Effect of cell size on epinephrine- and ACTH-induced fatty acid release from isolated fat cells

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ABSTRACT Free fatty acid release from fat cells, obtained from epididymal adipose tissue of rats of different sizes, was found to be dependent on the cell surface area, regardless of the age of the animals. The same result was found with cells of different sizes from the same animal. These results, when related to in vivo conditions, would indicate that for the same adipose tissue mass, activity would decrease with increasing cell size. On the other hand, the total activity of a given tissue would increase by increasing the size of its cells.

SUPPLEMENTARY KEY WORDS lipolysis · cell surface area · plasma membrane

In a previous communication (1) we showed that the incorporation of radioactive palmitate and radioactive glucose into TG of adipose cells in the normal rat is a function of the surface area of the cells. When related to surface area, no significant differences in activity were found between rats of different ages.

Most of the work dealing with lipolysis in adipose tissue has related this function to tissue weight (2), μmoles of TG (3), or DNA content (4). Since the release of FFA is one of the most important functions of adipose tissue, the dependence of this parameter on surface area was investigated. Results of such an investigation may be relevant to the problem of whether the total fatty acid releasing activity of adipose tissue in the body is determined by cell number, by tissue mass, or both, i.e., the total cell surface area.

Abbreviations: TG, triglycerides; FFA, free fatty acids.

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EXPERIMENTAL PROCEDURES

Materials

The epinephrine used was from the Teva Pharmaceutical Company, Jerusalem, Israel. ACTH was from N. V. Organon, Oss, Holland, and from the Armour Pharmaceutical Co., Chicago, Ill. All reagents used were of analytical grade.

Methods

Epididymal fat pads were obtained from male rats (82–307 g) of the Hebrew University strain. The animals were maintained on standard laboratory chow and were not fasted before they were killed.

Isolation of the adipose tissue cells was achieved by digestion of the tissue with collagenase (Worthington Biochemical Corp., Freehold, N.J.) according to Rodbell (5), except that no glucose was present. The cells were washed twice and then resuspended in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 4% bovine albumin fraction V (Armour) which had been dialyzed overnight at 4°C. A sample of the cell suspension was taken for observation under a light microscope. Cell size (volume) and surface area were calculated, taking into consideration the skewness in the distribution of radii. The formula used by Hirsch and Gallian (6) was employed, namely,

$$\text{cell volume} = \frac{\pi}{6}(3\sigma^2 + \bar{x}^2)\,\bar{x}$$

where σ^2 = variance and $\bar{x}$ = mean diameter. Similar considerations were observed in calculating cell surface area, thus, area $= \pi(\sigma^2 + \bar{x}^2)$. The incubation mixture contained 0.5 ml of cell suspension, and 1.5 ml of the

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above buffer, to which epinephrine or ACTH was added. All incubations were carried out in an atmosphere of 95% O_2 and 5% CO_2 for 30 min at 37°C.

At the end of the incubation period, 5 ml of the extraction mixture described by Dole and Meinertz (7) was added. 30 min later, 3 ml of heptane and 1 ml of water were added; the contents were thoroughly mixed and then centrifuged. A sample of the upper phase was taken for FFA determination by one of two equivalent methods: (*a*) a slight modification of Dole's procedure (8), in which the sample was dried under N_2, dissolved in alcoholic Nile blue indicator, mixed thoroughly, and titrated with 0.02 N NaOH; or (*b*) a method described by Duncombe (9), in which the sample was dried under N_2, dissolved in 5 ml chloroform, and the FFA determined by complexing the acids with a copper salt.

Another sample of the upper phase was taken for estimation of TG content by determination of ester bonds, using the method described by Stern and Shapiro (10).

RESULTS

Estimation of Cell Radius

The samples of cell suspension were observed by using a light microscope at 300 × magnification with a calibrated grid (American Optical Corp., Buffalo, N.Y.). The diameters of 150–200 cells from several fields were measured and the mean diameter was calculated. No covering slide was used because fat cells rupture on contact with glass (3). An example of the diameter distribution for cells from one animal is given in Fig. 1. It shows the sharp Gaussian pattern which was obtained in all cases.

Variation of Cell Radius with Animal Weight

As we have previously shown (1), in normal rat epididymal tissue cells there is a general trend for the cell radius to increase with increasing weight (age) of the animal, although there are exceptions (Fig. 2).

Dose Response Curves for Epinephrine and ACTH

The dose response curves for FFA release due to the effect of epinephrine and ACTH are shown in Fig. 3. The results are from one of two identical experiments which exhibited similar patterns.

As can be seen, the maximal response to epinephrine is reached at a dose of 0.5 μg/ml. In order to be in the maximal range, a dose of 1 μg/ml was therefore used in all experiments with epinephrine. With ACTH a plateau was reached at 5 mU/ml (5 μg, Armour), and in all the experiments with this hormone a dose of 10 mU/ml was used.

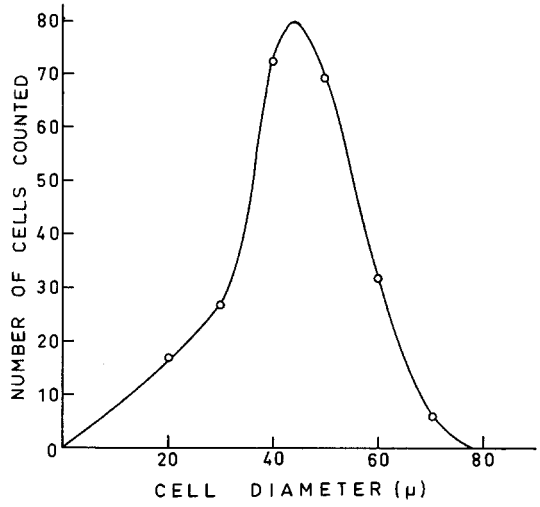

FIG. 1. Typical diameter distribution pattern of cells from one animal. The epididymal tissue was treated with collagenase, and a sample of the washed cell suspension was observed under 300× magnification with the aid of a calibrated grid.

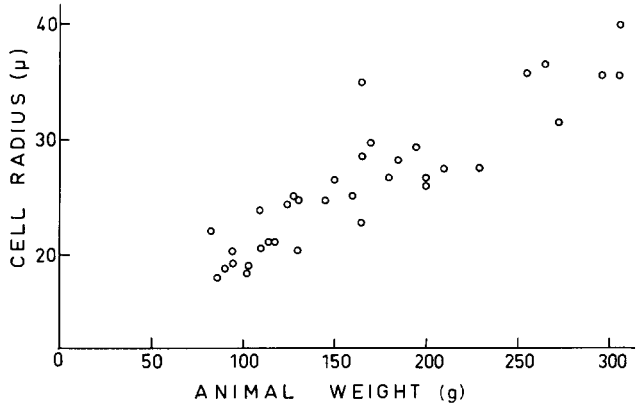

FIG. 2. Cell radius as a function of animal weight (age). Samples of cells from 35 animals of different sizes were observed as described in Fig. 1.

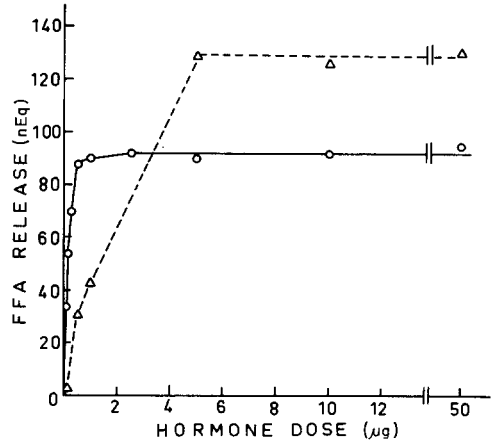

FIG. 3. Dose response curves for epinephrine and ACTH. Cell suspensions were incubated at 37°C for 30 min with rising amounts of epinephrine (O——O) or ACTH (△- - -△). Results are shown as nEq of FFA released per μmole TG in the system.

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Effect of Epinephrine

In most of our experiments epinephrine was used to promote FFA release. No FFA release was detected without the hormone, corroborating evidence presented by Rodbell (3) and Mosinger and Vaughan (2). Fig. 4 gives a graphic representation, as well as the algebraic function obtained by regression analysis (least possible squares), of FFA released from 37 samples of cells. The release was calculated in three ways: (*a*) per 10^5 cells; (*b*) per cm^2 surface area; and (*c*) per μmole of TG in the system.

It can be seen that there is considerable variation in individual experiments, but the general tendency, manifested in the statistical analysis, is apparent. As can be seen, calculation per cm^2 surface area gives an almost horizontal plot. FFA released per μmole of TG has a negative slope, while there is a positive slope when the data are plotted on a per cell basis. The slight negative inclination (-1 nEq of FFA/μ of cell radius) when the calculation is based on surface area could be due to the fact that cell rupture, which is already taking place during the 30-min incubation, would have a greater effect on the larger cells, which are more fragile.

In order to avoid individual variations due to age, weight, nutrition, etc., cells of different sizes were separated from the same tissue sample. Cells were prepared from the two epididymal fat pads of one or two rats of the same weight, and they were pooled and washed. The cells were then separated by flotation in cellulose (dialysis) tubing, 0.6 × 150 cm, using a modification of the method described by Björntorp and Karlsson (11). The separation was carried out in a humid room at 37°C, thereby eliminating clumping of cells caused by cooling of the suspension. Each fraction was isolated by two clamps 1 cm apart, which ensures a sharper separation of the cells according to size. A typical distribution pattern of cell size in the various fractions is shown in Fig. 5.

Fig. 6 shows the optimal linear function (calculated by regression analysis) obtained from the results of three separate experiments (two experiments with three fractions, and one with four) performed with the above method. As can be seen, the results corroborate, and are even more conclusive than, those obtained when using different animals for different cell sizes. In plot *B*, *P* was not significant, thus proving that there is no correlation between FFA release per cm^2 area and cell radius, and indicating that this parameter is independent of cell size. The plot of total FFA release as a function of total surface area in the system shows the direct proportionality between release and surface area (Fig. 7).

Effect of ACTH

In order to see whether the above effects were due to epinephrine, or whether they were of a more general nature whenever FFA was released, a series of experiments was undertaken with ACTH. In Fig. 8 are shown the results from seven animals, demonstrating the same patterns as were seen in the experiments with epinephrine. In plot *B*, as in Fig. 6*B*, a high value of *P* was found.

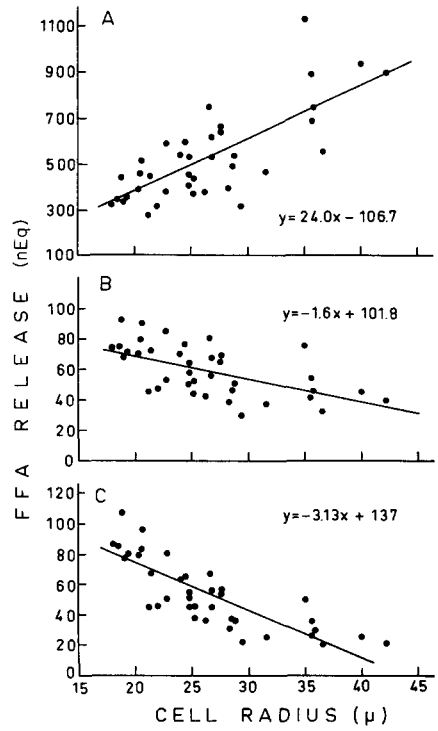

FIG. 4. Linear regression analysis of FFA release, as a function of cell radius, due to epinephrine (1 μg/ml) stimulation. Calculated *A*, per 10^5 cells; *B*, per cm^2 surface area; and *C*, per μmole of TG in the system. The plots show results of 37 experiments carried out with washed cells at 37°C for 30 min. In all plots $P < 0.01$.

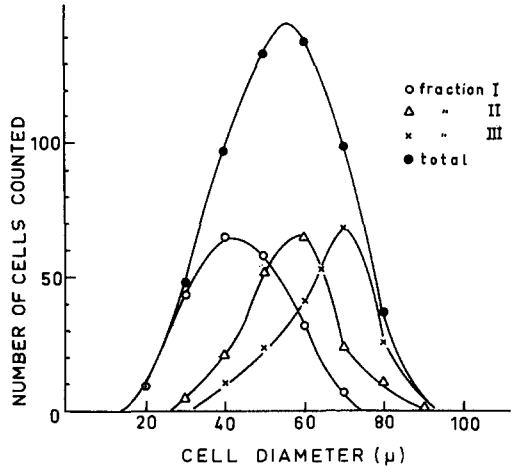

FIG. 5. Diameter distribution pattern of cell fractions from one animal, obtained by the flotation method of Björntorp and Karlsson (11).

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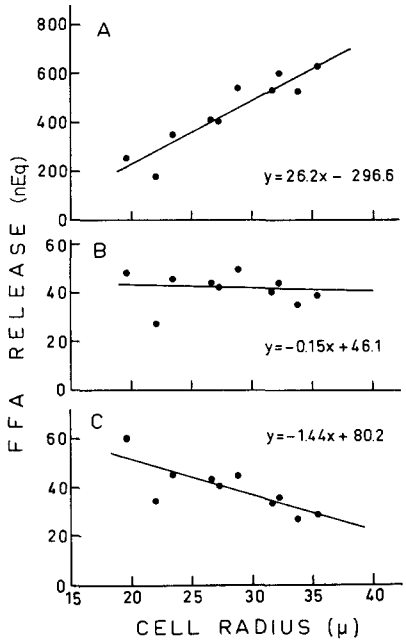

FIG. 6. Linear regression analysis as in Fig. 4, except that the points were obtained from various fractions of the same cell suspension. The plots are the total results of three experiments. In *A* and *C*, $P < 0.01$; in *B*, *P* was not significant.

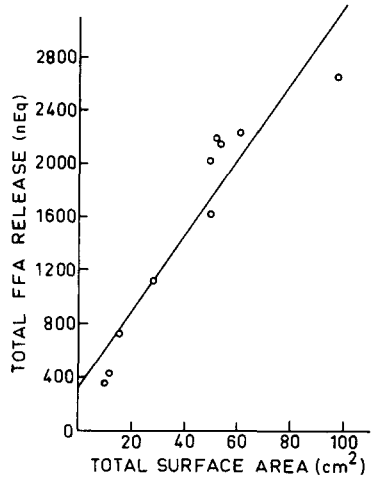

FIG. 7. Total FFA release as a function of total surface area in the system. The points were obtained from fractions of the same cell suspension, as in Fig. 6. Incubation was at 37°C for 30 min, using 1 μg/ml epinephrine. $P < 0.01$.

DISCUSSION

The accelerated release of FFA from adipose tissue due to the influence of epinephrine and ACTH is a well established fact (12–14) and has been confirmed in isolated adipose tissue cells (3, 5). Using these two hormones to promote lipolysis in fat cells, FFA production has been shown in the present study to be a function of the cell surface area. When cells of different diameters were obtained from rats of different sizes or were separated from

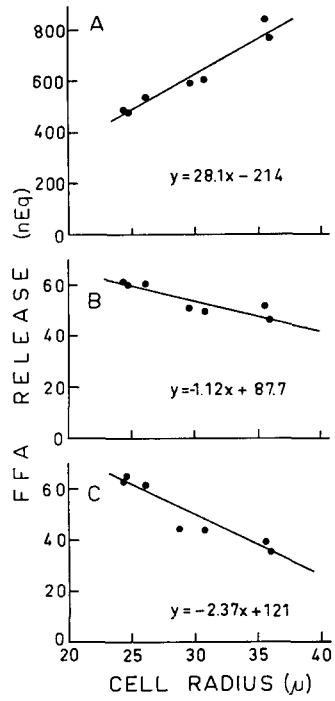

FIG. 8. Linear regression analysis of FFA release, as a function of cell radius, due to ACTH (10 mU/ml) stimulation. Calculated *A*, per 10^5 cells; *B*, per cm^2 surface area; and *C*, per μmole of TG in the system. Five experiments were carried out at 37°C for 30 min. In plots *A* and *C*, $P < 0.01$; in plot *B*, $P < 0.05$.

the same tissue sample, lipolysis per μmole of TG decreased as the cells became larger. This was due to the elevation in the ratio of cell volume to cell surface area. On the other hand, lipolysis per cell increased with increasing cell radius, in agreement with the observation that the degree of lipolysis is proportional to surface area.

Our former (1) and present findings are not in agreement with those obtained by Knittle and Hirsch (15) and recently by Greenwood, Johnson, and Hirsch (16). These investigators found that the triglyceride-synthesizing activity, as well as CO_2 production, from radioactive glucose, is constant for cells of different sizes (size measured as μg of lipid/cell) and is not proportional to the cell surface area. Differences in procedure and animal strain may be the basis for this difference. While Knittle and Hirsch (15) used cells of sizes corresponding to radii of 30–55 μ (by calculating the radius from μg of lipid/cell), in our experiments cell radii were from 15–40 μ (while animal weights were in the range of 100–300 g). When rats exceeding 300 g in weight (ca. 15 wk old) from our growth colony were used, results were difficult to assess because rapid rupture of cells took place before and during the incubation period and activity was not linear with time.

Greenwood et al. (16) used large and small cells obtained from mice which had passed the growth stage,

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while in our experiments the animals were in active growth.

It is feasible that as long as formation of new adipose cells persists (up to 15 wk of age, according to Hirsch and Han [17]) the observed increase in cell size is accompanied by an increase in cell plasma membrane and, consequently, by an increase in receptor sites for the hormones. When cell production and membrane formation stop, any increase in cell size will be due to stretching of the same membrane without changing the number of receptor sites, and activity per cell will remain constant.

The lipolytic effect of epinephrine and ACTH is exerted on the external membranes of the cells. Mosinger and Kujalova (18) found no effect of epinephrine on lipolysis in adipose tissue homogenates, whereas lipolytic activity was found in homogenates of tissues previously incubated with epinephrine. Similar evidence has been presented by Rubinstein, Daniel, Chiu, and Beck (19). The hormonal stimulation of lipase activity in the adipose tissue is mediated by a rise in the concentration of cyclic 3′,5′-AMP, as has been shown by Butcher, Ho, Meng, and Sutherland (20). Davoren and Sutherland (21) have shown that adenyl cyclase activity is found in the cell membranes of pigeon erythrocytes and of rat liver. More recently, Rodbell (22) has shown that this activity is also found in the membranes of isolated adipose tissue cells, and, as shown by Bär and Hechter (23), each hormone has distinctive sites on the membranes of rat fat cells.

If it is permissible to relate our results, obtained with isolated cells, to the activity of adipose tissue in the body, one could conclude that the total activity of adipose tissue in releasing fatty acids into the blood would decrease when the same tissue mass is made up of larger cells, and that the activity would rise with constant cell number by increasing cell size.

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