# Insensitivity of large rat adipocytes to the antilipolytic effects of insulin

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**Abstract** The ability of insulin to inhibit epinephrine-stimulated lipolysis was compared in large and small rat adipocytes. Large cells were obtained from older, obese animals (>12 months old and >500 g) and small cells were obtained from younger, leaner animals (4–5 weeks old, 140–160 g). When full insulin dose response studies were conducted it was found that large adipocytes were less sensitive to the antilipolytic effects of insulin. Thus, decreased insulin responses were seen at low insulin concentrations, while normal inhibition of lipolysis was seen at a maximally effective insulin concentration. In other words, the dose response curve for insulin's antilipolytic action was shifted to the right, and this is consistent with the previously reported decrease of insulin receptors in these cells. Furthermore, since the maximal antilipolytic response to insulin was fully normal in large adipocytes, the data also indicate that the post receptor antilipolytic system is intact in these cells.

**Supplementary key words** insulin resistance · lipolysis · epinephrine · obesity

It is well known that insulin initiates its cellular effects by binding to a plasma membrane receptor (1), and that adipocytes respond maximally to insulin when only a minority of all available insulin receptors are occupied (2–5). This observation has led to the spare receptor concept, which holds that adipocytes have an excess of receptors, all of which are fully functional, and that these spare receptors enhance the sensitivity of these cells to the relatively low insulin concentrations that prevail in vivo. According to this line of reasoning, if the number of insulin receptors per cell is decreased, the functional consequence will be a decrease in insulin action at low insulin concentrations, with normal effects at higher insulin concentrations, i.e., a right shift in the insulin dose response curve or decreased insulin sensitivity (6). This sequence has been clearly demonstrated by Kono and Barham (2) and El-Allaway and Gliemann (4), who studied glucose oxidation in adipocytes whose complement of insulin receptors had been decreased by mild trypsinization. Additionally, we have previously reported that large adipocytes from older, fatter rats have decreased numbers of insulin receptors (7, 8), and that the expected consequence of this defect, i.e., a rightward shift in the insulin–glucose transport dose response curve, could be observed (8).

Insulin markedly inhibits hormone-stimulated lipolysis in adipocytes, and this effect provides another convenient system in which to assess insulin sensitivity in these cells. In these studies we have compared insulin's antilipolytic effects in large and small adipocytes and have found that, while maximal antilipolytic effects are comparable in both groups of cells, the large cells are less sensitive to submaximal concentrations of insulin.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats were used for all experiments. Younger, leaner animals were 4–5 weeks old and weighed 140–160 g, and older, fatter animals were more than 12 months old and weighed more than 500 g. All animals were fed ad libitum until the morning of each experiment.

### Preparation of isolated adipocytes

All studies were begun between 8 and 9 AM. Animals were stunned by a blow to the head, decapitated, and epididymal fat pads were removed. Isolated fat cells were prepared by shaking at 37°C for 60 min in Krebs-Ringer bicarbonate buffer containing collagenase (3 mg/ml) and albumin (40 mg/ml), according to the method of Rodbell (9). Cells were then filtered through 250 $\mu$m nylon mesh, centrifuged at 400 rpm for 4 min, and washed two times

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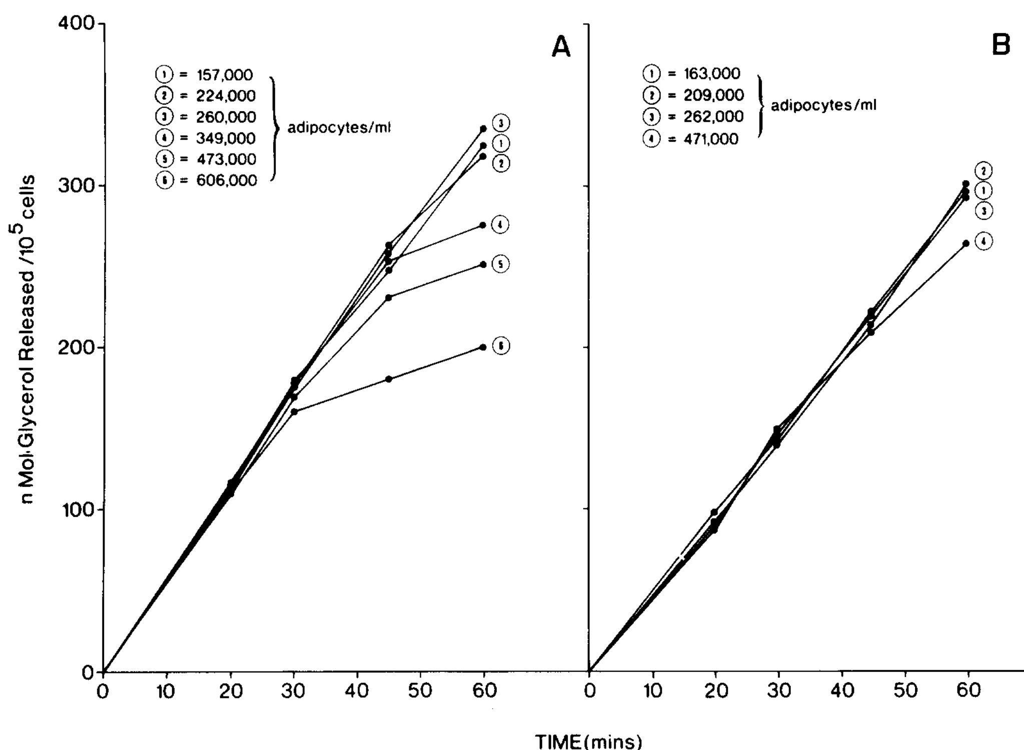


**Fig. 1.** Time course of glycerol release by small adipocytes from younger, leaner animals (*A*) and by large cells from older fatter animals (*B*). Epinephrine concentration was 0.3 $\mu$g/ml in all tubes. Four separate experiments were performed, each employing several different cell concentrations. The results in the figure are the data from a representative experiment. All data are normalized to $10^5$ cells/ml, and each line represents an incubation conducted at the indicated different cell concentration.

in buffer (4, 5, 7, 8). Adipocyte counts were performed according to a modification of method III of Hirsch and Gallian (10), in which the cells were fixed in 2% osmium tetroxide in 0.05 M collidine buffer (made isotonic with saline) for 24 hr at 37°C and then taken up in a known volume of 0.154 M NaCl for counting. Counting was performed using a Coulter Counter model ZB, with a 400 $\mu$m aperture. Aliquots for cell counts were obtained before and after the incubation period from test tubes containing cells treated identically to the incubates used for the lipolysis studies. The cell number used for each experiment was the mean of the two measurements made before and after the study. Furthermore, the mean cell counts before and after the incubation were comparable, indicating that cell breakage did not occur during the 1 hr lipolysis study. Adipocyte size was determined using a calibrated microscope according to the method of Di Girolamo, Medlinger, and Fertig (11).

### Lipolysis studies

Lipolysis was measured by previously described methods (12) in which the release of glycerol from adipocytes is taken as an index of lipolysis. Adipocytes (<250,000 cells/ml) were incubated in 2 ml of Krebs-Ringer bicarbonate buffer containing 1 mM glucose, pH 7.4, at 37°C for 60 min at the indicated concentrations of epinephrine and/or insulin. At the end of the incubation period the buffer was removed, deproteinated, and glycerol was enzymatically determined (12). Unless otherwise indicated, the epinephrine concentration used to stimulate lipolysis was 1.68 $\mu$M or 0.3 $\mu$g/ml.

## RESULTS

Schwabe, Ebert, and Erbler (13), Schwabe, Schonohofer, and Ebert (14), and Fain and Wieser (15) have demonstrated that during incubation adipocytes release adenosine which, itself, has antilipolytic effects. Thus, the higher the cell concentration, the lower will be the rate of lipolysis per cell due to the accumulation of this inhibitor in the incubation medium. Obviously, when insulin's antilipolytic action is assessed, it is important to avoid potentially confounding effects of released adenosine. Therefore, before measuring the antilipolytic effect of insulin, we studied the time course of epinephrine-stimulated



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glycerol release by large and small adipocytes as a function of cell concentration. These studies are summarized in **Fig. 1,** and it can be seen that the results confirm the observations of Schwabe et al. (13, 14) and Fain and Wieser (15). Thus, as the period of incubation became more prolonged, the rate of glycerol release became nonlinear (presumably due to adenosine accumulation), and this effect was more pronounced the higher the cell concentration. However, provided cell concentrations $<2.5 \times 10^5$ cells/ml were used and incubations were not carried out past 60 min, antilipolytic effects were not observed. These results were presumably due to the fact that sufficient medium concentrations of adenosine[2] did not accumulate under these conditions.

The ability of increasing concentrations of epinephrine to stimulate lipolysis in large and small adipocytes is presented in **Fig. 2**. As noted by other workers (16–19), these dose response curves are bimodal, and the reason for this is not entirely clear (16–19). The lipolytic peak occurring at lower epinephrine levels has been termed lipolysis I, while the second peak has been termed lipolysis II. Although both curves are bimodal, they are not parallel, and it is obvious that comparisons of epinephrine-stimulated lipolysis for both groups of cells will differ depending on the epinephrine level used. Furthermore, adipocyte sensitivity to insulin's antilipolytic effects decreases as the epinephrine concentration increases (17, 18). With these factors in mind, we decided to study insulin's antilipolytic effects in large and small adipocytes at an epinephrine concentration that produced submaximal rates of lipolysis in both groups of cells ($1.68 \times 10^{-6}$M). The dose response curves for insulin's antilipolytic effects are summarized in **Fig. 3**. It can be seen that, in the absence of insulin, the hormone-stimulated rates of lipolysis are reasonably comparable for large and small adipocytes. Furthermore, although a higher insulin concentration is needed to achieve maximal antilipolysis in the large cells, at these maximally effective insulin levels, lipolysis is 80% inhibited in both groups of cells. On the other hand, at submaximal levels of insulin, large cells are significantly less sensitive to the antilipolytic effects of insulin; in other words, the dose response curve is shifted to the right.

If the maximal absolute antilipolytic effect of insulin is taken as 100%, the percent of the maximal effect achieved at each insulin level can be calculated by the formula

$$\frac{\text{absolute effect at submaximal concentration}}{\text{absolute effect at maximal concentration}} \times 100$$

These results are presented in **Fig. 4**, and this analysis provides a better estimate of the insulin sensitivity of a metabolic process, particularly when comparisons are being made in which the basal or maximal values are unequal (7, 20). With this approach (Fig. 4) it is evident that the response curve for the large cells is still shifted to the right, and that half maximal insulin effects are reached at about 0.1 ng/ml in small cells and 0.2 ng/ml in large cells.

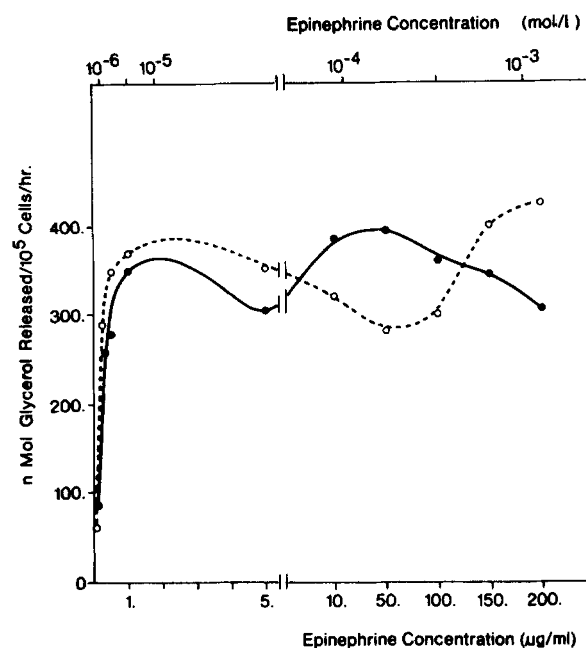


**Fig. 2.** Effect of increasing epinephrine concentration on glycerol release from large (○) and small (●) adipocytes. Data represent the mean of three separate experiments. The epinephrine concentrations below 5 $\mu$g/ml are 0.1, 0.3, 0.5, and 1 $\mu$g/ml.

## DISCUSSION

Decreased insulin binding has been found in a variety of tissues from obese animals and man (21–26), and we have recently reported that adipocytes from older, obese rats also have decreased insulin receptors (7, 8). Since adipocytes exhibit maximal insulin responses when only a minority of insulin receptors (10%) are occupied (2–5), the functional consequence of an absolute decrease in cellular insulin receptors should be a right shift in the insulin dose response curve (6, 27). Thus, providing the cellular effector system is intact, cells with fewer

[2] It should be noted that these studies simply show the accumulation of an inhibitor and its dependence on cell concentration. They do not specifically identify this inhibitor as adenosine, although recent reports indicate that this is likely to be the case (13–15). Clearly, this inhibitory effect could involve other substances.



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receptors will demonstrate decreased responsiveness at low concentrations of insulin but normal responses to maximal levels of insulin. In other words, insulin–receptor complexes are rate determining for insulin action until a certain fractional receptor saturation is reached (~10%). At receptor occupancies above this level, some step distal to the insulin receptor becomes rate determining. Direct experimental evidence for this spare receptor hypothesis has been provided by Kono and Barham (2) and El-Allaway and Gliemann (4) who found that when adipocyte insulin receptors were decreased by mild trypsin treatment the subsequent insulin–glucose oxidation dose response curves were shifted to the right. Furthermore, similar findings have been reported with large adipocytes from older, obese rats in which decreased insulin binding and a right shift in the insulin–glucose transport dose response curve was found (8).

These current studies clearly demonstrate that large adipocytes from older, heavier rats are less sensitive to the antilipolytic effects of low concentrations of insulin, compared to small cells from younger, leaner animals.

Thus, while insulin's maximal antilipolytic ability is the same in both groups of cells, the entire dose response curve is shifted to the right. Half maximal

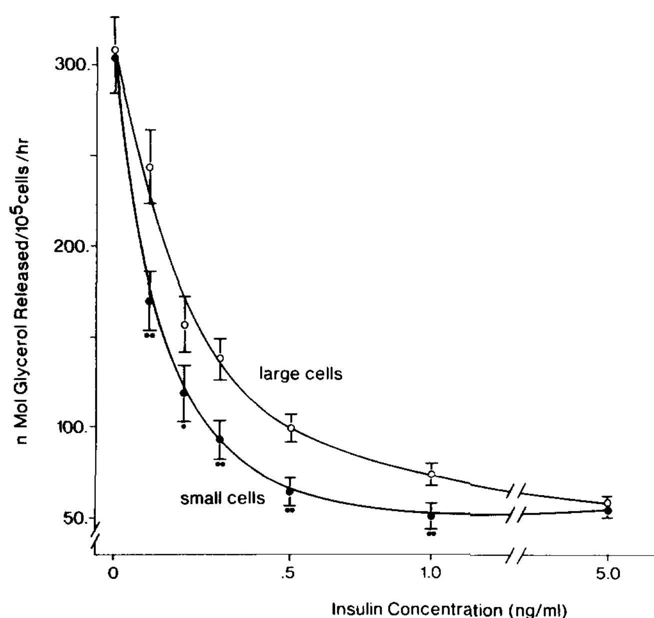


**Fig. 3.** Dose response curves for the antilipolytic effect of insulin in large (○) and small (●) adipocytes. Data represent the mean (±SE) at each point, and are the sum of seven separate experiments for each group. Epinephrine concentration used was 0.3 μg/ml. Two asterisks means $P < 0.01$, one asterisk means $P < 0.05$, no asterisk means lack of significant difference. The mean adipocyte volume was 63 ± 7 pl for the small adipocytes compared to 360 ± 31 pl for the large cells.

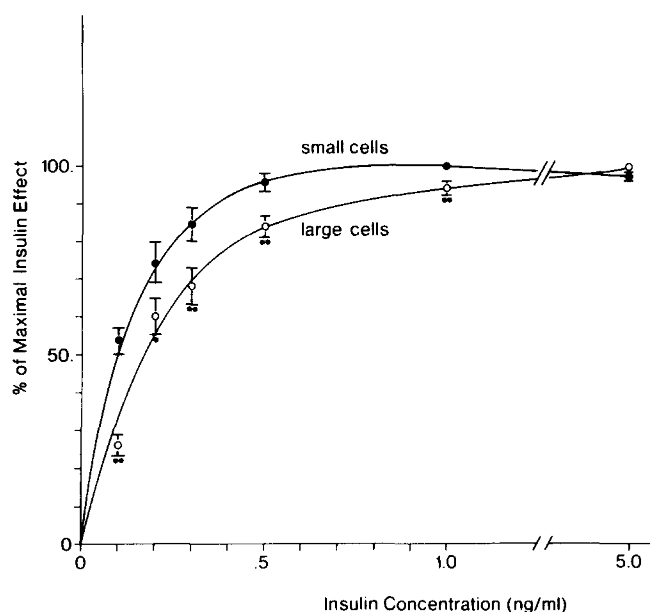


**Fig. 4.** Ability of insulin to inhibit epinephrine (0.3 μg/ml) stimulated lipolysis for large (○) and small (●) adipocytes. Data are derived from the results presented in Fig. 3 by dividing the decrease in glycerol release due to insulin at each concentration by the maximal decrement in glycerol release and multiplying by 100. Thus, the points represent the mean (±SE) percent of the maximal insulin effect at each insulin concentration. Two asterisks means $P < 0.01$, one asterisk means $P < 0.05$.

insulin effects occur at an insulin concentration of 0.08 ± 0.01 ng/ml in small cells and 0.18 ± 0.03 ng/ml in large cells. These differences are significant ($P < 0.01$) and it should be noted that this twofold increase in the half maximally effective insulin level correlates nicely with the previously reported 45% decrease in the number of insulin receptors on these cells (7, 8).

Since both groups of cells exhibit the same maximal responsiveness to high insulin levels, the postreceptor effector systems for insulin-induced antilipolysis are probably intact in the large adipocyte. Thus, although severe intracellular defects in glucose oxidation and fatty acid synthesis have been demonstrated in these cells, no evidence for an intracellular abnormality in the antilipolytic mechanisms is found.

Previous reports on the lipolytic effects of catecholamines[3] in large and small adipocytes have yielded conflicting results. For example, several groups have found that, in the absence of insulin, catecholamine-

[3] In the past, different groups have used either epinephrine or norepinephrine to stimulate adipocyte lipolysis, and these agents give essentially identical results (16). Therefore, no distinction will be made between these two agents in the remainder of the discussion, and the term catecholamine-stimulated lipolysis will be used.



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stimulated lipolysis is greater in large adipocytes (28–31) while others have found no effect of cell size or rat weight on stimulated lipolytic rates (17, 32–35). In the current studies, the catecholamine-stimulated lipolytic rates are comparable in both groups of cells and, therefore, our data agree more closely with the latter results (17, 32–35). No definitive explanation for these discrepancies is available, but it is possible that they may relate, in part, to the recently described antilipolytic effect of adenosine, which is normally released by cells into the incubation medium. The concentration of this inhibitor will obviously be related to the cell concentrations used and, if higher cell concentrations are employed when small cells are studied compared to large cells, the lipolytic rates observed with the small adipocytes may be spuriously low. This is a reasonable possibility since, when equal weights of fat tissue are studied (31), greater numbers of small cells will be employed than of larger cells. As seen in Fig. 1, attempts were made to avoid any potential effects of inhibitor accumulation in these current studies. Furthermore, as previously demonstrated by Miller and Allen (17), and as can be seen from Fig. 2, catecholamine-stimulated lipolysis can be increased, decreased, or normal in large adipocytes from older, fatter rats depending on the catecholamine concentration used. Finally, our studies were performed on large and small adipocytes from rats of different age, weight, etc., and the greatest effects of cell size were reported with different size adipocytes from the same animal ("within" animals studies) (30). Thus, the "between" animal variables could also contribute to the apparent lack of effect of cell size on rates of catecholamine-stimulated lipolysis.

Antilipolytic effects of insulin have also been previously compared in large and small adipocytes, again with conflicting results. Thus, either normal (36) or subnormal (17) responsiveness to maximal levels of insulin have been reported. Different amounts of adenosine accumulation could play a role in leading to these discrepancies, as could the concentration of catecholamine employed. For example, as has been shown by Miller and Allen (17) and Desai, Li, and Angel (18), lipolysis stimulated at lower levels of catecholamine (lipolysis I) is quite insulin sensitive, while lipolysis II is not inhibited by insulin. Therefore, in the current studies, submaximal levels of epinephrine were employed in order to optimize antilipolytic effects of insulin. In order to relate insulin's antilipolytic effects to insulin binding, detailed dose response curves must be constructed for both groups of cells. Two other workers have attempted this comparison. Gries (37) compared the insulin–antilipolysis dose response curves using adipocytes from normal and obese subjects, and found results essentially identical to those seen in Figs. 3 and 4, i.e., a right shift in the dose response curve of the cells from the obese patients. On the other hand, Jacobsson et al. (36), in a recent and excellent study, found no abnormality in the dose response curve for insulin's antilipolytic action on large human adipocytes. In the study by Gries (37), and in the current report, a relatively low catecholamine concentration was used (0.1 and 0.3 $\mu$g/ml, respectively), while Jacobsson et al. (37) employed a considerably higher level (10 $\mu$g/ml). Since adipocytes are much less sensitive to insulin's antilipolytic effects at higher catecholamine concentrations (17, 18), this difference in experimental design may account for the difference in results. It is also possible that the large adipocytes studied by Jacobsson et al. (36) were obtained from patients who were not much more obese than the controls and, therefore, may not have exhibited decreased insulin binding.

It is obvious that differences in cell concentration, catecholamine concentration, patient selection, and whether comparisons are made between large and small cells across individuals or within a single individual can all affect the experimental results. Thus, since the large and small cells in these studies were obtained from animals that differed in age, degree of obesity, caloric intake, plasma insulin level, etc., the results do not offer any insight into which of the above variables causes the observed differences. Nevertheless, the studies currently reported do demonstrate that adipocytes from older, fatter rats display a clear right shift in the insulin dose response curve for antilipolysis, and this is the predicted consequence of the decreased insulin binding, which we have previously demonstrated in cells from these animals.

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