Metabolism of Isolated Fat Cells

I. EFFECTS OF HORMONES ON GLUCOSE METABOLISM AND LIPOLYSIS

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Increased attention has been focused on the metabolism of adipose tissue and its marked sensitivity to various hormones (for reviews, see Vaughan (1) and Jeanrenaud (2)). In an attempt to obtain a homogeneous preparation of fat cells, it was found that if rat adipose tissue is treated with collagenase, fat cells are liberated. Owing to their high fat content, the fat cells can be separated from the more dense stromal-vascular cells by flotation.

The metabolism of glucose and the response to various hormones by free fat cells are reported in this paper.

EXPERIMENTAL PROCEDURES

Male Sprague-Dawley rats (160 to 210 g) were used in these studies and were fed, ad libitum, a high carbohydrate diet (3) consisting of: ground whole wheat, 66%; casein, 15%; whole milk powder, 10%; cottonseed oil, 5%; and required vitamins and salts.

Materials—Crystalline zinc insulin was obtained from Eli Lilly (Lot 288614, 26 units per mg). ACTH and Fraction V bovine albumin were Armour products. Purified TSH was from Lilly (Lot 288614, 26 units per mg). ACTH and Fraction V bovine albumin which had been dialyzed against the bicarbonate buffer. The same buffer was used for the metabolic studies.

The tissue was dispersed into small fragments within 1 hour of incubation with collagenase. Fat cells were liberated from the tissue fragments by gentle stirring with a rod. Liberation of the cells was manifested by an increased turbidity in the medium. Fragments of tissue still remaining after this treatment were removed with forceps. The suspension of cells was centrifuged in polyethylene centrifuge tubes1 for 1 minute at 400 x g. The fat cells floated to the surface, and the stromal-vascular cells (capillary, endothelial, mast, macrophage, and epithelial cells) were sedimented. The stromal-vascular cells were removed by aspiration, and the fat cells were washed by suspending them in 10 ml of warm (37°) albumin buffer containing the desired concentration of glucose and centrifuging for 1 minute at 400 x g.

1 This procedure was repeated three times. Stromal-vascular cells were absent, by histological examination, from the fat cell preparation after three washes. Fat droplets, which may have been formed from the breakage of the fat cells, floated more rapidly to the surface than the fat cells and were aspirated from the surface, after gently stirring the cell suspension.

For a set of experiments, fat cells were usually obtained from the pooled adipose tissue of three rats. The washed cells were suspended in 15 to 20 ml of albumin-bicarbonate buffer containing a given concentration of glucose, usually 3 μmoles per ml. The triglyceride concentration (fat cell content) was generally between 30 and 40 μmoles of triglyceride equivalent to about 50 to 60 mg of tissue) per ml of suspension. Just prior to dispensing the cells in the incubation vials, sufficient glucose-U-14C2 was added to the suspension to give a final specific activity of about 0.1 μc per μmole of glucose.

The following method was used for measuring and dispensing the cells. The cell suspension was swirled to ensure delivery of uniform suspensions of cells and was immediately drawn up into 18 cm of plastic tubing3 attached to a 2-ml calibrated syringe (lubricated to maintain a tight fit) and then discharged into plastic counting vials from the Packard Instrument Company. The volume of tubing was sufficient to contain 0.5 to 1.0 ml of cell suspension, the usual amounts measured. With this procedure, it was possible to dispense, without cell breakage, 0.5 ml of cell suspension into 40 vials within 3 minutes.

After the addition of hormones, etc., the vials were capped with rubber serum stoppers fitted with hanging glass wells purchased from the Kontes Glass Company. The wells contained cylinders of Whatman No. 1 paper rolled from 2- x 8-cm strips. Unless stated otherwise, incubations were carried out, with shaking, at 37° for 2 hours. The gas phase was 95%

1 The abbreviations used are: ACTH, corticotropin; TSH, thyroid-stimulating hormone.
2 Glucose-U-14C refers to the uniformly or randomly labeled compound.

1 No. 10 Transflex tubing, Irvington, Plastic Division, Prechold, New Jersey.

3 No. 10 Transflex tubing, Irvington, Plastic Division, Prechold, New Jersey.
Metabolism of Isolated Fat Cells. I

Vol. 239, No. 2

FIG. 1. Photomicrographs of free fat cells and stromal-vascular cells obtained from collagenase-treated rat epididymal adipose tissue. Suspensions of unfixed cells were stained with methylene blue. A, view of fat cells at low magnification (100X). Although not clearly visible on the photograph, stained nuclei were associated with each sphere. B, fat cells at higher magnification (210X) showing nuclei. C, intact blood vessels and other stromal-vascular cells (25X).

Analytical Procedures—Radioactivity was determined in a Packard model 314 EX liquid scintillation counter. $^{14}$C lipids and CO$_2$ were counted in a scintillation solution consisting of 0.4% 2,5-diphenyloxazole and 0.05% p-bis-2-(phenyloxazolyl)benzene in toluene. Bray's scintillation solution (5) was used for counting $^{14}$C-glucose in the incubation medium.

At the end of the incubation period, 0.2 ml of Hyamine-10X purchased from the Packard Instrument Company was injected onto the filter paper and 0.25 ml of 1 N sulfuric acid into the cell suspension. After the flasks were shaken for 15 minutes at room temperature, the paper strips were transferred to 10 ml of scintillation fluid and counted. Two drops of methanol were added to the counting vials to increase the solubility of the Hyamine-CO$_2$ in the scintillation fluid.

For the determination of $^{14}$C lipids, the cell suspensions were transferred to centrifuge tubes with glass stoppers and extracted with 5 ml of Dole's extraction mixture (6). After the mixture had stood for 15 minutes at room temperature, 3 ml of water and 3 ml of hexane were added, and the phases were allowed to separate. The lower phase was removed by aspiration and the upper phase was washed with 3 ml of water. Portions of the upper phase were analyzed for ester content (7) with tripalmitin as standard, free fatty acids by a slight modification of the method of Dole and Meinertz (8), and total lipid radioactivity. To determine radioactivity in triglyceride fatty acids, 1 ml of the upper phase was analyzed by refluxing for 1 hour with 2 ml of ethanolic KOH (1.0 ml of saturated KOH per 100 ml of 95% ethanol, freshly prepared). After 2 ml of water were added, the sample was neutralized to a bromcresol green end point and the fatty acids were extracted with 3 ml of hexane. A 2.0-ml aliquot of the latter was evaporated to dryness, and 10 ml of scintillation fluid were added to dissolve the residue. Fatty acid content was determined by titration (8) and generally agreed with the ester content of the original lipid extract. Since the lipids of adipose tissue are primarily triglycerides (9), the difference in radioactivity found in fatty acids and that in total lipid was assumed to represent radioactivity in the glycerol moiety and is referred to as glyceride-glycerol.

Glucose in the medium was measured, without deproteinization, by the glucose oxidase procedure (10). The quantity of glucose carbon converted to CO$_2$, glyceride-glycerol, and fatty acids was calculated from the initial specific activity of the glucose in the medium and the quantity of radioactivity in the products. Results are expressed as micromoles of glucose per mmole of triglyceride in the cell suspension.

RESULTS

The low density material released from adipose tissue by collagenase consisted of spheres which contained a nucleus and were 50 to 100 μ in diameter (Fig. 1A and B). The thin cytoplasmic rim usually seen surrounding that fat globule in thin sections of adipose tissue (11), could not be seen in the free fat cell suspensions. The fat cells were freely dispersed in the medium; there was no clumping of the fat cells (Fig. 1A).

The dense material which sedimented after treatment of adipose tissue with collagenase, contained mast cells, macrophages, connective tissue cells, and intact blood vessels and is referred to as stromal-vascular cells. A view of this material is shown in Fig. 1C.

Fat cells incubated in plastic vessels converted 29% of the $^{14}$C-glucose in the medium to CO$_2$, glyceride-glycerol, and fatty acids (Table I). No change in the gross structure of the cells was observed after 3 hours of incubation. When the cells were incubated in glass vessels, however, only 4% of the $^{14}$C-glucose was converted to the measured products. The loss in metabolic activity was primarily due to the rupture of the cells by incubation in the glass vessels. When the fat cells were homogenized, the fat was released from the cells and formed large droplets.

S. S. Chernick, unpublished procedures.
Glucone metabolism by free fat cells and stromal-vascular cells

<table>
<thead>
<tr>
<th>Incubation vessel</th>
<th>Glucose in medium converted to</th>
<th>CO₂</th>
<th>Glyceride-glycerol</th>
<th>Fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat cells</td>
<td>Plastic</td>
<td>9.6 ± 0.2</td>
<td>11.2 ± 0.5</td>
<td>6.0 ± 0.65</td>
</tr>
<tr>
<td>Fat cells</td>
<td>Glass</td>
<td>2.1 ± 0.5</td>
<td>1.2 ± 0.3</td>
<td>0.5 ± 0.7</td>
</tr>
<tr>
<td>Fat cells, homogenized</td>
<td>Plastic</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stromal-vascular cells</td>
<td>Plastic</td>
<td>&lt;0.1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Fat cells, containing 30 μmoles of triglyceride, were incubated for 2 hours at 37° in 1.0 ml of albumin-bicarbonate buffer, pH 7.4, containing 3 μmoles of glucose-U-14C per ml. Values are the mean of 4 replications ± standard error.

- Plastic counting vials, purchased from the Packard Instrument Company.

- Erlenmeyer flasks, 25-ml capacity, not siliconized.

- Fat cells, containing 150 μmoles of triglyceride, were homogenized at room temperature in a Potter-type glass homogenizer fitted with a Teflon plunger.

- Stromal-vascular cells represent the material sedimented at 400 × g after 2.5 g of adipose tissue was dispersed with collagenase, as described in “Experimental Procedure.” The cells were washed with albumin-bicarbonate buffer, and the entire amount was suspended in 1.0 ml of buffer containing 3 μmoles of glucose-U-14C per ml. Experimental conditions were the same as those employed with the fat cells, which bore no resemblance to the fat cell spheres shown in Fig. 1. Nuclei were not associated with the fat droplets. The homogenized preparations did not oxidize glucose or synthesize triglycerides from glucose. These results indicate that the preservation of cellular structure is required for the metabolism of glucose by the fat cells.

Table I also shows that the stromal-vascular cells incubated in plastic vessels oxidized less than 0.1% of the medium glucose and did not synthesize triglycerides. Insulin had no effect on the metabolism of glucose by the stromal-vascular cells (see below).

The quantity of glucose-U-14C oxidized to CO₂ was proportional to the amount of fat cells (Fig. 2) and oxidation proceeded at a constant rate for at least 3 hours (Fig. 3). Additions of insulin (1 milliunit per ml) caused a 2.5-fold increase in glucose oxidation, which also proceeded at a constant rate for 3 hours.

The effects of insulin on glucose uptake and its metabolism to carbon dioxide, glyceride-glycerol, and fatty acids are shown in Table II. Insulin increased proportionately glucose uptake and metabolism. Approximately 50% of the labeled glucose removed from the medium was accounted for as carbon dioxide and triglyceride. In these experiments, the over-all recovery of radioactivity in CO₂, glyceride-glycerol, and fatty acids was reproducible to within 10% for a set of six replicate flasks. Lactic acid and glycogen formation were not measured but probably accounted for the remainder of the products of glucose metabolism (12).

The oxidation of glucose-U-14C by the isolated fat cells was increased by adding insulin in amounts as small as 10 micro-units per ml; this was the lowest concentration tested (Fig. 4). A linear dose response was observed in the range of 10 to 100

**Table II**

<table>
<thead>
<tr>
<th>Glucose uptake</th>
<th>Conversion of glucose-U-14C to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂</td>
</tr>
<tr>
<td>Control</td>
<td>10 ± 0.4</td>
</tr>
<tr>
<td>Insulin</td>
<td>32 ± 0.2</td>
</tr>
</tbody>
</table>

Mean values obtained from 6 replications ± standard error.
micromunits of insulin per ml at a glucose concentration of 3 
μmoles per ml. Increasing the insulin concentration to 1 and to 
5 milliunits per ml did not cause a further increase in glucose 
oxidation. The same results were obtained when fatty acid 
synthesis was measured.

The effects of increasing the medium glucose concentration 
from 1 to 16.5 μmoles per ml on glucose metabolism are shown 
in Fig. 5. Maximal formation of CO₂ and fatty acids was ob-
erved when the glucose concentration was increased from 1 to 
6.5 μmoles per ml. Glyceride-glycerol formation was not 
changed by increasing glucose concentrations. At a glucose 
concentration of 1 μmole per ml, addition of insulin (1 milliunit 
per ml) increased the conversion of glucose to CO₂, glyceride-
glycerol, and fatty acids by about 3- to 6-fold. A further in-
crease in CO₂ and fatty acid formation occurred in response to 
insulin if the medium glucose concentration increased from 
1 to 3.5 μmoles per ml. Additional glucose in the medium did 
not change the amount of CO₂, glyceride-glycerol, and fatty 
acids that were formed in response to insulin.

![Graph showing glucose-U-14C metabolism](http://www.jbc.org/)

**Fig. 5.** Patterns of glucose-U-14C metabolism obtained by in-
creasing glucose concentration in absence and presence of added 
insulin. Four equal portions of fat cells obtained from epididymal 
fat pads of 3 rats were washed 3 times with 10 ml of albumin-bi-
carbonate buffer containing 1.0, 3.5, 6.25, and 16.5 μmoles of glu-
cose per ml, respectively, and finally suspended in 10 ml of buffer 
containing the same designated glucose concentration. One 
milliliter of the cell suspension (40 μmoles of triglyceride (TG) per 
ml) was incubated, in quadruplicate, for 2 hours at 37°C. Insulin 
added was 1 milliunit per ml. Each point represents the mean ± 
standard error (vertical bars). Solid line, insulin; dashed line, 
control.

![Graph showing glucose-U-14C metabolism by fat cells and adipose 
tissue](http://www.jbc.org/)

**Fig. 6.** Metabolism of glucose-U-14C by fat cells and adipose 
tissue obtained from fed rats and rats deprived of food. Fat cells 
and pieces of adipose tissue (45 μmoles of triglyceride per ml) were 
incubated in 1 ml of albumin-bicarbonate buffer, pH 7.4, for 2 
hours at 37°C. Glucose concentration was 3 μmoles per ml. In-
sulin added was 1 milliunit per ml. The data are expressed as the 
means of 4 replications for fat cells and for 3 pieces of adipose tis-
sue weighing approximately 50 mg each. Vertical lines at top of 
each column represent standard error of the mean. Group I re-
fers to rats (190 to 210 g) that were not fed overnight; Group II, 
rats (160 to 170 g) were fed. TG, triglyceride; GG, glyceride-glyc-
erol; FA, fatty acids.
The effect of insulin on the pattern of glucose metabolism in intact adipose tissue and isolated fat cells is shown in Fig. 6. Two groups of animals (3 rats per group) were used in this study. Rats in Group I (190 to 210 g) were maintained on the high carbohydrate diet for 2 weeks and were deprived of food for 24 hours prior to the experiment. Group II contained rats (160 to 170 g) which were fed the diet for 5 days. For the intact tissue studies, approximately 50 mg of tissue were cut from the distal portion of each fat pad and incubated for 2 hours at 37° in medium containing 3 pmoles of glucose-U-14C per ml. The two pieces of tissue from each animal were incubated with and without added insulin. The remainder of the adipose tissue from the three animals in each group was pooled and treated with collagenase for 1 hour, and the fat cells were isolated in the usual manner. The fat cells were incubated under the same conditions as described for the intact tissue.

In contrast to the results obtained with the isolated fat cells, the intact adipose tissues indicated a larger variation in the quantity of glucose converted to CO₂, glyceride-glycerol, and fatty acids. This was particularly noticeable when insulin was added to the medium. Although there appeared to be less statistically metabolized by the intact tissue, the difference was not statistically significant.

The "pattern" of glucose metabolism (glucose to CO₂, glyceride-glycerol, and fatty acids) in the tissue and free fat cells was similar in each group of rats. The cells and tissue from the fed rats (Group II), had a lower basal level of glucose metabolism and a larger percentage of conversion of glucose to CO₂ and fatty acids in response to insulin than those from fasted rats.

Several other hormones were examined for their effects on glucose metabolism and fatty acid release by free fat cells. The effects on glucose metabolism are shown in Table III. Oxytocin, like insulin, stimulated the formation of CO₂, glyceride-glycerol, and fatty acids from labeled glucose. The minimal effective concentration for the oxytocin effect was 0.2 μg per ml. Addition of ACTH, TSH, and epinephrine also increased the formation of CO₂ and glyceride-glycerol from glucose, but, unlike insulin and oxytocin, depressed fatty acid synthesis.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Conversion of glucose-U-14C toCO₂</th>
<th>Glyceride-glycerol</th>
<th>Fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.68 ± 0.18</td>
<td>1.86 ± 0.24</td>
<td>2.40 ± 0.12</td>
</tr>
<tr>
<td>Insulin, 0.02 μg per ml</td>
<td>5.13 ± 0.24</td>
<td>7.08 ± 0.18</td>
<td>5.70 ± 0.09</td>
</tr>
<tr>
<td>Oxytocin, 0.2 μg per ml</td>
<td>3.66 ± 0.36</td>
<td>3.48 ± 0.24</td>
<td>3.54 ± 0.24</td>
</tr>
<tr>
<td>TSH, 1.0 μg per ml</td>
<td>2.06 ± 0.12</td>
<td>6.06 ± 0.16</td>
<td>1.02 ± 0.12</td>
</tr>
<tr>
<td>ACTH, 1.2 μg per ml</td>
<td>3.48 ± 0.22</td>
<td>8.46 ± 0.42</td>
<td>1.56 ± 0.12</td>
</tr>
<tr>
<td>Epinephrine, 1 μg per ml</td>
<td>4.41 ± 0.18</td>
<td>7.20 ± 0.51</td>
<td>1.86 ± 0.12</td>
</tr>
</tbody>
</table>

* Mean ± standard error for 4 replications. 1.0 ml of cell suspension (cell triglyceride = 40 μmoles) incubated for 2 hours at 37° in albumin-bicarbonate buffer, pH 7.4, containing 3 μmoles of glucose-U-14C per ml.

**TABLE IV**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Net change in free fatty acids μg/μmol triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Insulin</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>-2.0 ± 0.7</td>
</tr>
<tr>
<td>TSH</td>
<td>40.5 ± 6.0</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>73.8 ± 6.0</td>
</tr>
<tr>
<td>ACTH</td>
<td>54.0 ± 2.0</td>
</tr>
</tbody>
</table>

* Same concentrations of hormones, cells, and incubation conditions as described in Table III. Initial free fatty acid concentration in medium, 0.2 μeq per ml.

**Discussion**

These results indicate that fat cells separated from adipose tissue maintain the intrinsic metabolic characteristics of this tissue. The similarity in the patterns of glucose metabolism (CO₂, glyceride-glycerol, and fatty acid formation) by both isolated fat cells and by intact adipose tissue indicates that the major pathways of glucose metabolism have been preserved in the isolated cells. The isolated fat cells account for essentially all of the glucose metabolism observed in adipose tissue.

The dependence of CO₂ and fatty acid synthesis on the medium glucose concentration in isolated fat cells has also been found in studies with epididymal fat pads (13). Glyceride-glycerol formation was unaffected by increasing the medium glucose concentration. This has also been observed with intact tissue (13, 14) and probably reflects the relatively decreased availability of substrate to the Embden-Meyerhof pathway as more substrate becomes available to the other pathways. In this regard, the quantity of glucose available for phosphorylation intracellularly was strongly dependent on the presence of insulin as evidenced by the finding that an increased glucose gradient in the medium was not sufficient, at least at concentrations from 20 to 200 mg/100 ml, to cause an elevation of glucose metabolism to the levels attained with insulin at low concentrations. Studies with epididymal adipose tissue have shown that increasing glucose concentrations to 2000 mg/100 ml simulates the effect of insulin on fatty acid synthesis and CO₂ formation in the presence of low glucose concentrations (13, 14).

In view of the exquisite sensitivity of rat adipose tissue to insulin, it was of particular interest to find that the free fat cells responded to insulin over the same dose response range as that...
reported for intact adipose tissue (15, 16). The magnitude of the insulin effect on glucose metabolism in isolated cells was as great as that in the tissue. These findings suggest that the isolated fat cells may be useful for insulin assay studies.

Hormones other than insulin also increased the metabolism of glucose by the fat cells. The insulin-like effect of oxytocin on glucose metabolism may be related to the disulfide bridge which is common to both hormones, as suggested by Pittman et al. (17) in their studies with adipose tissue. However, insulin at 0.1 the concentration was more effective than oxytocin. The effects of lipolytic hormones on glucose metabolism and lipolysis in the free fat cells were the same as those observed in intact adipose tissue (ACTH (12,18-21); TSH (19,22); and epinephrine (12, 17, 23-27)).

**SUMMARY**

Studies were made on the metabolism of isolated fat cells and stromal-vascular cells prepared by collagenase treatment of rat epididymal adipose tissue.

The isolated fat cells metabolized glucose by the same pathways as intact adipose tissue and accounted for essentially all of the glucose metabolism observed in this tissue. Free fat cells also maintained the different metabolic characteristics observed in adipose tissue from fasting and fed rats.

Increasing the medium glucose concentration to a maximal level of 6.5 μmoles per ml stimulated the formation of CO₂ and fatty acids from glucose. Insulin caused a further 3-fold increase in the uptake of glucose and its utilization to CO₂, glyceride-glycerol, and fatty acids. As little as 10 microunits of insulin per ml stimulated glucose metabolism by free fat cells; the insulin dose response range was 10 to 100 microunits per ml.

Oxytocin, at 10 times the maximal effective concentration of insulin, caused an insulin-like effect on the metabolism of glucose by free fat cells. Corticotropin, thyroid-stimulating hormone, and epinephrine stimulated the conversion of glucose to CO₂ and glyceride-glycerol and the release of free fatty acids; they suppressed fatty acid synthesis from glucose. Insulin, but not oxytocin, caused a net disappearance of free fatty acids from medium and cells.

It is concluded that isolated fat cells retain the ability to metabolize both glucose and triglycerides and respond to several hormones that have been shown to affect the metabolism of adipose tissue.

**Acknowledgments**—I would like to express my appreciation for the excellent technical assistance given by Miss Ann Butler during the course of this study.

**REFERENCES**
