

Effect of cell size on lipolysis and antilipolytic action of insulin in human fat cells

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Abstract The lipolytic response to catecholamines and the antilipolytic effect of insulin were studied as a function of adipose cell size and number. The results show that cellular enlargement is associated with an increase in the basal lipolysis as well as the release of glycerol induced by salbutamol (a β_2 -receptor agonist), noradrenaline, adrenaline, and isopropyl-noradrenaline. The glycerol release induced by all these agents seems to be more favorably correlated with cell surface area than with cell volume or diameter.

Under the incubation conditions used with glucose in the medium, the antilipolytic effect of insulin on the basal as well as on the adrenaline- and isopropyl-noradrenaline-stimulated lipolysis was not consistent at any cell size studied. However, in the presence of noradrenaline and salbutamol, insulin exerted a consistent antilipolytic effect. The results show that the larger adipose cells are at least as sensitive to the antilipolytic effect of insulin as the smaller cells.

The results imply that the previously reported diminished responsiveness to insulin shown by large adipose cells is exerted only on the side of lipid accumulation. It is suggested that the negative correlation between cell size and responsiveness to insulin on the side of lipid accumulation may be one way to control adipose cell enlargement.

Supplementary key words adipocyte size · adipocyte number · catecholamines

RECENT INVESTIGATIONS have shown that adipose cell size is an important parameter for the rate of lipid synthesis from glucose (1-3) as well as for the basal rate of lipolysis (4-7). In addition, it is now well established that enlarged adipose cells are less responsive than small cells to the stimulating effect of insulin on the metabolism and uptake of glucose and exogenous fatty acids (1, 3, 8-10). The reason for this diminished responsiveness is presently unknown. An increased rate of

lipolysis (11, 12) or alteration of the insulin receptors associated with cellular enlargement (8) have been suggested as possible reasons.

In the present study the antilipolytic effect of insulin in human adipose cells of different sizes was studied. In addition, the effect of cell size on lipid mobilization in response to lipolytic hormones was investigated.

MATERIAL AND METHODS

Source of tissue

Biopsies of subcutaneous adipose tissue were obtained from patients undergoing surgery for cholecystolithiasis or exploratory laparotomy. Patients with diabetes mellitus, jaundice, or malignant disease were excluded. All patients were fasted overnight. Anesthesia was induced with a short-acting barbiturate and maintained with halothane, nitrous oxide, and oxygen. The biopsies were usually obtained at the beginning of the operations. Clinical data of the patients are shown in Table 1. It is clear from these data that the mean size of adipose cells taken from patients of the same weight may differ greatly. Thus, the biopsy with the largest mean cell size was not obtained from the fattest patient.

Immediately after excision the biopsy specimens were placed in a vessel containing 20 ml of Parker's medium 199 (for composition see Ref. 13; Statens Bakteriologiska Laboratorium, Stockholm, Sweden) modified to a glucose concentration of 1.0 mM.

Tissue preparation and cell isolation technique

The mean cell size of the biopsies was determined as previously described (3). Briefly, smaller specimens weighing about 20-30 mg each were dissected from different parts of the biopsies. The fat cells were isolated

TABLE 1. Data of patients in the metabolic studies

Patient	Age	Sex	Height	Weight	% of Ideal Body Weight ^a	Mean Cell Size
	yr		cm	kg		$\mu\text{m} \pm \text{SEM}$
1	68	M	182	78.0	107	84.9 \pm 1.95
2	40	F	166	76.0	134	110.1 \pm 2.29
3	36	M	185	105.0	140	105.8 \pm 2.90
4	49	M	179	96.0	136	99.4 \pm 2.40
5	53	M	175	84.5	125	95.5 \pm 1.69
6	50	M	173	75.0	114	76.0 \pm 1.72
7	59	M	158	70.5	125	96.6 \pm 1.89
8	52	M	174	80.5	121	99.6 \pm 1.92
9	73	F	147	52.5	115	66.8 \pm 0.92
10	25	M	186	72.5	95	79.7 \pm 1.48
11	43	M	181	116.0	161	97.3 \pm 2.59
12	31	M	168	76.0	122	110.3 \pm 2.07
13	76	F	158	73.0	141	113.5 \pm 1.75
14	72	F	159	67.0	129	101.9 \pm 2.05
15	52	M	178	79.0	114	100.4 \pm 2.04

^a Calculated from desirable weight (Metropolitan Life Insurance Co.).

from the stroma by incubating the specimens with collagenase (Sigma type I, Sigma Chemical Co., St. Louis, Mo.) for 60 min. The diameters of 100 cells were measured with a calibrated ocular in a Zeiss photomicroscope, and the mean cell diameter was determined.

Assay procedure

Fragments from the tissue samples (total weight 300–500 mg) were incubated in 2 ml of Parker's medium 199 (modified to a glucose concentration of 1.0 mM) in the presence or absence of maximal concentrations (5×10^{-5} M) of noradrenaline, adrenaline (Astra AB, Södertälje, Sweden), isopropylnoradrenaline (Riker Laboratories, Loughborough, England), α_1 -(*t*-butylaminoethyl)-4-hydroxy-*m*-xylene- α_1, α_3 -diol (salbutamol; Allan and Hanburys Ltd., Ware, England), and the indicated concentrations of insulin (recrystallized pork insulin, Vitrum AB, Stockholm, Sweden).

All incubations were performed in tightly stoppered siliconized glass vials for 2 hr at 37°C and at pH 7.4 ± 0.2 . The gas phase was air. Aliquots of the incubation media were taken for glycerol determinations at 0 min and after incubation for 120 min. Glycerol was determined enzymatically as described by Laurell and Tibbling (14). The release of glycerol was taken as an index of the lipolysis.

The tissue lipids were extracted with chloroform-methanol 2:1 (v/v) as described by Folch, Lees, and Sloane Stanley (15). Aliquots of the chloroform phase were taken for the determination of the glyceride-glycerol according to the method described by Carlson (16).

Determination of fat cell volume, number, and cell surface area

The mean cellular volume was calculated on the assumption that the cells were spherical, using the formula suggested by Goldrick (17):

$$V = \frac{\pi}{6}(3\sigma^2 + \bar{x}^2)\bar{x}$$

in which V = volume, σ^2 = variance of mean cell diameter, and \bar{x} = mean cell diameter.

The mean cellular weight was calculated on the assumption that the density of fat cells is that of triolein (18). When the glyceride-glycerol content of the specimens is known, the number of fat cells can be calculated.

The mean cell surface area was calculated as suggested by Zinder and Shapiro (19) according to the relationship: mean surface area = $\pi(\sigma^2 + \bar{x}^2)$.

RESULTS

Effect of cell size on lipolysis

The basal rate of glycerol release was significantly correlated with cell diameter (Fig. 1; $r = 0.685$, $P < 0.01$). The effect of cell diameter on the glycerol release induced by salbutamol, noradrenaline, adrenaline, and isopropylnoradrenaline was then studied. The absolute increase in glycerol release induced by all these agents was significantly correlated with cell diameter ($r = 0.530$, $P < 0.05$; $r = 0.536$, $P < 0.05$; $r = 0.579$, $P < 0.05$; $r = 0.645$, $P < 0.01$, respectively). As an example, the effect of isopropylnoradrenaline is shown in Fig. 2. A positive correlation between cell diameter and the lipolytic effect of isopropylnoradrenaline is noted. However, as shown in Fig. 2a, the regression line does not pass through the origin. When the glycerol incre-

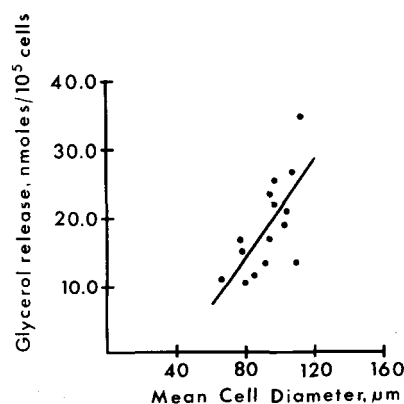


FIG. 1. Effect of cell size on the basal rate of glycerol release. Specimens of human adipose tissue were incubated for 2 hr in 2.0 ml of Parker's medium 199 modified to a glucose concentration of 1.0 mM.

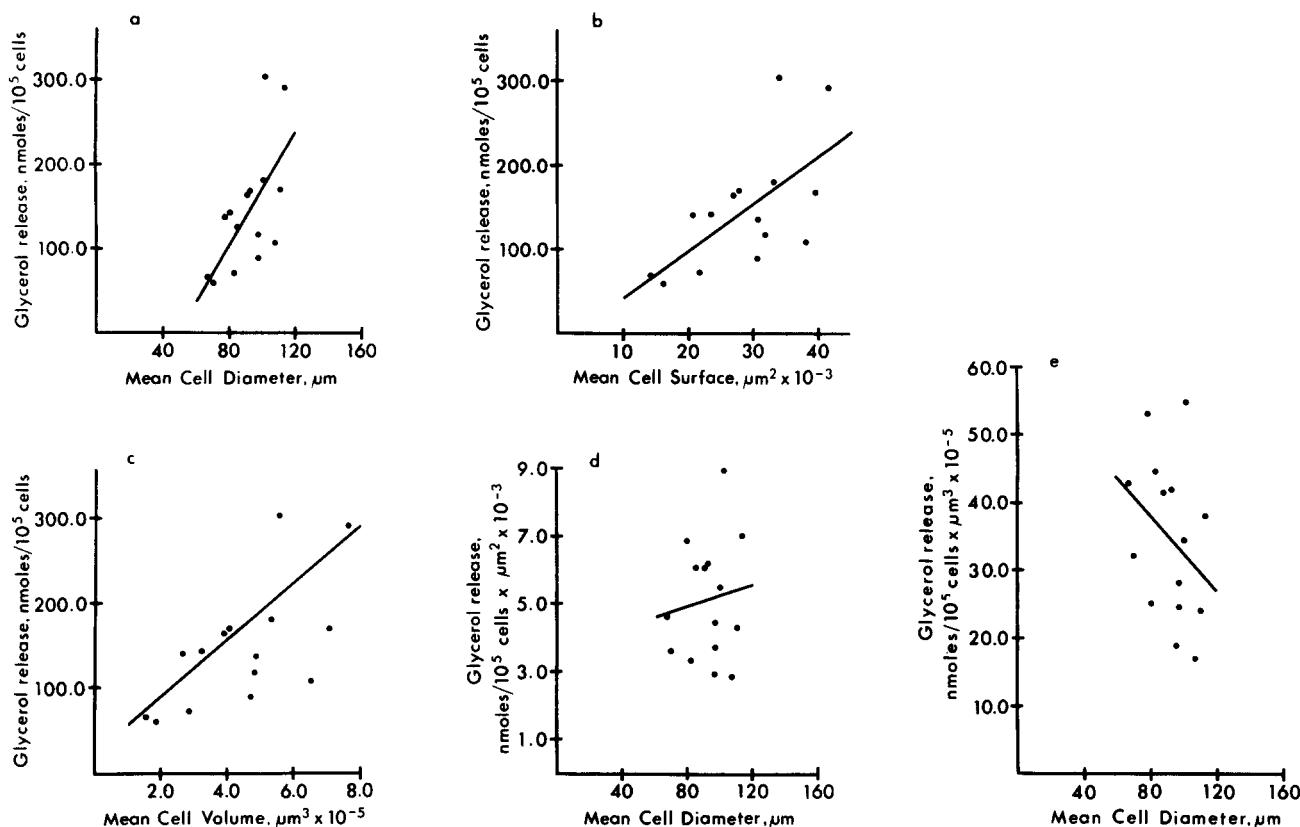


FIG. 2. Effect of cell size on the isopropylnoradrenaline-stimulated lipolysis. *a*, glycerol increment vs. mean cell diameter; *b*, vs. cell surface; *c*, vs. cell volume; *d*, glycerol release per unit surface area; *e*, glycerol release per unit volume. The specimens were incubated for 2 hr in the presence of isopropylnoradrenaline at a concentration of 5×10^{-5} M.

ments were related to mean cell surface area, the regression line intercepts close to the origin (Fig. 2*b*). When mean cell volume was used, the regression line intercepted the *y* axis (Fig. 2*c*). The increment in glycerol release induced by isopropylnoradrenaline and expressed per unit surface area is similar at any cell diameter studied (Fig. 2*d*; $y = 0.017x + 3.56$, $r = 0.131$, $P > 0.1$). However, when the values were expressed per unit volume, a negative regression line was obtained (Fig. 2*e*; $y = -0.268x + 59.51$, $r = 0.317$, $P > 0.1$).

Antilipolytic effect of insulin

Under the conditions used, the effect of large concentrations of insulin on the basal glycerol release was not consistent at any cell size studied. In some experiments insulin exerted a slight antilipolytic effect, but in most cases insulin actually enhanced the lipolysis. As shown in Table 2, the slight stimulating effect of insulin on glycerol release seemed to be a function of the concentration of insulin used.

Since there was no consistent effect of insulin on the basal lipolysis, the antilipolytic effect in the presence of isopropylnoradrenaline, adrenaline, noradrenaline, and salbutamol was studied. Insulin did not exert a consistent antilipolytic effect on the isopropylnoradrenaline-

adrenaline-stimulated lipolysis. In fact, analogous to the results obtained with basal lipolysis, large concentrations of insulin (10^5 μ U/ml) tended to stimulate the lipolytic effect of the catecholamines; this was most pronounced in the presence of adrenaline. However, in the presence of salbutamol and noradrenaline, insulin was found to exert a consistent antilipolytic effect which was maximal at a concentration of about 10^3 μ U/ml (Table 3). The

TABLE 2. Effect of insulin on basal glycerol release by human adipose tissue

Insulin Concentration	Glycerol Release	<i>P</i> Level ^a
μ U/ml	nmoles/ 10^6 cells	
0	18.4 ± 1.6	
10^2	18.9 ± 2.0	NS ^b
10^3	20.6 ± 2.7	NS
10^4	22.3 ± 2.1	<0.05
10^5	24.4 ± 3.0	<0.05

Specimens of human adipose tissue were incubated in 2.0 ml of Parker's medium 199, modified to a glucose concentration of 1.0 mM, for 2 hr with the indicated concentrations of insulin. The results are the means \pm SEM of duplicate determinations on specimens from six patients.

^a *P* values as compared with basal value were calculated according to Student's *t* test.

^b NS, not significant.

TABLE 3. Antilipolytic effect of insulin on the noradrenaline-stimulated lipolysis

Insulin Concentration	Glycerol Release	<i>P</i> Level ^a
$\mu\text{U/ml}$	$\text{nmoles}/10^6 \text{ cells}$	
0	110.4 \pm 17.7	
10 ²	84.3 \pm 9.6	<0.05
10 ³	69.1 \pm 9.3	<0.025
10 ⁴	67.5 \pm 12.3	<0.01
10 ⁵	73.4 \pm 11.1	<0.025

Specimens of human adipose tissue were incubated in 2.0 ml of Parker's medium 199, modified to a glucose concentration of 1.0 mM, for 2 hr in the presence of noradrenaline at a concentration of 5×10^{-5} M and with the indicated concentrations of insulin. The results are the means \pm SEM of duplicate determinations on specimens from six patients. The specimens were obtained from the same patients as were used for the studies shown in Table 2.

^a *P* values as compared with basal value were calculated according to Student's *t* test.

effect of cell size on the antilipolytic action of insulin was then studied. As shown in Fig. 3, the inhibitory effect of insulin on the noradrenaline-stimulated lipolysis was not significantly correlated with cell surface or cell diameter whether the results were expressed on a percentage basis (Fig. 3) ($r = 0.01$, $P > 0.1$; $r = 0.033$,

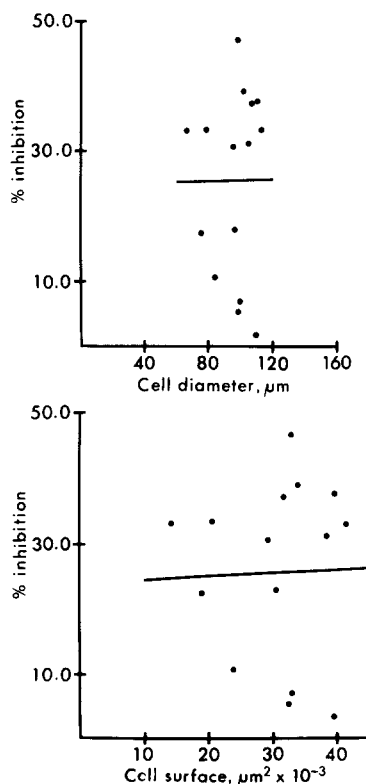


FIG. 3. Antilipolytic effect of insulin in relation to mean cell diameter (*top*) and mean cell surface (*bottom*). Specimens of human adipose tissue were incubated with or without the addition of noradrenaline and insulin at a concentration of 5×10^{-5} M and $10^5 \mu\text{U/ml}$, respectively. The results shown represent the antilipolytic effect of insulin in percentages of basal values.

$P > 0.1$, respectively) or in terms of the absolute decrease ($r = 0.370$, $P > 0.1$; $r = 0.399$, $P > 0.1$, respectively). Similar results were obtained in the presence of salbutamol. Although the data were not significant in the present study, the positive correlation coefficient between cell diameter and the absolute decrease in the noradrenaline-stimulated lipolysis indicates that the larger adipose cells are at least as sensitive as the smaller adipose cells to the antilipolytic effect of insulin.

DISCUSSION

In the present study it was found that cellular enlargement was associated with an increase in the basal lipolysis, confirming the results of previous investigations with human adipose tissue (4-7) and rat adipose tissue (20). However, the results obtained with catecholamine-stimulated lipolysis have been more controversial. Positive correlations between cell size of human adipose tissue and the lipolytic effects of adrenaline (4) and noradrenaline (6) have been found, while other investigators have reported that no such correlation was present (7). Discrepant results have also been obtained with rat adipose tissue. Zinder and Shapiro (19) found a positive correlation between cell size and the adrenaline- and ACTH-induced release of fatty acids, but Hartmann et al. (20) recently reported that there was no correlation between the size of the adipose cells and the lipolytic effect of noradrenaline. In the present study it was found that cellular enlargement was associated with an increase in the lipolytic effect of salbutamol, a β_2 -receptor agonist, noradrenaline, adrenaline, and isopropylnoradrenaline. Methodological differences may be one reason for the differences in results to those reported by Goldrick and McLoughlin (7) and Hartmann et al. (20). Goldrick and McLoughlin (7) used isolated cells of different sizes obtained from the subcutaneous as well as the omental tissue; in the investigation by Hartmann et al. (20), a rather restricted range of adipose cell size was studied (smaller than about $70 \mu\text{m}$). In addition, the differences in incubation media used may contribute to the differences under discussion (5). The results obtained by Balasse (21) indicate that, also in vivo, subjects with obesity, who have enlarged fat cells (cf. Ref. 22), have increased responsiveness to the lipolytic effect of parenterally administered catecholamines.

For the range of mean cell diameters studied (about 70 - $115 \mu\text{m}$), the glycerol release induced by the lipolytic agents seems to be more favorably correlated with cell surface area than with cell diameter or volume, which is in accord with the suggestions of Zinder et al. (1, 19) and Björntorp and Sjöström (6). This is supported by the observation that the catecholamine-stimulated glycerol release, expressed per unit surface area, was similar at

any cell size studied. When the values were calculated per unit volume, a negative regression line was obtained. The greater responsiveness of the larger adipose cells to the catecholamines may thus be due to an increase in cell surface area accompanied by an increase in the total number of receptor sites available to the hormones.

A negative correlation between cell size and responsiveness to pharmacological concentrations of insulin, determined as increase in glucose oxidation to CO₂ and increase in glucose incorporation into the lipids, has recently been demonstrated with human adipose tissue (3, 8) as well as with rat fat pads (9, 10). Enlarged rodent adipose cells are also less responsive to the stimulating effect of insulin on the uptake and esterification of exogenous fatty acids (1). However, the effect of cell size on the antilipolytic effect of insulin does not seem to have been studied previously. The results of the present investigation show that the larger adipose cells are at least as responsive as the smaller cells to the antilipolytic effect of insulin on the noradrenaline- and salbutamol-stimulated lipolysis. The same results were obtained whether the results were calculated on a percentage or an absolute basis. The results imply that cellular enlargement per se does not lead to an alteration of the insulin receptors (8), which could account for the diminished responsiveness of the larger adipose cells on the side of lipid accumulation as discussed above. Furthermore, since the lipolytic effect of a given concentration of noradrenaline and salbutamol was more pronounced in the larger adipose cells, it does not seem probable that an increase in the lipolytic rate per se leads to a decrease in the insulin responsiveness as suggested by other investigators (11, 12).

In the present system with glucose in the incubation medium, the inhibitory effect of insulin on the basal lipolysis was inconstant at any cell size studied. In fact, large concentrations of insulin tended to stimulate the basal lipolysis, and this effect increased with increasing concentrations of insulin. Although not discussed, similar results were obtained in the study by Solomon, Brush, and Kitabchi (23). Burns and Langley (24) reported that the effect of insulin on the basal lipolysis was variable. The mild stimulatory effect of insulin on basal lipolysis is presumably due to the presence of glucose in the incubation medium, since it is not seen in a glucose-free medium (25) and glucose or products of glucose metabolism may exert a direct effect on the lipolytic process (26, 27). Similarly, the adrenaline- and isopropylnoradrenaline-stimulated lipolysis was not consistently antagonized by the large concentrations of insulin used. Similar results have been reported by some investigators (26, 28–30) while others have found a significant antilipolytic effect of insulin on the adrenaline-stimulated lipolysis in the presence of glucose (7, 24).

The reason for this discrepancy between the investigations under discussion is unclear at present, but may partly be due to differences in the ages of the donors (31) and in the concentrations of insulin and lipolytic agents used (11, 23, 24).

The results of the present study make it tempting to speculate that the correlation between cell size and responsiveness to insulin is exerted only on the side of lipid accumulation and is one way for the adipocytes to control cellular enlargement and, thus, cell size.

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