Cellularity of rat adipose tissue: effects of growth, starvation, and obesity

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ABSTRACT The size, number, and rate of formation of mature adipocytes were studied in the epididymal pads and retroperitoneal adipose depots of the Sprague-Dawley rat. Early growth of these depots was accompanied by progressive enlargement of adipose cells as well as by increases in number. Beyond the 15th wk of life, the depot grew exclusively by the process of cellular enlargement, with no further change in cell number. Severe starvation during the 6th wk of life followed by normal feeding had no lasting effect on cell size or cell number; prolonged semistarvation beginning in the 15th wk greatly reduced cell size while cell number was unaffected. Likewise, extreme increases in depot size produced by hypothalamic lesions did not change cell number, but only cell size. The concept of a fixed number of mature adipocytes in the adult organism may be of central importance in caloric and metabolic equilibrium.

SUPPLEMENTARY KEY WORDS adipocyte · cell counting · cell size · cell number · hypothalamic obesity

MATURE ADIPOSE CELLS are often considered to be "nonmitotic" cells and therefore may have a fixed number in the adult animal, even though each cell remains capable of great change in size (1). A study of the possibility of fixed adipose cell number in adult organisms is of general biologic interest as well as of clinical relevance, since it has been shown that some obese individuals have a great increase in cell number (2) and, also, that cell size and cell number may be important parameters of adipose tissue metabolism (3).

Previous studies of this question have given equivocal results. Reh, in his histologic studies of human adipose tissue, concluded that nutritional effects induce great changes in cell size, thus emphasizing cell enlargement or hypertrophy as a mechanism for changing the size of the adult adipose organ (4). Enesco and Leblond (5), Peckham, Entenman, and Carroll (6), as well as Zingg, Angel, and Steinberg (7), concluded that changes in both cell size and cell number could occur in adult rats. These investigators mainly used DNA content of the tissue as a measure of cellularity. However, their findings must be reevaluated, since the use of methods recently made available indicates that a large proportion of adipose tissue DNA is present in the supportive tissue matrix and not in the adipocytes (8). In fact, recent unpublished observations in the senior author's laboratory indicate that about three-fourths of adipose DNA is found outside of adipocytes. Goldrick (9) made histologic studies of rat epididymal pad growth and concluded that there was a linear relationship between adipose cell volume and the total fat content of the epididymal pad, which suggests that this adipose depot grows by cellular enlargement.

Recently a method has been made available for the rapid and accurate determination of adipose cell size and cell number in any sample of adipose tissue (10). The present communication describes the application of this method to a study of cell size and cell number during growth and development of rat adipose tissue and the effects of changing the size of the adipose depot by experimentally-induced obesity or starvation.

METHODS

Animals and Feeding

Male, weanling Sprague-Dawley rats purchased from the Blue Spruce Farms, Altamont, N.Y. were used for all studies. The animals were housed, three to five to a cage, in a temperature-controlled room where they had unlimited access to water and a Purina chow diet. During starvation periods food cups were removed, but there was unlimited access to water. At various times, groups of four animals were killed under diethyl ether anesthesia.

Experimental Obesity

Obesity was induced by bilateral electrolytic lesions of the ventromedial hypothalamic nuclei (11). The lesions were produced under sodium pentobarbital anesthesia. A stainless steel electrode was guided by a stereotaxic instrument to coordinates appropriate for bilateral placement in the ventromedial hypothalamus and 1.5–2.0 ma was applied for 15–20 sec. Sham-operated animals had the same operation without the insertion of electrodes.

Tissue Isolation and Processing

The epididymal pads were removed just distal to the major blood vessels in the base of the pads. In addition, a triangular area of retroperitoneal fat was removed from each side. The triangle was always dissected with the vertex in the inguinal region, the base at the lower pole of the kidney, one side at the midline and the other side extending into the lateral retroperitoneal reaches as far as fat was visible. The tissues were washed free of adherent oil droplets with warm, isotonic saline, placed on a tared, circular, nylon sieve, and then blotted and weighed to provide a measure of wet weight. One piece was placed in chloroform–methanol 2:1 for extraction of lipid, and another piece (approximately 100 mg, wet weight) was used for a determination of adipose cell size. Distilled water (0.2 volume) was added to the chloroform–methanol extract and aliquots for lipid determination were taken from the chloroform phase. Lipids were determined as carboxyl ester by the hydroxamate reaction (12).

Cell Counting

The tissue sample used for cell counting was placed on a tared, nylon sieve, repeatedly washed with warm saline, then blotted and weighed. The details of cell counting have been previously described as method III for cell size and number determination (10). With this method the tissue is fixed in osmium tetroxide, and the fixed, separated cells are then counted in a Coulter Counter to give the number of cells obtained from a known wet weight of tissue and, hence, from a known amount of lipid. Cell size is expressed in μg of lipid per cell. The lipid content is a close estimate of cell size, since in most instances the lipid content of the tissue is close to 85% of the wet weight, and the lipid is found in one large droplet comprising the major portion of the cell volume.

Calculations

The above procedures yield the following data: wet weight of the tissue under consideration (epididymal pad or retroperitoneal fat), lipid to wet weight ratio, and number of cells per unit of wet weight. Average cell size is calculated as follows:

$$\mu\text{g of lipid per cell} = \frac{\text{prefixation wet weight of osmium-fixed tissue (mg)}}{\text{number of cells in osmium-fixed tissue}} \times \frac{\text{lipid in unfixed tissue (mg)}}{\text{wet weight of unfixed tissue (mg)}} \times 10^3.$$

Cell number in the epididymal pad (or retroperitoneal fat) was calculated as follows:

$$\text{adipose cell number per pad} = \frac{\text{wet weight of total pad}}{\text{wet weight of tissue for osmium fixation}} \times \text{number of cells obtained from osmium-fixed tissue.}$$

RESULTS

The data presented were obtained by sampling four adipose sites in four animals at various times; the four sites (right and left epididymal pads and right and left retroperitoneal areas) were each examined as to lipid content, adipose cell size, and adipose cell number. Examination of these data for all animals under all treatment conditions by the paired "t" test revealed significant differences ($P < 0.05$) between the right and left retroperitoneal areas, particularly in cell number and total lipid content (right greater than left), but no such differences between the right and left epididymal pads. Hence, the epididymal pad data were pooled, such that two pads from each of four animals gave eight observations at each time, from which a single mean was obtained. The retroperitoneal adipose data were separated into four observations from the right side and four from the left. In a few instances, the data shown are from three rather than four animals, but all critical comparisons are shown as mean ± SEM.

Cellularity during Growth and Development

Fig. 1 shows the growth curve of animals observed from 6 to 26.5 wk of life. Adipose cellularity was determined eight times during these 5 months. It is clear that during weeks 6 to 8 there is a marked increase in cell number as well as size, but as the animals and their lipid depots continue to grow, the contribution of cell number to growth diminishes. In the epididymal pads, this decrease in growth by hyperplasia is evident by the 8th wk, and by the 12th wk there is no further significant change in cell number. The retroperitoneal areas show a continuing slow increment in cell number up to and including the observations made at the 15th wk. Yet in all sites observed the great increase in lipid content from the 20th to the 26th wk is accomplished exclusively by a change in cell size.

The relative contributions of cell number and cell size to adipose depot growth are shown in Fig. 2. The cell

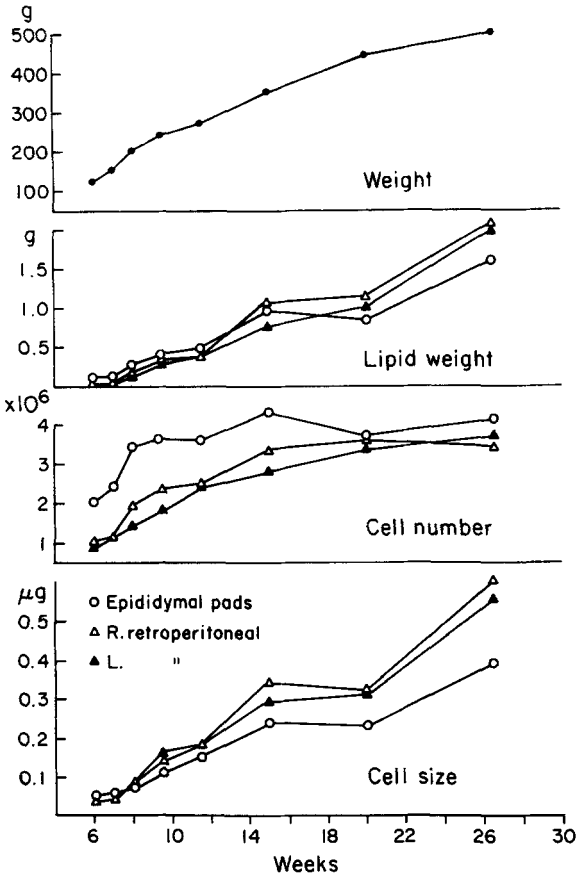

FIG. 1. Observations of adipose cellularity at eight different times during growth and development of Sprague-Dawley rats. The large increase in fat accumulation between 19 and 26.5 wk is clearly related to change in cell size, rather than to an increase in cell number. In earlier intervals, both processes are involved in the growth of the depot.

number and cell size data from Fig. 1 were plotted as a function of depot lipid content shown on the ordinate. It can be seen that from 6 to 9.5 wk the increases in depot size are highly correlated with both cell number and cell size increases. From 9.5 to 15 wk the contribution of new adipose cells to growth of the depot falls off and is less than that of cell size ($r = 0.67$ *vs.* $r = 0.88$), and in the final period beyond 15 wk the cell size changes are sufficient to explain all of the further growth of the depot; changes in cell number are only the result of the variance of measurement and make no contribution to the enlarging depot. It is interesting that cell size and lipid content of the depot are linearly related throughout growth and development, even during the early period when the increase in cell number also contributes to growth.

Reduction of Depot Size

The size of the adipose depot was reduced in two ways: by early, acute starvation or by later, more prolonged starvation.

Acute, Early Starvation. The results shown in Fig. 3 were obtained from animals starved to the point of inanition for a 1-wk period beginning at the 6th wk of life. The mean body weight of these animals decreased by a third, and the lost weight was completely restored by the 10th wk. Both cell size and cell number showed a prompt decline after the onset of starvation, but both were quickly restored with feeding, to the same levels as in non-fasted animals; the last two observations (11.5 and 15 wk) show no differences between the adipose cellularity of the previously starved and the control animals.

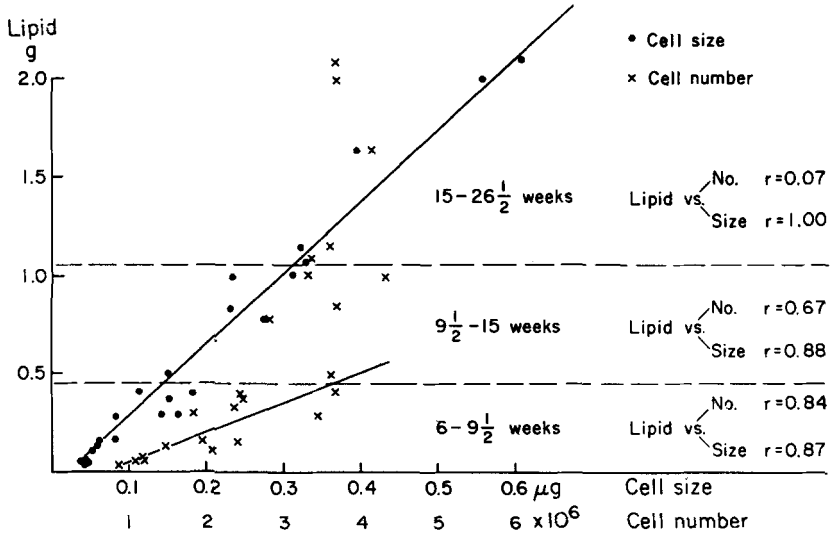

FIG. 2. Correlation coefficients are shown for three time intervals relating the accumulation of lipid in epididymal pads and retroperitoneal depots to cell size and cell number. The data used are the same as those shown in Fig. 1. The correlation coefficients offer a rough numerical index as to the relative contributions of cell size and cell number to the accumulation of depot lipid.

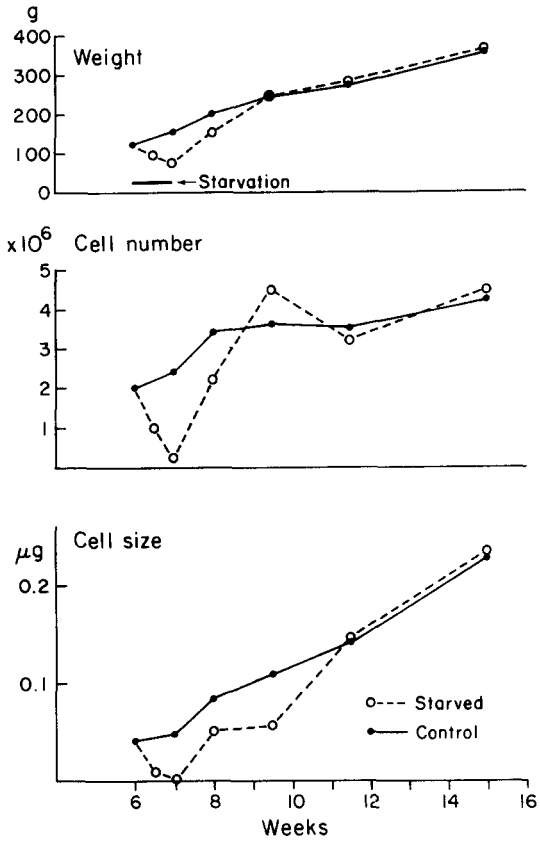

FIG. 3. The effect of 1 wk of starvation (acute, early) on cell size and cell number in epididymal pads of the rat. The effect on cellularity does not last beyond the 11th wk.

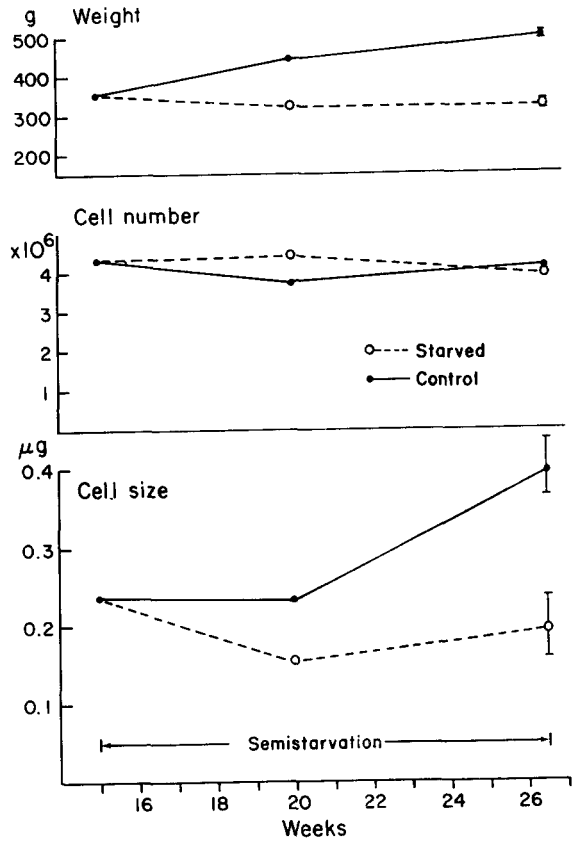

FIG. 4. Chronic, mild starvation shows a persistent and unequivocal reduction of adipose cell size lasting for approximately 3 months, with no significant change in adipose cell number.

Starvation reduced the lipid content of the epididymal pads from a mean of 105 mg of lipid per pad to 14 mg after 3 days, and to only 0.3 mg of lipid per pad after 7 days. At this point the lipid content was only 3% of the wet weight, as compared to 80% for unstarved animals of this age. With so much of the lipid depleted, the counting technique using osmium-fixed cells undoubtedly has serious shortcomings, since cells with a low lipid content are insufficiently fixed to be separable from tissue debris and counted. Thus, it remains unclear whether starvation really has an immediate effect on adipose cell number. Evidently there is no lasting effect of such starvation on either cell size or cell number.

Chronic, Late Starvation. Fig. 4 summarizes a study of the effect on adipose cellularity of a prolonged period of semistarvation. Animals were fed approximately one-half of the caloric intake of controls maintained on an ad libitum chow diet. This semistarvation was begun at the 15th wk of life and continued for 11.5 wk. Beginning at 350 g of body weight, the starved animals had no further increase of weight, while control animals on ad libitum feeding gained an additional 150 g. At 26.5 wk of age, the cell numbers of the two groups were not significantly different, but there was a large difference in cell size, which demonstrates that cell size can be chronically reduced without necessarily affecting cell number.

In both this experiment and the preceding experiment on the effects of acute starvation, changes in the retroperitoneal fatty tissues were concordant with those shown for the epididymal pads; thus there is no reason to expect that changes in different adipose sites would be qualitatively different from those changes found with the epididymal pads.

Increase in Depot Size

Experimental obesity was produced by the destruction of the ventromedial hypothalamic nuclei. This was done both early, at 7.5 wk of life, and later, at the 13th wk of life. Figs. 5 and 6 show the effects of this procedure on adipose cellularity compared to the results with sham-operated animals. Animals operated on early or late have a marked increase in body weight, much of which can be accounted for by massive increases in fat deposits. These deposits are approximately equally distributed in the various depots such as the epididymal fat pads and the retroperitoneal areas. The data shown in Fig. 5 and 6 clearly establish that this increase in adipose tissue is not in any way related to changes in cell number. The more

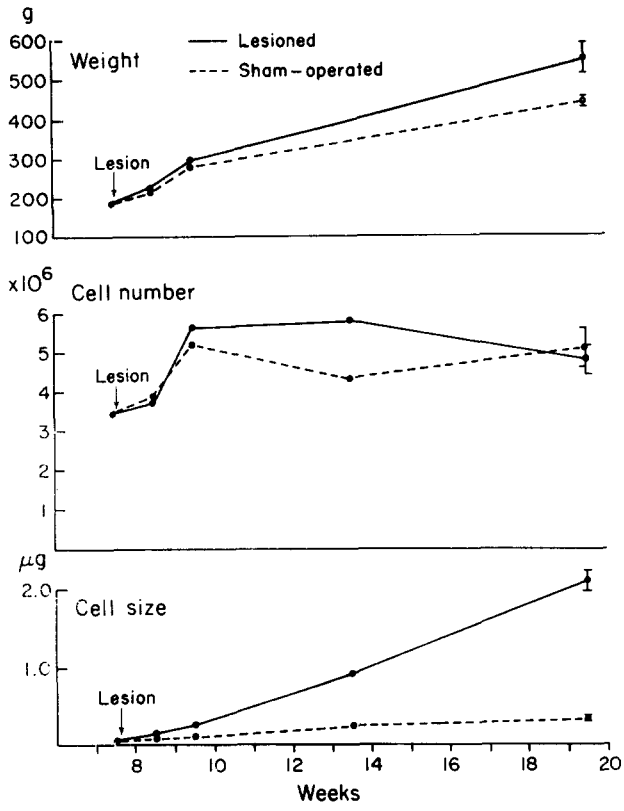

FIG. 5. Obesity produced by ventromedial hypothalamic lesions during the 7th wk of life is accompanied by marked adipose cell enlargement, with no change in cell number.

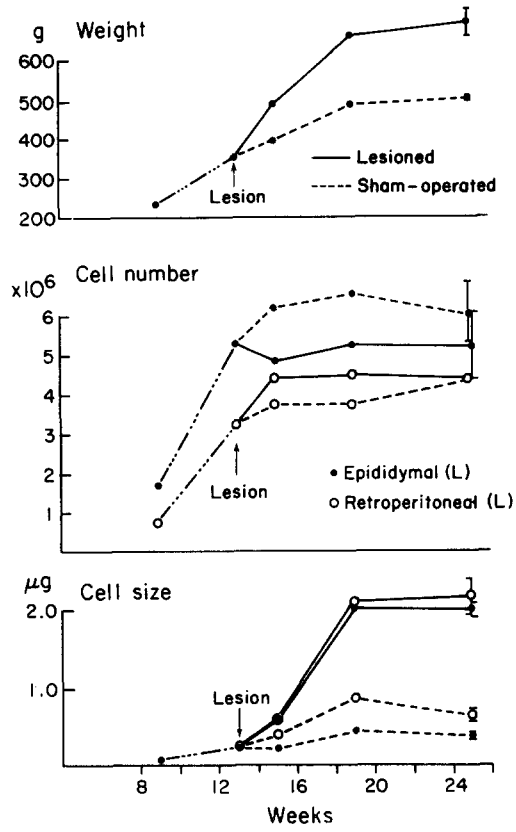

FIG. 6. Obesity produced by ventromedial hypothalamic lesions during the 13th wk of life leads to adipose cell enlargement, with little change in cell number, both in epididymal pads and in retroperitoneal depots.

than fourfold increase in cell size in lesioned animals is the morphologically important change in explaining the mechanism of this type of experimental obesity. Incidentally, the large cells of the experimental animals (roughly 2 μg of lipid per cell) are the largest adipose cells that have been observed in this laboratory in human, mouse, or rat adipose tissue under many clinical and experimental circumstances. As with acute starvation, findings in retroperitoneal fat are no different from those in the epididymal pads (Fig. 6).

DISCUSSION

These results indicate that adipose tissue grows in the young rat by an increase in both cell number and cell size. By the 15th wk of life the cell number becomes fixed, and further changes in the size of the tissue occur by an increase in cell size. Extreme changes in depot size induced by starvation or experimental obesity produce no change in final cell number, even when these alterations are made as early as the 6th wk of life, when cell number is still rapidly increasing. In other work recently reported from this laboratory (13), it was shown that much earlier nutritional effects can change the adult adipose cell number of the rate. When rats were nutritionally deprived during the first 21 days of life, prior to weaning, they were stunted in nearly all aspects of growth and development, and in spite of ad libitum feeding following weaning the adipose depot remained small, with a low cell number and small cell size.

It seems likely, then, that with age adipose tissue progressively loses the ability to grow by hyperplasia of the adipocytes. In this respect, adipose tissue is intermediate between cells such as neurons, which do not multiply or regenerate after early life, and hepatocytes, which are continuously capable of regeneration (1). Adipose tissue shows new cell formation, but in diminishing amounts, for roughly 4 months after the birth of the rat.

The above considerations depend on the concept that a fixed cell number means that no new cells are being formed. It is, of course, possible that a certain number of new cells are being formed and the same number of cells are dying and being replaced. However unlikely this may be, it cannot be ruled out without precise studies of DNA metabolism in the adipocyte. The present method of evaluating cell number has the shortcoming of counting only those cells that contain enough lipid to be fixed by osmium tetroxide and thus to be detected

by the counting method. If new, fat-laden cells were formed from some precursor cells with a low fat content, the precursor cells would not be counted, and yet the rate of formation of mature adipocytes may be a function of the hyperplastic potential of such precursor cells, as well as of the rate at which these cells can accumulate lipid.

The fixed number of mature adipocytes, unaffected by extreme and long-term changes in depot size, favors the idea that adipocytes or their precursor cells are formed early in life by a process that is responsive only to very early influences. Once formed, these adipocytes can change size but do not change in total number. If this idea proves correct, it will focus attention on early modification of adipose cellularity as an important feature of caloric and metabolic equilibrium, since it can be shown that some metabolic events in adipose tissue are a function of cell number and are apparently unaffected by changes in cell size. This is true for in vitro measures of the rate of glucose oxidation to CO_2 (3). When, however, one measures the enhancement of this phenomenon by the addition of insulin to the medium, it is clear that this "insulin effect" is closely related to cell number and also cell size. Larger adipose cells are less sensitive to insulin than are smaller cells. This phenomenon has been used to explain the carbohydrate intolerance of extremely obese subjects who have larger and more numerous adipose cells (3). With weight reduction the cells regain insulin sensitivity, they are reduced in size, and the carbohydrate intolerance is greatly lessened.

The concept that adipose tissue cellularity is achieved during the early life of the organism, can be permanently changed by very early influences, and is an important aspect of metabolic regulations should be explored further, particularly with reference to human obesity and experimental obesity in animals.

The authors gratefully acknowledge the technical assistance of Florence Oetjen, Madeline Riccio, and Anna Hamlet.

This study was supported in part by U.S. Public Health Service Grants AM-HD 09360, AM 11731, and HD 02761 from the National Institutes of Arthritis and Metabolic Disease and the National Institute of Child Health and Human Development, as well as Grant 15930 of the National Science Foundation.

Manuscript received 3 July 1968; accepted 26 September 1968.

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