Relationship of Age and Cellularity to Metabolic Activity in C57B Mice[1] (34600)

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The size of adipose tissue depots, the composition and metabolism of adipose tissue, and its response to hormonal influences have all been reported to change with age (1, 2). More specifically, Benjamin *et al.* (1) reported that epididymal adipose tissue from young rats had a higher rate of glucose-1-^{14}C oxidation than did the tissue from old rats. Since Hirsch and Han (3) have reported that after the 60th day of life in the rat the cell number in adipose depots becomes fixed and further changes in the size of the tissue come about by an increase in cell size, it appeared pertinent to reinvestigate metabolic activity in aging animals as a function of cell number and cell size. We chose to study the conversion of glucose-1-^{14}C to $^{14}CO_2$ in adipose cells isolated from the epididymal pads of C57B mice.

Methods and Materials. Male C57B mice from a highly inbred strain were used in this study. The mice all weighed the same but were divided into two age groups: young mice, 3–4 months old, and postreproductive mice, 12 months old. In the tables and figures, these groups are designated young and old, respectively.

Epididymal fat pads were excised from animals of both ages. Representative samples of tissue were fixed in 2.0% OsO_4 for 48 hr and counted in the Coulter counter using Method III, as described by Hirsch and Gallian (4). Excised adipose tissue samples were extracted by the method of Folch (5). Aliquots were taken for triglyceride (TG) determination by the method of Hatch and Lees (6). Isolated adipocytes were prepared from epididymal fat pads of both age groups using the collagenase[2] digestion method described by Rodbell (7). The isolated cells were suspended in Krebs-Henseleit buffer, pH 7.4 (1/3 Ca^{2+} concentration) containing D-glucose at a concentration of 1 mg/ml, and 5% bovine serum albumin. To measure glucose conversion to CO_2, a 1-ml aliquot of cells was added to 1 ml of albumin buffer containing 2 μCi glucose-1-^{14}C/ml. The cell suspension was incubated at 37° for 2 hr. A 1-ml aliquot of each cell suspension was taken for chloroform:methanol extraction and lipid analysis. The incubation was stopped by injection of 6*N* H_2SO_4 into the medium; Hyamine hydroxide [*p*-(diisobutylcresoxyethoxyethyl) dimethylbenzylammonium hydroxide] was injected into hanging glass wells for absorption of CO_2. After mild shaking for 1 hr, the hanging wells were removed, placed in Liquiflor solution (0.4%PPO and 0.05%POPOP in toluene) in scintillation vials, and counted in a Packard Tri-Carb scintillation counter. To determine the insulin-mediated increase in glucose-1-^{14}C conversion to $^{14}CO_2$ of the isolated cells, glucagon-free insulin was added to the incubation medium at a concentration of 1250 μunits/ml. Data were analyzed statistically by Students' *t* test. Calculations to determine cell size and cell number in the epididymal pad are described elsewhere (4).

Results. The body weight of the C57B mouse reaches a plateau at about 3 months of age, but the epididymal fat pad weight continues to increase (Fig. 1). The body weights of the young and old animals used in this

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[2] Collagenase was obtained from Worthington Chemicals (Lot No. 145w/mg). Glucose-1-^{14}C was obtained from New England Nuclear. Glucagon-free insulin was kindly supplied by Dr. Mary Root, Eli Lilly Company.

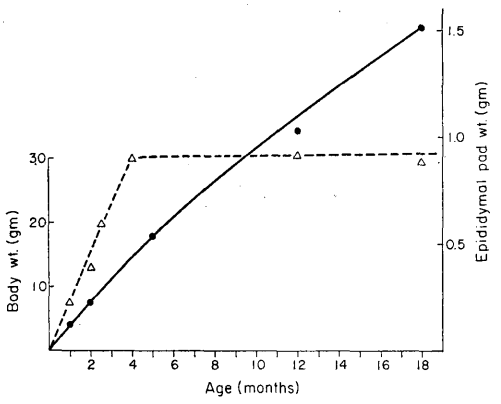

FIG. 1. Relationship between body weight and fat pad weight in C57B mice. Growth curve of C57B mice is indicated by the broken line. Epididymal fat pad weight is indicated by the solid line. Each point represents the mean from 8–10 mice.

study were the same (Table I). The cell number of the epididymal pads from young mice was not significantly different from that of old mice, but the cell size differed markedly (Table I). The mean cell size of the older mice was nearly double that of the young mice. When the glucose-1-^{14}C conversion to $^{14}CO_2$ by the isolated adipocyte preparation is expressed as cpm/mg TG, the cells from older mice showed only 37% of the activity of the cells from young mice (Fig. 2). If the metabolic data is corrected for surface area by the method of Zinder *et al.* (8), the difference between old and young is reduced to 53% of activity (Fig. 2). If the glucose conversion to CO_2 is expressed on an individual cell basis, however, no significant difference exists in the activity of adipocytes isolated from old animals as compared to that of cells isolated from young animals. As shown in Fig. 3, the insulin-mediated increase in glucose conversion to CO_2 was greatly diminished in cells derived from old mice when compared to young mouse cells, confirming previous reports (1, 2).

TABLE I. Cell Size and Number in Mouse Epididymal Pad.[a]

	Body weight (g)	No. × 10^3	Size (μg lipid/cell)
Young	29.0	1027 ± 81[b]	0.14 ± 0.01[b]
Old	29.0	1187 ± 140[b]	0.27 ± 0.01[b]

[a] There is no significant difference in body weight or cell number. Cell size values are significantly different ($p < .01$).

[b] SEM.

Discussion. Previous studies on the metabolic activity and response of epididymal adipose tissue utilizing whole pads, segments of pads, or isolated cells have expressed the data as cpm/mg TG, cpm/mg total lipid, cpm/mg N or cpm/mg wet weight. Data are seldom expressed on a per cell basis. DNA is sometimes used as a measure of cellularity, but as much as two-thirds of the DNA in adipose tissue is contained in the tissue matrix and not in the adipocytes; hence, DNA measurements can be misleading (4). In similar fashion and as might be expected, a high percentage of adipose tissue protein is contained in the stromal fraction, and thus protein determinations on whole tissue reveal little about adipocyte protein content. Since Rodbell (7) has clearly shown that the adipocytes are responsible for essentially all of the metabolic activity of adipose tissue, it appears imperative to base activity on a reliable expression of adipocyte cellularity. While measurements of isolated adipocyte protein should be useful in assessing metabolic activity, such values are often artifically elevated because of the adherence of the collagenase used in the isola-

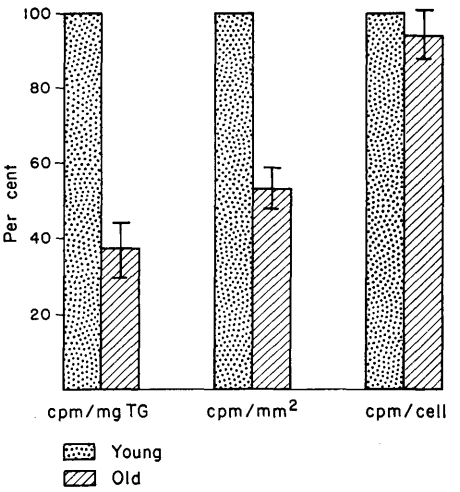

FIG. 2. The effects of age on glucose-1-$^{14}C \rightarrow {}^{14}CO_2$. Values are reported as percent young.

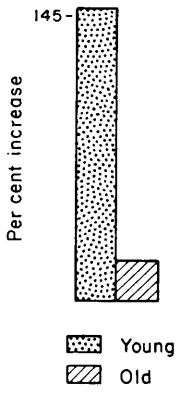

FIG. 3. The effects of age on insulin response (glucose 1-$^{14}C \rightarrow {}^{14}CO_2$). Values are reported as percent above basal level.

tion procedure. Experiments in this laboratory with tritium-labelled collagenase have shown significant contamination of adipose cells with radioactivity after copious washings of the isolated cells. When the number of cells in the tissue sample, or in the cell suspension, is not known, erroneous conclusions regarding cell activity can occur. In fact, our data support the conclusion that just such an artifact has occurred in the assessment of glucose metabolism of aging mice.

The mice in this study differed by a year in age, but weighed the same (Fig. 1). A comparison of epididymal pad weight between groups showed that the older mice had much heavier fat pads. As can be seen in Table I, the change in pad weight is largely accounted for by an increased cell size in the older mice, which is nearly double the cell size of young mice. The cell number of the pads from the two groups is the same, but, as these data indicate, 1-ml aliquots taken from different cell preparations or tissue slices of comparable size, *i.e.*, containing the same amount of lipid, can vary in the number of cells. When the glucose-1-^{14}C conversion to $^{14}CO_2$ was expressed as cpm/mg TG, cells from older mice showed 37% of the activity of cells from young mice, confirming findings reported on whole tissue and tissue fragments (1, 2). Expression of data in this way, however, does not relate metabolic activity directly to the cell, but rather to a selected cellular component, *e.g.*, lipid, which is not constant between young and old mouse adipocytes. When glucose conversion to CO_2 is expressed on the basis of cell surface area, a substantial difference in activity (47%) still exists between young and old mouse adipose cells. The surface area expression should be pertinent particularly to adipose cell glucose metabolism, since the rate-limiting step is glucose transport (9, 10) across the plasma membrane, which forms the outer surface of the fat cell. Larger cells might be thought then, to exhibit greater transport per cell than smaller cells, but our data do not support this assumption. In fact, the larger cells from the older mice are less active per unit of surface area, contrary to the findings reported by Zinder *et al.* (8). When the data are expressed as cpm/cell (Fig. 2), the differences in glucose-1-^{14}C conversion to $^{14}CO_2$ disappear. The apparent difference in activity is seen to be a function of the number of cells in the aliquot.

When insulin is present in the incubation mixture, cells from older mice show only a slight increase in activity above basal levels, while cells from young mice show a marked increase in glucose-1-^{14}C conversion to $^{14}CO_2$. It would be tempting to suggest that reduced sensitivity to insulin is age-related. That insulin sensitivity is age-related remains a possibility, but it has been reported that insulin responsiveness decreases as cell size increases in obese humans, genetically obese mice, and hypothalamically lesioned rats (3, 11). Since age differences did not occur in the aforementioned experiments, it seems most likely that the observed decreased insulin response is related to the enlarged cells found in the older mice and not to the age of the mice, *per se*.

Summary. Our results indicate that isolated adipose cells from older mice convert glucose to carbon dioxide as rapidly as cells from young mice. Therefore, a slowed basal glucose conversion rate in isolated cell preparations is not exclusively age-related, but depends on the number of cells in the aliquot. Our data do not substantiate an age difference in rate of glucose conversion to CO_2 per cell by adipocytes in C57B mice.

Decreased insulin response in adipocytes may represent cell size changes rather than direct age-related changes.

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