Lipolytic response and adenyl cyclase activity of rat adipocytes as related to cell size

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ABSTRACT The number of fat cells contained in the rat epididymal fat pad was found to increase rapidly as the rats grew to a weight of about 300 g. Additional increases in cell number above this weight were minimal. By contrast, cell size, as measured by the amount of triglyceride per cell, increased linearly until the rats reached about 600 g. Glycerol release per 10^6 cells in response to norepinephrine in vitro was observed to be independent of cell size. Basal release expressed in this manner showed a slight but significant positive correlation with increasing cell size. When the rate of lipolysis was based either on the amount of triglyceride in the incubation medium, as is the usual custom, or on the cell surface area, lipolysis was inversely related to cell size. In addition to these observations on lipolysis, it was also demonstrated that norepinephrine-activated adenyl cyclase activity expressed per 10^6 cells was unaffected by cell size. This leads to the suggestion that the number of adrenergic receptors in the fat cell is fixed and is independent of the size of the cell; as the cell enlarges, these receptors are merely distributed over a greater surface area.

SUPPLEMENTARY KEY WORDS norepinephrine · glycerol release · adipocyte number

THE REPORT IN 1964 of the use of isolated fat cell suspensions by Rodbell (1) was an important contribution to the study of adipose tissue metabolism. Subsequent to this, methods were devised to determine the size and triglyceride content of isolated fat cells either in the fixed state with the aid of a Coulter counter (2) or microscopically in the unfixed state with the aid of an ocular micrometer (3–5). The combination of metabolic studies on isolated fat cells with the concomitant determination of their size and triglyceride content have enabled correlations to be drawn between various metabolic functions and cell size (5–9).

In the case of lipolysis, a seeming paradox may exist between the clinical and basic research literature with regard to the lipolytic potential of the fat cells from both animals and patients with different degrees of adiposity. In vitro studies utilizing rat adipose tissue slices or isolated fat cells, with one exception (10), have indicated that the fat tissue of older animals is less sensitive to catecholamines than fat tissue of younger animals (11–14). Since the size of the fat cells of the rat increases throughout growth (5, 7, 8, 12, 15), one implication of these metabolic studies might be that fat cells lose their sensitivity to the lipolytic effects of catecholamines as they enlarge. If this concept is extended to the obese individual who has fat cells of increased size, then one might expect to find the same apparent metabolic deficit. This, in fact, was observed in vivo in obese individuals to whom epinephrine was administered parenterally (16–19), although other investigators (20–24) have been unable to detect any diminution in responsiveness to catecholamines in obese subjects. The studies in which no increase in plasma FFA levels were elicited by catecholamines were interpreted as demonstrating a lipolytic defect in the obese individual. Several studies in the last few years, however, have called into question the role of the adrenergic nervous system in the regulation of lipolysis under normal physiologic situations and starvation (23, 25, 26).

More recently, clinical investigations in the obese have been extended to include careful determinations of the plasma FFA turnover rate. The results of these studies have indicated that instead of exhibiting a deficiency in lipolysis and FFA turnover, obese individuals have in reality a greatly increased turnover rate which

Abbreviations: FFA, free fatty acids; NE, norepinephrine; Tris, tris(hydroxymethyl)aminomethane.

is more than adequate to supply the energy demands of the individuals (27–29). Similarly, in vitro studies with adipose tissue fragments indicate a similar or increased rate of basal lipolysis in the obese as compared with lean individuals (30–32).

In view of the apparent discrepancy between the animal and clinical data, we decided to reinvestigate the in vitro relationship between the size of rat epididymal fat cells and lipolysis in both the basal state and in the presence of various doses of NE. In addition, we also studied the activity of adenyl cyclase in these cells since this enzyme is believed to be associated with the adrenergic receptor at the fat cell plasma membrane (33–37). The results indicate that there is no impairment in lipolysis when measured by glycerol release in response to NE as the cells increase in size. In addition, there is no alteration in NE-stimulated adenyl cyclase activity when results are based on activity per 10^6 cells. A preliminary report has appeared (38).

METHODS

All animals used in these studies were male rats of the Sprague-Dawley strain (Em-Jay Animal Laboratories, Averill, N. Y.). Food (Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.) and water were provided ad lib. Animals were killed by decapitation and the epididymal fat pads were quickly removed and placed in warm 0.9% saline. Fat cells were prepared from the thin distal portion of the pad by the method of Rodbell (1). In very young rats the thin and thick portions of the fat pad could not be differentiated, in which case the whole fat pad was used. In vitro studies were conducted in Krebs-Ringer bicarbonate buffer at 37°C under an atmosphere of 95% O_2–5% CO_2 at pH 7.4 for 90 min. Preliminary studies conducted on fat cells from rats weighing 400 g indicated linear release of both glycerol and FFA for 2 hr in response to NE. To the buffer was added glucose (1 mg/ml) and bovine serum albumin (fraction V, Pentex, Inc., Kankakee, Ill.) (40 mg/ml) that had been made fatty acid free by the method of Chen (39), followed by extensive dialysis of the protein solution against distilled water with subsequent lyophilization. When norepinephrine (*l*-arterenol bitartrate, Sigma Chemical Co., St. Louis, Mo.) was used it was added in a volume of 100 µl or less from aliquots of a previously frozen stock solution. Concentrations are reported as µg/ml of NE base. The final volume of the incubated samples was 2.0 ml. Glycerol in the incubation media was determined by the enzymatic method of Wieland (40). The triglyceride content of the incubated samples and adipose tissue homogenates was determined by the method of Rapport and Alonzo (41) after preliminary lipid extraction by the method of Dole (42).

The distribution of fat cell sizes from the fat pads used in these studies was determined on washed fat cell suspensions isolated according to the method of Rodbell (1); in this procedure 100 cells were sized by two or three individuals using a microscope and an ocular micrometer. The distribution of cell sizes obtained by the different individuals were averaged and from this the average cell diameter, the standard deviation, and standard error of the mean were calculated. From the average diameter and standard deviation the average volume and triglyceride content of the cells could be calculated as discussed by Hirsch and Gallian (2) and Nestel, Austin, and Foxman (5). In addition, the surface area was also calculated using the relationship:

$$\text{surface area} = \pi \frac{\Sigma d^2}{n}$$

where: d = diameter in microns
n = number of observations.

The number of fat cells per fat pad was also calculated using the average cellular triglyceride content and the total triglyceride content of the fat pad (2, 5, 12).

Adenyl cyclase activity of isolated fat cells was determined by a modification of the method of Krishna, Hynie, and Brodie (43). Instead of using the whole fat pad to prepare the homogenate for the assay, the isolated and washed fat cells from 1 g of tissue were homogenized in 3 vol of 8×10^{-2} M Tris buffer at pH 7.3 containing 7×10^{-3} M $MgSO_4$ and 2×10^{-2} M theophylline. The cells were prepared and washed in Krebs-Ringer bicarbonate buffer without serum albumin or glucose present. An aliquot of the washed cells was retained for the determination of cell size, cellular triglyceride content, and surface area. In almost every case the distribution of cell sizes approximated a bell-shaped curve, indicating that there was no appreciable specific loss of cells due to breakage but that cell rupture was random. When the effect of NE on adenyl cyclase was tested, it was added in 0.2 ml of the Tris buffer to provide the final concentration desired. To this was generally added 0.2 ml of the fat cell homogenate and 0.2 ml of 6×10^{-3} M adenosine-8-^{14}C-triphosphate (Schwartz BioResearch, Inc., Orangeburg, N.Y.) in Tris buffer, with a specific activity of 6–7 $\times 10^5$ dpm/µmole. Incubation time was 15 min. The remainder of the assay procedure was performed as described by Krishna et al. (43). In addition, aliquots of the cell homogenate were taken for triglyceride (41) and protein (44) analyses. The triglyceride concentration of the homogenate combined with the separately determined concentration of triglyceride per cell and surface area

allowed the calculation of the total number of broken cells and surface area of plasma membrane used in the adenyl cyclase assay.

Radioactive counting was performed with a Nuclear-Chicago Mark I liquid scintillation system. Aliquots of 2–3 ml of the supernatant from the $ZnSO_4$–$Ba(OH)_2$ precipitation step of the adenyl cyclase method (43) were counted in 15 ml of Bray's solution (45) for 20–40 min. Counting rates were corrected for quenching using a ^{133}Ba external standard and the channels ratio method. The final calculation for the conversion of adenosine-8-^{14}C-triphosphate to cyclic AMP was based on the specific activity of the substrate used in the incubation.

The calculations of the average cellular diameter, triglyceride content per cell, and cell surface area from the frequency distribution of cell sizes were performed using a specially prepared program for the IBM 1800 computer. Curve fitting was done using the method of least squares. The 95% confidence limits for the slopes of regression lines were calculated as described by Goldstein (46). Correlation coefficients for the regression analyses were similarly calculated according to Goldstein (46) by the IBM 1800 computer.

RESULTS

In conjunction with our studies on the correlation between lipolysis and cell size, we reexamined the relationships between both the size of adipocytes and the number of fat cells in the epididymal fat pad as a function of body weight. Fig. 1 shows that cell size as expressed by the amount of triglyceride per cell increased linearly as the animals increased in weight from about 100 g to 600 g. The correlation coefficient for this relationship was 0.88. In addition, we observed that the number of cells per pad increased rapidly until the rats reach about 300–350 g (Fig. 2), after which the rate of increase was less rapid or reached a plateau. These data are in essential agreement with those previously published by other groups (5, 7, 8, 12).

In our initial studies on lipolysis, complete dose-reponse curves to NE were run on fat cells from rats of various sizes. Typical dose-response curves of isolated fat cells from groups of rats of four different weights are seen in Fig. 3, in which the glycerol release is reported on the basis of the cellular triglyceride present in the incubation media. It will be observed that the cells from the rats averaging 96 g, which are also the smallest in size, have the greatest apparent sensitivity to NE. As the cells increased in size, the lipolytic response apparently decreased. These results could not be explained by the fact that the amount of triglyceride per flask in the incubations varied widely, since this was essentially

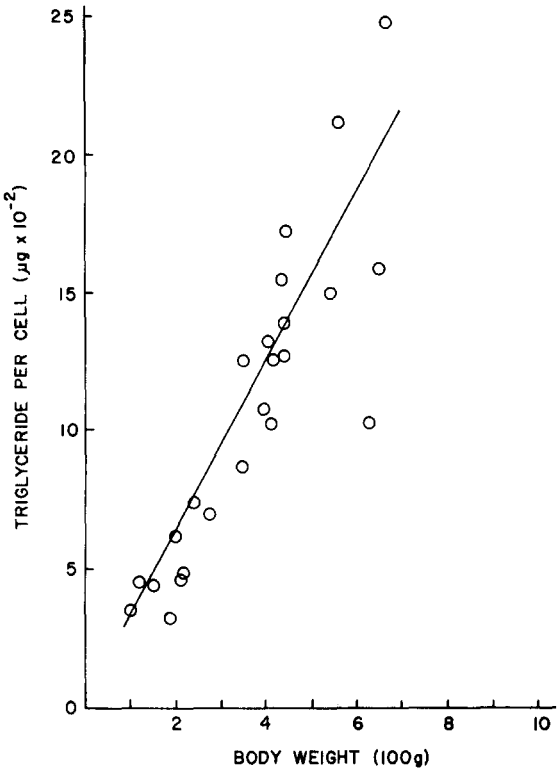

FIG. 1. Relationship between adipocyte triglyceride content and body weight. Each point represents the average cellular content of pooled cells from 2–8 animals.

the same in all four experiments and averaged 19.5 ± 3.0 μmoles.

Additional studies were performed using fat cells from rats of various sizes and NE doses of 0.038, 0.075, and 0.375 μg/ml. The lipolytic activity of these cells expressed as glycerol release per μmole of triglyceride in response to the 0.375 μg/ml dose of NE is presented in Fig. 4 as a function of the size of the incubated fat cells. This curve provides a clearer representation of the relationship between the lipolytic rate expressed per mmole of triglyceride and cell size than the dose-response curves in Fig. 3. These results are typical of the relationships found at the other two doses. It will be observed that the glycerol release is inversely related to cell size by a curvilinear relationship. When the basal release of glycerol was expressed in this manner, glycerol release was directly proportional to cell size, and the best fit was described by a straight line with a correlation coefficient of 0.27. This correlation was not significantly different from zero. Qualitatively similar results for both basal and NE-stimulated lipolysis were obtained when glycerol release was expressed per square centimeter of surface area. The relationship for the 0.075 μg/ml dose based on surface area is shown in Fig. 5. This means of expression was chosen because Zinder, Arad, and Shapiro (3) claimed that when data were expressed in this manner,

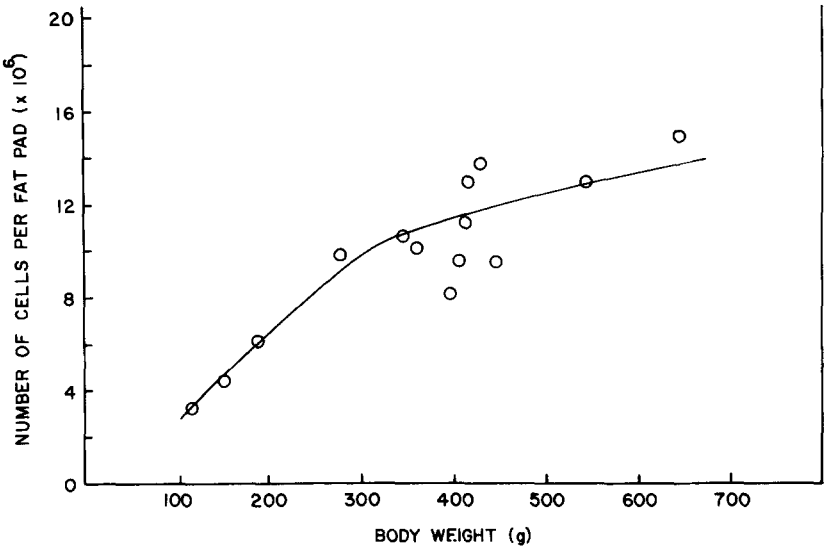

FIG. 2. Number of fat cells per epididymal fat pad as a function of body weight. Each point represents the average value determined for 2–8 animals.

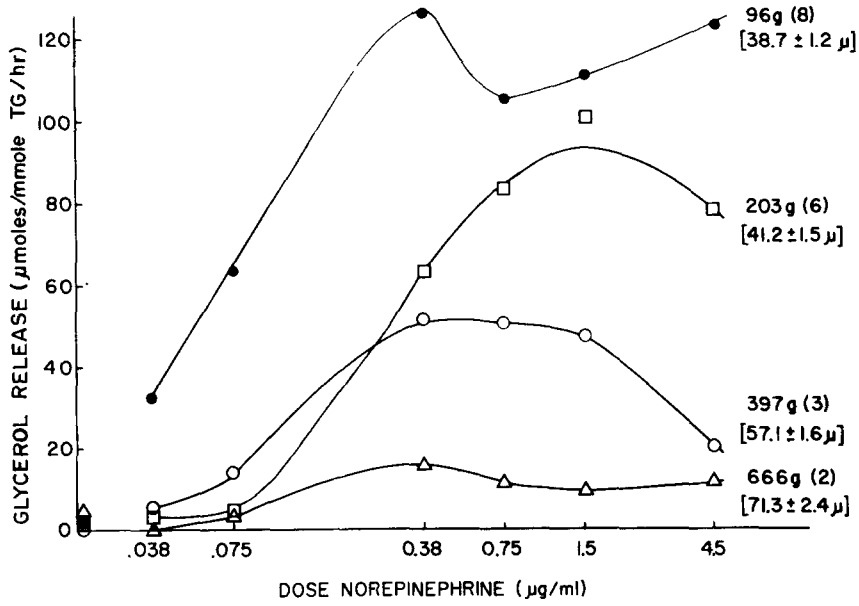

FIG. 3. The influence of cell size on the lipolytic sensitivity to NE. Body weights for the animals used are indicated; the number in parentheses indicates the number of animals from which tissue was pooled to provide cells for the incubation. The numbers in brackets indicate the mean diameter in microns ± standard error of the mean. The isolated cells were incubated in duplicate in Krebs-Ringer bicarbonate buffer for 90 min under an atmosphere of 95% O_2–5% CO_2. In addition, the incubation media contained glucose (1 mg/ml) and fat-free bovine serum albumin (40 mg/ml).

equal rates of incorporation of radiopalmitate into adipocyte triglyceride were exhibited by cells of different sizes under the influence of insulin. From the results shown in Fig. 5 it is obvious that this means of expressing the data does not hold for lipolysis.

Since the cells used in these studies differed widely in their triglyceride content, it was obvious that the number of cells per unit weight of triglyceride would also differ widely. For this reason, the lipolytic results were also calculated on the basis of glycerol release per 10^6 cells. When the basal release was expressed in this manner, glycerol release was proportional to cell size (Fig. 6); the slope of the line is significantly greater than zero ($P < 0.05$), with a correlation coefficient of 0.63. It is entirely possible that this correlation would be even better if one considered only cells which contained more

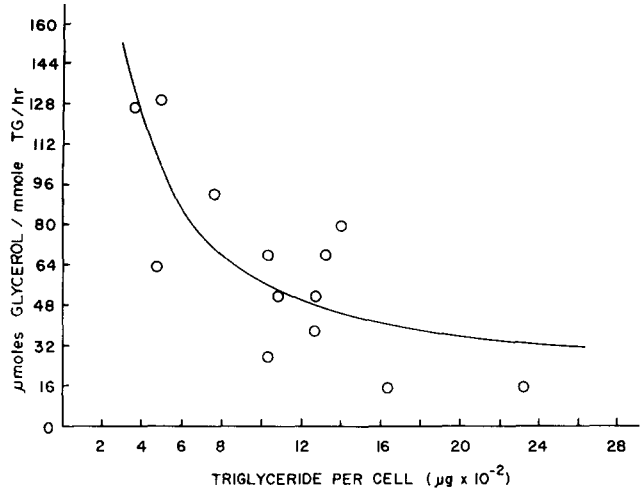

FIG. 4. Inverse curvilinear relationship between cell size and the lipolytic response of adipocytes per unit weight of triglyceride in response to 0.375 µg/ml NE. Each point represents the average of duplicate cell incubations at this dose of NE. Cells at each point were derived from the pooled epididymal fat pads of 2–8 rats. Incubation conditions were the same as described for Fig. 3. The curve plotted is the least squares line of best fit.

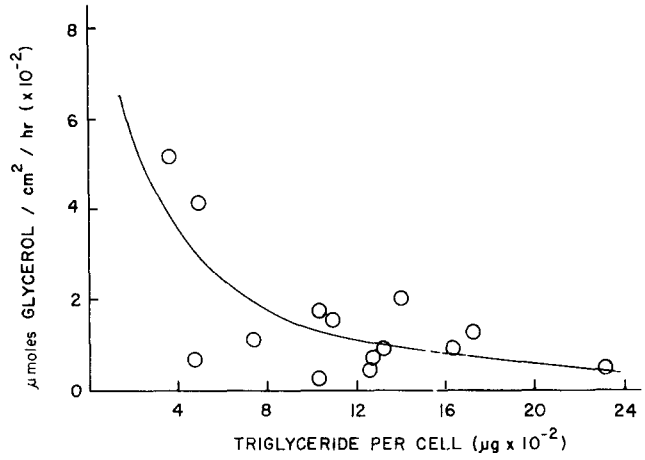

FIG. 5. Inverse curvilinear relationship between cell size and the lipolytic response of adipocytes per cm² of cell surface area in response to 0.075 µg/ml NE. Each point represents the average of duplicate cell incubations with cells derived from at least two rats at this dose of NE. Incubation conditions were the same as described for Fig. 3. The curve plotted is the least squares line of best fit.

than 10–12 × 10^{-2} µg of triglyceride/cell, since up to this size range there appears to be little correlation between cell size and basal glycerol release. In view of the paucity of data for cells exceeding this size, however, this hypothesis for the present must remain speculative. In contradistinction, when the three test doses of NE were added to the incubation medium, the lipolytic rate expressed per 10^6 cells was independent of cell size; the results for the 0.375 µg/ml dose, which was the only maximally stimulating dose tested, is shown in Fig. 7.

The slopes and correlation coefficients of the regression lines for basal lipolysis and three test doses of NE are shown in Table 1. With the exception of the basal release as already discussed, the slopes of the regression lines for lipolysis stimulated by NE for all three doses were not significantly different from zero, indicating that lipolysis was independent of cell size.

In agreement with the results on lipolysis, stimulation of fat cell adenyl cyclase with a maximally stimulating dose of NE gave a response which was also independent of cell size (Fig. 8). This dose was used since we were interested in determining the total cyclase activity associated with the NE receptors. Preliminary dose-response curves with this enzyme activator gave responses

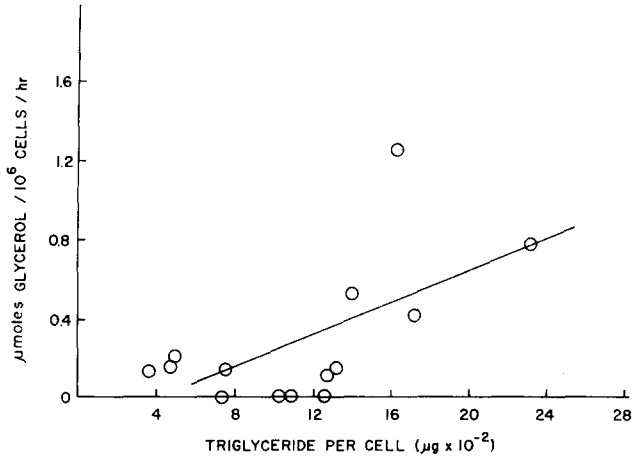

FIG. 6. Correlation between basal glycerol release per 10^6 fat cells and cell size. Each point is the mean of duplicate incubations. Cells at each point were derived from the pooled fat pads of 2–8 rats. Incubation conditions were the same as described for Fig. 3, with the exception that NE was excluded. The line is the least squares regression line of best fit.

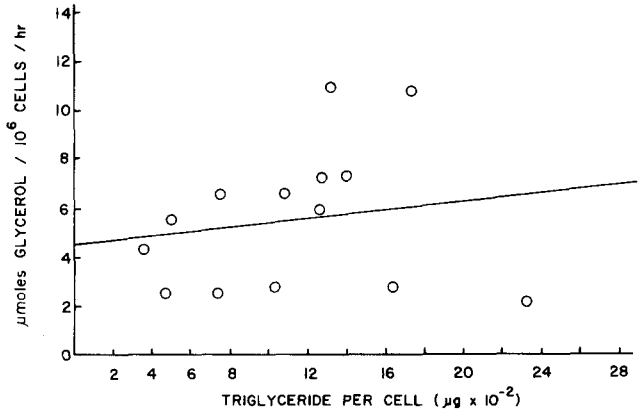

FIG. 7. Lipolytic response per 10^6 fat cells to 0.375 µg/ml NE as a function of cell size. Each point represents the mean of duplicate incubations. Cells at each point were derived from the pooled tissue of 2–8 rats. Incubation conditions were the same as described for Fig. 3. The line drawn is the calculated least squares regression line.

TABLE 1 CORRELATION COEFFICIENTS AND SLOPES OF LEAST SQUARES REGRESSION LINES FOR THE RELATIONSHIP BETWEEN LIPOLYSIS* AND CELL SIZE

Dose Norepinephrine	Correlation Coefficient†	Slope‡
$\mu g/ml$	r	
0	0.63 ($P < 0.015$)	0.041 ± 0.029
0.038	−0.15 ($P > 0.05$)	−0.015 ± 0.057
0.075	−0.30 ($P > 0.05$)	−0.041 ± 0.075
0.375	0.17 ($P > 0.05$)	0.090 ± 0.303

* μmoles Glycerol/10^6 cells/hr.

† Values in parentheses indicate the probability that the correlation coefficient reported is not different from zero.

‡ The slopes are reported as the slope of the least squares regression line of best fit ± the 95% confidence limits for the slope.

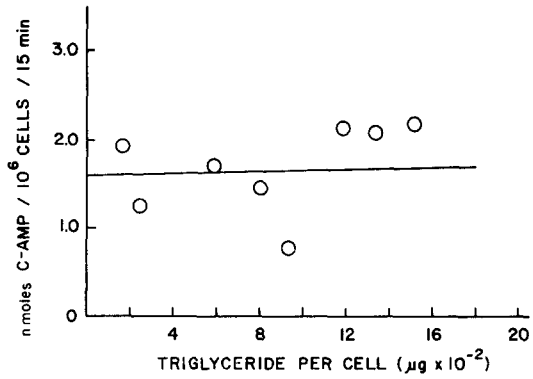

FIG. 8. Cyclic AMP production per 10^6 cells in response to the addition of a maximally stimulating dose of NE as a function of cell size. Homogenates of isolated fat cells derived from at least two rats were incubated for 15 min at 30°C in 8×10^{-2} M Tris buffer containing 6×10^{-3} M 8-^{14}C-ATP ($6–7 \times 10^5$ dpm/μmole), 2×10^{-2} M theophylline, 7×10^{-3} M $MgSO_4$, and 0.124×10^{-3} M (20.9 μg/ml) NE. The data are expressed as nmoles ^{14}C-cyclic AMP produced per 10^6 broken cells in the incubation.

which were similar to those obtained by Birnbaumer and Rodbell (35) for epinephrine. Expression of the adenyl cyclase activity per unit of cell surface area resulted in an inverse curvilinear relationship between enzyme activity and cell size.

DISCUSSION

In the course of our studies on the relationship between cell size and lipolysis, we have reaffirmed the results first reported by Hirsch's group (7, 8), and later by others (5, 12), regarding the number of fat cells contained in the epididymal fat pads of rats. The number of cells was observed to increase until the animals approached a weight of 300–350 g, after which they reached an apparent plateau level. In contrast to the number per fat pad, the size of the cells continued to increase linearly until the animals attained a weight of about 600 g. In the few animals we have studied above 600 g, we have noted either a biphasic cell size distribution containing a large number of small as well as large cells or a shift in the distribution to smaller sizes. Enough animals have not been studied to determine which of these two alternatives is correct, or of what physiological importance this may be.

When lipolysis in response to NE was studied in relation to cell size and the lipolytic rate was expressed in the conventional manner on the basis of the millimolar amount of triglyceride contained in the incubated cells, the rate was inversely related to cell size. This is in agreement with the results of Benjamin, Gellhorn, Wagner, and Kundel (11) and others (13, 14) on adipose tissue slices and with Therriault, Hubbard, and Mellin (12), who used isolated fat cells of the rat. These results show very clearly (Fig. 4 compared with Fig. 7) that when lipolysis is to be compared in adipocytes or tissue from rats of different body weight or differing experimental treatment, the comparison should be made on the basis of cell number and not on the basis of tissue weight or triglyceride content. When the same lipolytic data in our studies were expressed per cm^2 of cell surface area, as suggested by Zinder et al. (3), a similar inverse relationship between lipolysis and cell size was observed.

When NE-stimulated glycerol release was expressed per 10^6 cells, lipolysis was found to be independent of cell size. Similar results on lipolysis have been demonstrated in a very limited number of patients by Björntorp and Hood (30), Björntorp (31), and in a much larger series of patients by Goldrick and McLoughlin (32). In Björntorp's studies, pieces of adipose tissue surgically removed from the abdominal wall of obese and normal-weight individuals were employed. Their results showed that NE-stimulated lipolysis per μg of DNA or per 10^4 cells was the same for obese and controls. They also showed that the basal glycerol release in the obese, although increased 65%, was not significantly different from that observed in controls. Unfortunately, these studies do not provide a true picture of lipolysis per 10^4 fat cells as determined by DNA analysis, since the total DNA of the fragments represents the content of many cell types, which may vary in composition from sample to sample. On the other hand, the results of Goldrick and McLoughlin (32), who used isolated cells prepared from omental and subcutaneous adipose tissues, have demonstrated that a highly significant positive correlation exists between basal lipolysis and adipocyte volume. They also showed a lack of correlation between epinephrine-stimulated lipolysis and cell size. In our studies, basal glycerol release per 10^6 cells was slightly but significantly increased with cell size.

The alterations observed in the lipid metabolism of the fat cell as it enlarges may indicate that a control system is operating from within the cell at the direction of its

own genetic profile and is modified by outside influences such as circulating levels of various hormones. It has been reported that as the adipose cell increases in size the lipoprotein lipase activity per cell falls (5). In addition, the esterification mechanism for fatty acids presented as either FFA (3) or chylomicrons (5) is depressed as the cells enlarge. Furthermore, the ability of the fat cell to oxidize glucose-1-^{14}C to carbon dioxide under the influence of insulin is decreased with increasing cell size (9), and the conversion of glucose to fatty acids is also depressed (47). These metabolic functions are all concerned with increasing or maintaining the size of the adipose depot and, significantly, their activities per cell have all been shown to be depressed the larger (older) the cell becomes. Of all the metabolic processes of the adipocyte so far studied, the lipolytic mechanism appears to be different and is unaltered in activity as the cell enlarges (ages). Thus, as the cell enlarges in size, those processes which are involved in the acquisition of more lipid, either per se or by de novo synthesis, are depressed, whereas the lipolytic process remains unaltered. Thus, it might be argued that under normal circumstances these opposing forces are kept in check within certain prescribed limits, and body weight is maintained. In the dynamic phase of obesity this balance is upset either by the shear magnitude of the influx of carbohydrate and lipid and/or as a result of a decreased rate of lipid utilization. Perhaps as a compensatory mechanism to decrease the adipose mass in the case of human obesity, the turnover rate of FFA is increased in the fasting state (27–29).

Although in this study we have reported only the rate of glycerol release from isolated fat cells, it is possible that there could be a net decrease in mobilization of FFA due to increased reesterification. This does not appear to be the case, however, since in our experience with large rats such as those used in some of these studies, the fatty acid to glycerol ratio in response to these test doses of NE approaches 3:1.[1] Thus these data indicate that both gross and net lipolysis are unaffected by an increase in adipocyte size.

It is also conceivable that larger fat cells contain a decreased content of cellular hormone-sensitive lipase similar to that reported for lipoprotein lipase (5). To account for the results of NE-stimulated lipolysis one could postulate either an increased rate of cyclic AMP formation to more fully stimulate the existing lipase or a decrease in the activity of phosphodiesterase with a resultant increase in the cellular half-life of cyclic AMP. Since adenyl cyclase activity is unaffected by cell size, it is apparent that any alterations in the intermediate reactions are so offset as to lead to no net effect on the lipolytic rate. Furthermore, our data on NE-stimulated adenyl cyclase activity indicate that alterations in cellular size with growth produce no change in the number of receptors for the catecholamine and probably little if any alterations in the activity of the hormone-sensitive lipase or phosphodiesterase. It is thought that as the cells increase in size the fixed number of adrenergic receptors are interspersed over a greater surface area.

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