous adipose tissue were cut from the freshly excised tissues with

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a razor blade. Tissue slices and fragments of intramuscular adipose tissue were rinsed in 0.154 M NaCl (isotonic saline) at 37°C to remove free lipid released from adipose cells ruptured during slicing. The adipose tissue (200–300 mg) was dried on filter paper, weighed, and placed in a 25-ml Erlenmeyer flask and incubated for 2 hr with 3 μ Ci of [1- 14 C]acetate (20). The slice of tissue was removed from the Krebs-Ringer buffer and rinsed in isotonic saline at 37°C to remove surface [14 C]acetate. The slice was placed in a scintillation counting vial containing 8 ml of 3% osmium tetroxide in 50 mM collidine-HCl buffer at pH 7.4 (21). A minimum of 72 hr in a well-ventilated fume hood was required to completely fix the adipose cells. Osmium tetroxide is volatile and highly toxic and should be used with caution. Adipose cells were isolated using 350 and 25 μ m nylon screens (21) and placed in isotonic saline for separation into sizes on the basis of their diameter.

Separation of adipose cells was facilitated using plastic Nalgene filter units (No. 245/0045) with the Millipore filters and the bottom 1 cm of the plastic bases removed. After this modification to the filter units they can be fitted together such that the base of the upper filter fits into the top of the lower unit, holding in place a circular nylon screen of specified mesh size (**Fig. 1**). Thirteen nylon screens (Swiss Screen Pty Ltd., Sydney) of specified mesh size (Table 1) were placed in order of decreasing mesh size on the plastic grids of each of the filter units. The filter units were then fitted together (**Fig. 1**). The isolated adipose cells were washed onto the upper screen (335 μ m) and eluted through the screens with copious amounts of isotonic saline. A final rinse was given with 50 ml of distilled water. In this way adipose cells were serially separated into groups on the basis of their diameter.

The number of cells retained on each screen was then measured by two methods. First, the cells were washed from the nylon screen and transferred to a beaker containing a known volume of isotonic saline. The number of cells was then counted for each group of cells using a Coulter counter (Model Z_B). Second, the adipose cells from each screen were washed into weighed scintillation counting vials with distilled water and after the cells had settled to the bottom, most of the water was removed using a Pasteur pipette. The remaining water was removed by drying overnight at 37°C. From the weight of cells in each vial, the number of cells per vial was calculated from the assumed density of osmium-treated cells (1.1 g/ml) and the calculated average volume of cells collected between screens of known mesh size. Since the percentages

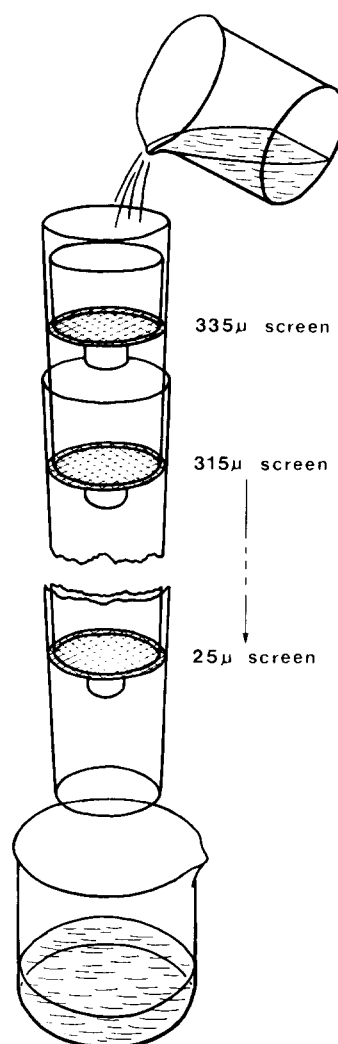


Fig. 1. Schematic diagram of the arrangement of the thirteen filter units used in the separation of osmium tetroxide-fixed adipose cells. Progressively smaller nylon mesh (Table 1) was used in succeeding filter units.

of cells in each diameter range and the relative counts per cell (Table 1) were the most informative methods of data expression, the exact density of the fixed cells was not critical when the data were averaged or compared. To overcome the effects of quenching in the scintillation counting system, the cells were decolorized with hydrogen peroxide (19). After decolorization, the total lipid was either assayed for radioactivity in a liquid scintillation spectrometer (Packard 2650) or separated into lipid and glyceride-glycerol using a method which has previously been described (20). The incorporation of radioactive substrate into individual cells with known diameter ranges was calculated from the number of cells per vial and the radioactivity present in each scintillation vial.

TABLE 1. Screen sizes and a comparison of methods of calculating the number of adipose cells in each diameter range

Mesh Size of Nylon Screens μm		Cells in Each Diameter Range (%)		Counts/ Min/Cell	Relative Counts/Cell ^b
Lower Screen	Upper Screen	Weight	Coulter Counting ^a Before After		
315	335	0.01		3.68	10.00
280	315	0.01	0.01 0.01	3.18	8.64
250	280	0.25	0.10 0.09	2.88	7.83
223	250	0.55	0.38 0.39	2.00	5.43
202	223	0.70	1.02 0.98	1.28	3.48
183	202	1.60	2.00 2.01	0.86	2.34
153	183	6.81	7.88 8.62	0.47	1.28
130	153	31.10	27.93 27.65	0.14	0.38
102	130	23.12	21.92 21.23	0.23	0.62
80	102	14.20	14.47 14.55	0.16	0.43
53	80	12.66	13.58 13.42	0.12	0.33
25	53	8.99	10.68 11.05	0.01	0.03

^a The percentage of cells in each diameter range was measured using a Coulter counter on the population of cells before separation and then after separation by counting the number of cells trapped on each screen.

^b Calculated from the preceding column, by assigning 10 to the highest value in that column.

RESULTS AND DISCUSSION

The adipose cells studied were derived from sheep with large fat depots. Subcutaneous fat thickness over the rump region was as high as 51 mm and as much as 58% of the carcass meat was fat. Some of the subcutaneous cells were so large they would not pass a 250 μm screen, necessitating the use of screens greater than the 250 μm mesh size that were used in previous studies of sheep adipose cells (6).

Providing the adipose cells are rinsed through the filter units with sufficient saline and water, the distribution of adipose cells calculated on a weight basis is comparable to that measured with the Coulter counter both before and after separation of the adipose cells. Typical results for subcutaneous adipose tissue from one sheep are shown in **Table 1**. The results from the Coulter counter were slightly higher for the small cells which is probably due both to electronic noise when the instrument is working at the lower limit of sensitivity and to fragments of connective tissue which are sometimes retained by the lower 25 μm screen. The weight of adipose cells withheld by each screen is the more satisfactory method of calculating the number of cells in each diameter range, since the same cells can be treated with hydrogen peroxide and the incorporation of radioactivity into total lipid or lipid fractions and glyceride-glycerol can be measured. The volatilization of osmium tetroxide with hydrogen peroxide did not result in a loss of radioactivity in the total lipid fraction. Results from the two adipose tissues of the three

sheep showed that 97% of the radioactivity was found in the lipid fraction rather than the glyceride-glycerol fraction when acetate was used as the substrate. Similar findings have been reported for bovine adipose tissue (20). Significant incorporations of radioactivity into glyceride-glycerol would be expected in tissues other than ruminant adipose tissue, particularly if glucose was used as substrate. For all samples analyzed, more than 94% of the weight of the fixed adipose tissue slice was recovered as fixed cells.

Data obtained using these procedures are shown in **Fig. 2**. Although not shown on **Fig. 2**, most of the variability in the results was due to differences in the degree of fatness of the sheep rather than to methodology, since fat sheep have larger adipose cells than thin sheep (6). In agreement with earlier reports for cattle (7, 22) and pigs (23), the average diameter of adipose cells in the intramuscular tissue is smaller than that in the subcutaneous tissue. Furthermore, the shape of the distribution curves of cell diameter was different between the two tissues. Most of the intramuscular adipose cells were <100 μm and most of the subcutaneous cells were >200 μm in diameter. However, in contrast to a previous study on cattle (7), we did not find a bimodal distribution of adipose cells in the intramuscular adipose tissue of the longissimus dorsi.

Lipogenesis, as measured by [¹⁴C]acetate incorporation per cell, was considerably higher (about four times) in subcutaneous cells than in intramuscular cells. The most lipogenic cell groups were in the 220–240 μm range for subcutaneous and 100–130

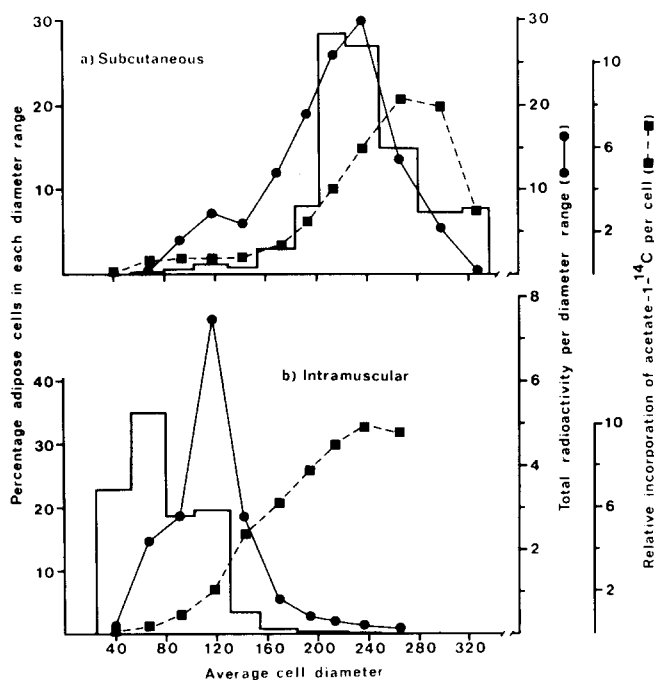


Fig. 2. The number of adipose cells in each diameter range and their incorporation of $[1-^{14}\text{C}]$ acetate into lipid in ovine intramuscular and rump subcutaneous adipose tissue. The data are the mean of three sheep.

μm range for intramuscular adipose tissue. On a per cell basis smaller adipose cells from both tissues were the least lipogenic. Lipogenesis per cell increased linearly over the diameter range 160–250 μm for subcutaneous and 100–240 μm for intramuscular adipose cells. In both tissues $[^{14}\text{C}]$ acetate incorporation per cell declined in the very largest cells (280 μm for intramuscular and 300–335 μm for subcutaneous). The reason(s) for this decline in activity of the larger cells could not be deduced from this experiment. It is possible that this response was biological but the possibility that it was artifactual, perhaps due to grouping of smaller cells, cannot be ignored. It is evident that in studies of lipogenesis in adipose tissue consideration must be given to adipose cell size.

Although the technique is described using adipose tissues from obese animals, it is also suitable for small animals and serial studies, providing approximately 50 mg of adipose tissue can be obtained. If the adipose tissue contained adipose cells with an average diameter of 100 μm , approximately 100,000 fixed cells could be isolated from 50 mg of tissue.

After either in vivo administration or in vitro incubation of radioactive substrate this technique may also be suitable for studies on the effect of hormones or nutritional treatments on lipogenesis in cells of specified size in a single population of cells. For example, the technique may be helpful in studying

the response of cells of different size to insulin and in determining if there is a relationship between cell surface area and number of insulin receptor sites. The procedure is sufficiently versatile to permit many studies on the relationship between adipose cell diameter, volume, and surface area and rates of synthesis in individual adipose cells within a population of cells, rather than comparing activities in adipose tissues with different mean sizes of cells which have been isolated from different animals. ■

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