SURFACE STRUCTURE CHANGES OF RAT ADIPOCYTES
DURING LIPOLYSIS STIMULATED BY
VARIOUS LIPOLYTIC AGENTS

A Scanning Electron Microscopic Study

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ABSTRACT
A qualitative and quantitative electron microscopic study was performed on rat adipocytes during stimulation of lipolysis by various agents. Scanning electron microscopy of control cells revealed a spherical cell with a textured glycocalyx surface exhibiting small irregular projections. Globular surface evaginations or protrusions measuring 8–18 μm in diameter were seen on cell hemispheres, and there was an average of one protrusion for every two hemispheres examined. Distribution analysis showed that 60% of the hemispheres had no protrusions, and 25, 10, and 5% of the hemispheres had one, two, or three protrusions, respectively. Thin-section and freeze-fracture electron microscopy of the protrusions showed a small triglyceride droplet surrounded by a thin cytoplasmic rim that was continuous with the main cytoplasmic matrix. The glycocalyx coating and plasma membrane extended from the cell surface onto, and over, the protrusion. Scanning microscopy of cells stimulated by lipolytic agents, including epinephrine, adrenocorticotropic hormone, theophylline, and dibutyryl cyclic AMP, revealed a dose-dependent increase in the number of protrusions per cell hemisphere. Maximal concentrations of lipolytic hormones caused an average 2.5-fold increase in the number of protrusions per hemisphere without changing the average size of the protrusions. Only 40% of the stimulated cell hemispheres exhibited no protrusions; over 15% of the cells contained three or more, and a number of the protrusions were multilobulate. Insulin prevented the increase in the number of protrusions and the change in distribution caused by the lipolytic hormones but did not prevent the increase caused by theophylline and dibutyryl cyclic AMP. The data suggest that the protrusions are a structural feature of the cell and may be related to the lipolytic pathway. These observations may help explain some of the discrepant biochemical data relating to hormonal stimulation of lipolysis.

KEY WORDS adipocytes • lipolysis • insulin • catecholamines • scanning electron microscopy

Adipose tissue plays a major role in fatty acid homeostasis by cycling fatty acids into and out of triglyceride storage depots (18). This metabolic
process is under multiple hormonal control, with insulin being the major hormone stimulating lipogenesis and preventing lipolysis. A number of hormones acting through the adenylate cyclase-cyclic AMP system (catecholamines, adrenocorticotropic hormone [ACTH], glucagon, etc.) stimulate lipolysis (6). Steinberg and Khoo (18) have elucidated the molecular mechanism by which the lipolytic hormones activate the hormone-sensitive lipase of adipose tissue via the cyclic AMP-sensitive protein kinase system. However, a major discrepancy, not completely explained by biochemical data, has been found between the magnitude of activation of the hormone-sensitive lipase (5, 18, 19) of rat adipocytes and the extent to which lipolysis is stimulated (6, 15, 22). A partial explanation for this discrepancy was provided by Khoo et al. (10), who found that hormone stimulation of lipolysis resulted in redistribution of the activated lipase with preference for endogenous substrate. Wise and Jungsas (21) have confirmed activation of a hormone-sensitive lipase intimately associated with the endogenous substrate and have suggested that a second mechanism exists for activation of lipolysis.

Morphological studies of the lipolytic process have primarily been qualitative thin-section electron microscopic studies. Williamson and Lacy (20) have summarized most of the earlier studies, which have shown an increase in the amount of triglyceride found in the cytoplasmic matrix and in the number of invaginated flask-shaped vesicles and micropinocytotic-like vesicles. A number of other investigators have studied the effects of various lipolytic and antilipolytic agents on adipocyte morphology (for a review, see reference 17). Recently, Carpenter et al. (3), using freeze-fracture techniques, have demonstrated that the number of invaginations per adipocyte remains constant as the concentration of invaginations per unit area changes, under various conditions that decrease the size of the cell through lipolysis.

In our study, we have used scanning electron microscopy to qualitatively and quantitatively document surface alterations in the adipocyte during stimulation and inhibition of lipolysis.

**MATERIALS AND METHODS**

Intact adipocytes were isolated from the epididymal fat pads of Sprague-Dawley rats (120-140 g) as described by Rodbell (15) with minor modifications. The fat pads from 2 to 50 rats were removed, rinsed in normal saline at 37°C, minced, and placed in a single polypropylene flask containing, per rat, 1.5 ml of Krebs-Ringer phosphate buffer with 3% bovine serum albumin and 0.2% dextrose at pH 7.4 (KRP I) to which had been added 0.5 mg of collagenase/ml. The pads were digested for 40 min at 37°C in a Dubnoff metabolic shaker (GCA/Precision Scientific Group, Chicago, Ill.) operating at 120 oscillations/min. The contents of the flask were filtered through a silk screen to remove connective tissue and undigested fat tissue. The cell suspension was washed three times by resuspension in KRP I at 37°C, centrifugation at 800 g for 30 s, and aspiration of the infranates. The final cell cake was resuspended in KRP I to yield about 10^6 cells/ml. The cells were allowed to equilibrate for 15 min at 37°C before the addition of hormones or other agents. This method yielded ~1.8 × 10^6 cells/rat.

1 ml of the adipocyte suspension was added to a 12-ml polyethylene centrifuge tube containing the hormone or agent to be tested, and the cells were incubated at 37°C for 5 or 30 min with gentle shaking. At the conclusion of the incubation, the cells were quickly diluted in Krebs-Ringer phosphate buffer, pH 7.4, at 37°C and centrifuged at 800 g for 30 s. The cell cake was resuspended in 1 ml of the same buffer at 37°C. An equal volume of 2% glutaraldehyde in phosphate-buffered saline, pH 7.4, was slowly added with constant gentle mixing to prevent clumping of the cells. After 5 min at 37°C, the cells were allowed to come to room temperature. After a 30-min fixation in glutaraldehyde, the cells were diluted with 10 ml of phosphate-buffered saline and allowed to float to the top of the solution. The infranate was removed, and the cells were similarly washed with 0.1 M sodium cacodylate-HCl buffer (NaCac), pH 7.4. The cell cake was resuspended in 1 ml of 0.1 M NaCac, and an equal volume of 2% OsO₄ in 0.1 M NaCac was added dropwise with constant mixing. The cells sank rapidly and were allowed to osmicate for 30 min, after which they were washed twice with 0.1 M NaCac and once briefly with deionized water.

Dehydration of the cells was attempted by several methods, including treatment with a graded series of alcohol or acetone and treatment with acidified dimethoxypropane (13). The best results were obtained by treating the cells with ethylene glycol at concentrations of 30, 60, 95, and 100% for 3–5 min/concentration, followed by two 5-min rinses in 100% ethylene glycol monomethyl ether (14). The cells sank rapidly in 30 and 60% ethylene glycol, but centrifugation at 1,000 g for 1 min was required to recover the cells from 95 and 100% ethylene glycol. The cells suspended in ethylene glycol monomethyl ether were filtered through a silk screen to remove the few clumps of cells caused by the fixation procedure. A monolayer of cells was applied to a Gelman AN-5000 filter (Gelman Instrument Co., Ann Arbor, Mich.) under very gentle vacuum, and care was taken not to allow the cells to air-dry. The filters were transferred to a critical-point drying apparatus and dried with CO₂ (1).

The dried filters were cut into 2 × 8-mm rectangles and attached to specimen holders with double-sided tape or silver paint. A 100- to 200-Å Au/Pd coating was applied by sputter coating in an argon atmosphere. The cells were examined in a JEOL 100CX electron microscope with an ASID-4D scanning attachment at 20 kV. Between 350 and 900 cells were examined for each experimental condition, and each experiment was performed at least twice.

For quantitative analysis, the cells were examined at a mag-
mification of × 800 at a tilt angle of 0°, and only the protrusions on the "upper hemisphere" of each cell were counted. Data are presented in terms of "per hemisphere" rather than "per cell." The diameter of the protrusions was measured at their widest point directly on the SEM screen, and these data were converted to micron dimensions.

Adipocytes were prepared for thin-section analysis as previously described (9). Adipocytes for freeze-fracture analysis were fixed for 1-2 min in 0.5% glutaraldehyde and washed in phosphate-buffered saline before freezing. Freeze-etch replicas were prepared and cleaned as described by Carpentier et al. (3).

Male Sprague-Dawley rats were purchased from Eldridge Laboratory Animals, Barnhart, Mo. Collagenase, bovine serum albumin, ethylene glycol, and ethylene glycol monomethyl ether were obtained from Sigma Chemical Co., St. Louis, Mo. Glutaraldehyde and osmium tetroxide were purchased from Polysciences, Inc., Warrington, Pa. All other reagents and materials were obtained from standard sources.

RESULTS

Adipocyte Preparation

It was necessary to develop a technique for preparation of adipocytes to be examined by scanning electron microscopy. To this end, most routine fixation and dehydration techniques were tried. Poor results were obtained with all polar organic solvents used for dehydration. Although high-magnification observation of the cell surface suggested that the membrane surface itself had been adequately preserved, these reagents produced collapse and, apparently, lipid depletion of the cells (not illustrated). This unacceptable artifact was overcome by using the graded series of ethylene glycol and ethylene glycol monomethyl ether treatments described. These reagents required the use of a nonreactive membrane filter such as the Gelman AN series to prevent artificial surface contamination of the cells with filter residues. Cells prepared by the described method of dehydration showed little, if any, signs of collapse or shrinkage (Fig. 1). High-magnification observation demonstrated that the cell surfaces were virtually identical, regardless of the solvent-dehydration system used (not shown).

Critical-point drying prevented the partial collapse or flattening of the cells and the cracking of the glycocalyx that was observed in 15-20% of the air-dried cells.

Adipocyte Morphology

The adipocytes from 120-140-g rats varied in diameter from 25 to 125 μm and appeared as

![Figure 1](https://example.com/figure1.jpg)

**Figure 1** A low-magnification scanning electron micrograph of isolated adipocytes. The cells were primarily spherical and ranged from 25 to 125 μm in diameter. The surface of the cell is seen as textured, with several small (<3 μm in diameter) surface irregularities (arrows). Bar, 10 μm. × 950.
spheres with a textured coating (Fig. 1). This coating probably corresponds to the glycocalyx coating seen by thin-section transmission electron microscopy (8, 9). Occasional, small, irregular projections were seen. About half of the hemispheres examined had a large, globular surface evagination or protrusion (Table I and Fig. 2). Distribution analysis showed that 60% of the cells had no such structures, and that 25, 10, and 5% had one, two, and three, respectively (Fig. 3). These protrusions appeared to be randomly distributed on the cell surface and varied in size from 8 to 18 μm in diameter. When viewed at an appropriate angle (Fig. 2), the protrusions seemed to be projecting from the cell surface, yet the glycocalyx coating of the protrusion was continuous with that of the cell.

At high magnification (Fig. 2), numerous small irregularities in the glycocalyx were seen. Some of these gave the appearance of being localized thickenings of the glycocalyx whereas others resembled strands of material associated with the cell coat.

The protrusions were infrequently seen in con-

**Table 1**

Effect of Insulin and Lipolytic Agents on the Formation of Surface Protrusions

| Lipolytic agent       | Mean protrusions/adipocyte hemisphere |
|-----------------------|--------------------------------------|
| Insulin               |                                      |
| None                  | 0.49 (1,112)*                        |
| 100 mU/ml ACTH        | 1.11† (1,271)                        |
| 1.25 μg/ml epinephrine| 1.04‡ (1,303)                        |
| 2 mM theophylline     | 1.15‡ (1,375)                        |
| 1 mM DBcAMP           | 0.96‡ (1,055)                        |

* Numbers in parentheses are the total number of hemispheres observed in two experiments.
† Significantly different from control as determined by Student's t test, P < 0.001.
‡ Significantly different from control as determined by Student's t test, P < 0.01.

**Figure 2** A scanning electron micrograph demonstrating the globular evagination or protrusion on the surface of the adipocyte. The protrusion is covered with the same textured surface as the cell and shows many points of continuity with the cell. Because the magnification is higher than in Fig. 1, the small irregularities on the textured surface can be better visualized. Bar, 2 μm. × 5,000.
control cells by both thin-section (Fig. 4) and freeze-fracture (not shown) electron microscopy. Fig. 4 shows a protrusion composed of a large triglyceride droplet surrounded by a thin rim of cytoplasm that is continuous with the cytoplasm surrounding the main triglyceride droplet. Occasionally, various organelles (mitochondria, for example) were seen in the thin cytoplasmic rim, but they were not seen as frequently as in the main cytoplasmic matrix of the cell. Higher magnifications (not shown) revealed that the glycocalyx coating and plasma membrane of the cell extended onto and over the protrusion.

Effects of Lipolytic Agents and Insulin on Adipocyte Morphology

The effect of various lipolytic agents, including epinephrine, ACTH, DBcAMP, and theophylline, on the surface structure of adipocytes was tested. Each of these agents caused more than a doubling of the average number of protrusions per adipocyte hemisphere (Table I), which represents a significant increase ($P < 0.001$). The diameter of 100 randomly selected protrusions from each incubation condition was measured (Table II). There was a slight, statistically insignificant increase in the mean diameter of the protrusions in each set of treated cells. This small increase was a result of the multilobulate structures seen in treated cells but not in control cells. Fig. 3 shows that a marked change in distribution of the protrusions occurred with treatment with the various lipolytic agents. Only 40% of the cell hemispheres examined had no protrusions, and a much larger proportion of the cell hemispheres exhibited three to five protrusions per hemisphere. No apparent difference in the number or distribution of the protrusions was observed among the various lipolytic agents. A small, but significant, relationship of protrusion formation to concentration was seen when submaximal and maximal concentrations of lipolytic agents were tested (Table III). However, no time-related increase between 5- and 30-min incubations was seen (data not shown). Fig. 5 is a scanning electron micrograph demonstrating the increase in protrusions caused by ACTH and showing examples of multilobulate protrusions. The other lipolytic agents caused similar structural changes (not shown). The multilobulate protrusions were not seen on control cells. Thin-sections of cells treated with lipolytic agents showed structures identical to those in Fig. 4, including multilobulate protrusions (Fig. 6).

Insulin in the absence of lipolytic agents had no effect on the distribution or mean number of protrusions per adipocyte hemisphere (Table I and Fig. 3). Insulin also had no effect on the size of the protrusions (Table II). However, insulin completely prevented alteration in the number and distribution of protrusions by epinephrine and ACTH. Insulin did not prevent the effects of theophylline or DBcAMP (Table I and Fig. 3).
A thin-section electron micrograph through a protrusion, demonstrating the apparent continuity of the cell plasma membrane (PM) and glycocalyx with those of the protrusion. The lipid droplet (L₁) of the protrusion is surrounded by a cytoplasmic matrix (C₁) that is continuous with the cell's cytoplasmic matrix (C₂), which borders the main lipid droplet (L₂). Bar, 2 μm. × 7,000.

Findings are similar to previously reported biochemical data on lipolysis and protein synthesis in the adipocyte (6).

**TABLE II**

| Lipolytic agent | Mean diameter: | +Insulin (100 μU/ml) |
|----------------|---------------|----------------------|
|                | Mean diameter |                      |
| None           | 7.89 ± 0.13   | 7.89 ± 0.13          |
| 100 mU/ml ACTH | 7.99 ± 0.12   | 8.05 ± 0.12          |
| 1.25 μg/ml epinephrine | 7.99 ± 0.13 | 8.01 ± 0.13          |
| 2 mM theophylline | 7.95 ± 0.13 | 8.01 ± 0.14          |
| 1 mM DBCAMP   | 7.99 ± 0.14   | 7.94 ± 0.13          |

100 protrusions were measured for each incubation condition. No significant differences were found.

**DISCUSSION**

In this report we describe a method for preparing adipocytes for scanning electron microscopy. The fragility of the isolated fat cell and its high lipid content necessitated the development of special preparation techniques for both thin-section (9) and freeze-fracture (3) electron microscopy. The large, centrally located lipid depot is primarily

**TABLE III**

| Lipolytic agent | Mean protrusions/ adipocyte hemisphere |
|----------------|---------------------------------------|
|                |                                       |
| None           | 0.48 (559)*                           |
| 100 mU/ml ACTH | 1.02 (445)                            |
| 400 mU/ml ACTH | 1.26 (452)§                           |
| 0.125 μg/ml epinephrine | 0.86(453) |                           |
| 1.25 μg/ml epinephrine | 1.30 (411)$                          |
| 2 mM theophylline | 1.09 (411)                           |
| 10 mM theophylline | 1.22 (473)$                          |
| 1 mM DBCAMP   | 1.05 (433)                            |
| 5 mM DBCAMP   | 1.19 (456)$                           |

* Numbers in parentheses are the number of hemispheres observed in a single experiment. Results at maximal concentration are significantly different from results at submaximal concentration as determined by Student's t test.

¶ P < 0.01.
§ P < 0.005.
responsible for the spherical shape of the isolated adipocyte. Extraction of this lipid by organic solvents during dehydration causes severe shrinkage and collapse of cells. The use of the nonpolar solvents ethylene glycol and ethylene glycol monomethyl ether allowed retention of the lipid and thereby the shape of the cell. The ability to observe these cells by scanning electron microscopy has enabled the qualitative and quantitative study of the cell surface of a large number of adipocytes. Similar studies by thin-section or freeze-fracture techniques would be impractical.

The discovery of globular surface evaginations or protrusions adds new insight into possible structural-functional aspects of adipocyte metabolism. These structures are small relative to the whole cell and occur infrequently on the surface of control adipocytes. The infrequency of occurrence and relatively small size make routine observations of these structures by thin-section or freeze-fracture electron microscopy unlikely. However, on several occasions, small, lipid-filled structures attached to or in close proximity to adipocytes have been seen by both these techniques. Scanning microscopy has helped in the interpretation of the relationship between these structures and the larger "parent" cell. Transmission electron micrographs document the composition of the protrusions and their relationship to intracellular structures.

The data presented here suggest that these structures may be involved in, or related to, the lipid homeostatic mechanism of the adipocyte, particularly the lipolytic phase. Various lipolytic agents, including the hormones epinephrine and ACTH and the cAMP phosphodiesterase inhibitors theophylline and DBcAMP (4, 7), caused a significant doubling in the number of protrusions per cell. Not only was the number doubled, but some of the protrusions seen in the stimulated lipolytic phase were multilobulate forms not observed in controls. Insulin, an antilipolytic agent, prevented the hormones from causing an increase in protrusion formation but did not affect the increase caused by theophylline or DBcAMP. This finding is consistent with previous observations on the effect of insulin on stimulation of lipolysis by these same agents at the same concentrations (6). The inability of insulin to overcome the effects of theophylline and DBcAMP under the conditions of the present and past (6) studies is not under-

![Figure 5](https://jcb.rupress.org/figure-5)  
**Figure 5** A scanning electron micrograph of cells treated with 100 mU/ml ACTH showing increased protrusion formation. Protrusions ranged in size from 8 to 18 µm in diameter. In many instances, multiple, closely associated protrusions were seen (arrows). The small surface irregularities were not significantly different from those seen in control adipocytes (Fig. 1). Bar, 15 µm. × 700.
stood. It may relate to the ability of these agents to block cAMP phosphodiesterase (4, 7), which insulin stimulates (11, 12). These agents may act beyond the insulin-sensitive step, whereas epinephrine and ACTH may be elevating cAMP by stimulating adenylate cyclase at an earlier stage. Also consistent with earlier studies (6) is the finding that the number of protrusions is related to the concentration of the lipolytic agent. The number of protrusions observed with a given concentration of lipolytic agent was the same after incubation periods of 5 and 30 min, agreeing with the biochemical data that indicate that lipolysis is activated to a constant rate within 1 or 2 min after addition of the lipolytic agent and remains at this rate for up to 1 h (6).

The formation of protrusions during activated lipolysis may be part of the mechanism by which the cell increases the rate of lipolysis, and their discovery may help explain discrepant biochemical data. It has been demonstrated that epinephrine increases the rate of lipolysis at least 20-fold (6), but the activity of hormone-sensitive lipase in the cytosol is increased twofold, or less (5, 18, 19). A partial explanation of this discrepancy was provided by Khoo et al. (10), who found that a partial redistribution of hormone-sensitive lipase occurred after epinephrine treatment of adipocytes. The lipase became associated with the fat cake of the cell homogenate and preferentially hydrolyzed endogenous substrate. It is possible that as the protrusions are formed, activated cytosolic hormone-sensitive lipase associates with the protrusions. This would explain the redistribution findings of Khoo et al. (10). These protrusions would certainly fractionate with the fat cake during centrifugation, as a result of either spontaneous release from the cell surface or mechanical release during cell homogenization, because of their high fat to nontriglyceride ratio. This is supported by the data of Benjamin and Clayton (2), which show that membrane components are associated with the fat cake. Recent data of Wise and Jungas (21) demonstrate that epinephrine treatment of adipose

FIGURE 6  A thin-section electron micrograph of an adipocyte incubated with 1.25 μg of epinephrine/ml for 30 min. This protrusion has the same general characteristics as the control cell shown in Fig. 4, except for the small lipid droplets (small arrows) in the rim of cytoplasm seen here. Several of these droplets may coalesce to form a larger droplet, resulting in a multilobulate protrusion (large arrow). Continuity of the cell plasma membrane with that of the protrusion was not demonstrable in this section but was seen in other serial sections of this protrusion. Bar, 2 μm. × 9,000.
tissue increases the rate of lipolysis of endogenous glycerides in homogenates threefold but has no effect on hydrolysis of exogenous triglyceride.

Our study suggests that during activation of lipolysis, the main triglyceride storage depot is broken into smaller triglyceride droplets creating the protrusions we have observed. This would provide a larger lipid surface area for the activated enzyme. In addition, these protrusions, being located at the cell surface, could presumably facilitate egress of the free fatty acids, a hypothesis related to the theory proposed by Scow (16) for movement of free fatty acids out of the cell. There is no clear evidence that the protrusions are the result of secretion from the cell surface or that they represent secretory routes of the free fatty acids from the main triglyceride droplet. Although there was no evidence that the protrusions are continuously turning over, with a steady state being rapidly reached for each concentration of lipolytic agent, this is a likely possibility. Thus, epinephrine and other lipolytic agents may stimulate lipolysis by initiating both biochemical and structural changes, resulting in an increase in lipase activity and substrate availability. These multiple, small alterations could result in a much larger overall increase in the rate of lipolysis.

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