Effects of Glucocorticoids on Metabolism of Adipose Tissue in Vitro*

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The adrenal cortex is necessary for development of fatty liver (2, 3) and increased plasma free fatty acids in numerous situations (4–6). It has been difficult, however, to demonstrate direct effects of glucocorticoids on fat metabolism. The role of the adrenal cortex in fat metabolism is generally considered to be a permissive one.

Pancreatectomized rats deprived of either the pituitary or adrenal glands develop severe ketosis and fatty livers when given glucocorticoids (7). There is a close relationship between the amount of steroid given and the severity of lipid disturbances. Either insulin administration or depletion of body fat stores prevents the above effects of glucocorticoids. It has been suggested that development of ketosis and fatty livers in diabetes is the result of a direct action of glucocorticoids on adipose tissue in the absence of insulin (7).

In a recent study, adipose tissue taken from adrenalectomized rats treated with dexamethasone released fatty acids at a faster rate, in vitro, than did tissue from operated controls (8). The accelerated release of fatty acid was associated with a suppression of glucose uptake. This paper reports that addition of dexamethasone in vitro had similar effects on adipose tissue from normal female rats.

EXPERIMENTAL PROCEDURE

Female Sprague-Dawley rats were fed, for at least 1 week, a synthetic diet (No. 4370) consisting of casein, 20%; L-cystine, 0.3%; dextrin, 66.7%; corn oil, 8%; salts, 4%; and all vitamins needed by the rat (9).

After deprivation of food for 18 hours, rats weighing 145 to 175 g were decapitated. The parametrial and mesenteric adipose tissues were immediately removed and placed in a Petri dish containing buffer solution at room temperature. The adipose tissue was cut into 5 to 10 pieces, and 80 to 125 mg of tissue were put into 3 ml of buffer solution in a 25-ml flask. The buffer solution contained dialyzed bovine Fraction V albumin, 4%; glucose, 0.05%; KCl, 2.7 mM; NaCl, 137 mM; CaCl2, 1.4 mM; MgCl2, 0.5 mM; NaHCO3, 12 mM; Na2HPO4, 4 mM; penicillin G, 220 units per ml; and dihydrostreptomycin sulfate, 0.22 mg per ml. Unless stated otherwise, the tissues were incubated for 4½ hours at 37°. The gas phase was 5% CO2-95% O2.

Glucose in the medium was measured without deproteinization by the glucose oxidase procedure (10). Fatty acids were measured by a modification2 of the procedure of Dole and Meinertz (11). The fatty acid content of the buffer solution was 0.20 to 0.27 μmole per ml.

In some experiments, 0.24 μc of uniformly labeled glucose-C14 was added to each flask. After gassing, the flasks were sealed with rubber serum stoppers. The amount of labeled carbon dioxide produced was determined as follows. Ten minutes before the end of the incubation period, 0.25 ml of 1 m Hyamine was injected through the rubber stopper onto a strip of filter paper, 20 × 80 mm, in a hanging well. Ten minutes later 0.1 ml of 2 N H2SO4 was injected into the incubation medium, and the flask was allowed to sit at room temperature for 30 minutes. The filter paper was then placed in a vial with 10 ml of scintillator solution containing 10% P-bis[2-(5-phenyloxazolyl)]-1-benzene in toluene. Radioactivity was measured in a Packard 314 EX liquid scintillation instrument.

The amount of radioactive glucose added was determined in a 25-μl aliquot of the medium taken from the zero time control flasks; it was added to scintillator solution containing 10% absolute ethanol.

For determination of lipid C14, the adipose tissue was rinsed three times in water, blotted, and placed in a centrifuge tube containing 10 ml of chloroform-methanol (2:1 by volume). When the extraction of lipids was completed several hours later, the tissue residue was removed, 2.4 ml of water were added, and the tube was shaken vigorously. The tube was then centrifuged at low speed to separate the chloroform and water layers. After the upper phase was aspirated, anhydrous powdered sodium sulfate was added to remove traces of water from the lower phase. Two milliliters of the chloroform lipid extract (total lipid) were evaporated to dryness in a counting vessel, and the toluene scintillator solution then was added. Another 2-ml aliquot was heated to remove the chloroform, and the lipid was saponified by refluxing it for 1 hour with 2 ml of alcoholic KOH.
(1 ml of saturated KOH, 20 ml of ethanol, freshly prepared). After neutralization to the bromocresol green end point, 2 ml of water were added and the fatty acids were extracted with 4 ml of Skellysolve B. A 3-ml aliquot of the latter was evaporated to dryness, and 10 ml of scintillator solution were added. Radioactive samples were corrected for quenching by use of internal standards.

The steroids tested were used as the free alcohols. The more polar steroids were dissolved in water; the others were dissolved in dilute alcohol (1 to 3%). This amount of alcohol had no effect on control tissues. 2α-Methylcortisone was a gift from Dr. Grant Liddle of Vanderbilt University, and 2α-methylocorticosterone, from the Upjohn Company.

The protein hormone preparations studied were Wilson oxycecl ACTH (Lot 102201, 90 subcutaneous units per mg) and porcine crystalline zinc insulin (Lot 499667, 26 units per mg), a gift from Dr. Otto K. Behrens at Eli Lilly and Company.

**RESULTS**

**Effect of Dexamethasone on Fatty Acid Release and Glucose Uptake**—Dexamethasone increased the release of fatty acid by incubated parametrial adipose tissue (Fig. 1). The minimal effective concentration was 0.004 μg per ml (10⁻⁸ M). There was a dose response relationship between concentrations of 0.0016 and 0.016 μg per ml. Higher concentrations, up to 0.16 μg per ml, did not cause a greater effect (Fig. 2).

Fatty acid release was also increased by corticosterone, but a greater amount of the steroid was needed (Fig. 1). Deoxycorticosterone, testosterone, β-estradiol, and pregnanediol had no effect when tested at 0.16 μg per ml. Deoxycorticosterone produced a small increment in fatty acid release at a concentration of 1.6 μg per ml, but not at 3.2 μg per ml (Fig. 1).

Dexamethasone also reduced glucose uptake in parametrial adipose tissue (Fig. 2). Glucose uptake was decreased by the same steroid concentrations that increased fatty acid release.

Similar effects of dexamethasone on fatty acid release and glucose uptake were observed in mesenteric adipose tissue (Table I). When compared on a wet weight basis, the mesenteric adipose tissue was slightly more sensitive to dexamethasone than was parametral adipose tissue.

**Reversal of Dexamethasone Effects by Insulin**—The effects of dexamethasone on fatty acid release and glucose uptake were prevented by addition of a small amount of insulin (4 milliunits per ml) (Fig. 2). When the hormones were added together, the effects of insulin predominated. In both control and dexamethasone-treated tissues, insulin increased glucose uptake 2- to 3-fold.

**Effects of Dexamethasone Compared with Those of ACTH and Epinephrine**—Glucose uptake was reduced in dexamethasone-treated tissues. Other lipolytic hormones, however, stimulate glucose uptake when the tissues are incubated in albumin buffer (12, 13).

Table II presents a comparison of effects of dexamethasone with those of ACTH and epinephrine. The maximal release of fatty acid observed was much greater in the case of the latter two hormones, which increased glucose uptake not only when release of fatty acid was high but also when release was similar to that induced by glucocorticoids. Effects of simultaneous addition of dexamethasone and ACTH were additive, in that the release of fatty acid was greater and the glucose uptake was less than in the presence of ACTH alone.
TABLE II

Comparison of effects of dexamethasone with those of ACTH and epinephrine

<table>
<thead>
<tr>
<th>Additions</th>
<th>Fatty acid released</th>
<th>Glucose uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td></td>
<td>(μmol/g/100 mg/1 hr)</td>
<td>(μmol/100 mg/1 hr)</td>
</tr>
<tr>
<td>None</td>
<td>+0.09 ± 0.07</td>
<td>1.25 ± 0.61</td>
</tr>
<tr>
<td>Dexamethasone, 0.016 μg per ml</td>
<td>1.03 ± 0.07</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>ACTH, 0.15 μg per ml</td>
<td>+0.76 ± 0.30</td>
<td>1.90 ± 0.18</td>
</tr>
<tr>
<td>Dexamethasone, 0.016 μg per ml + ACTH, 0.15 μg per ml</td>
<td>1.22 ± 0.28</td>
<td>0.99 ± 0.14</td>
</tr>
<tr>
<td>Epinephrine, 0.1 μg per ml</td>
<td>+1.19 ± 0.49</td>
<td>1.83 ± 0.58</td>
</tr>
<tr>
<td>Epinephrine, 1.0 μg per ml</td>
<td>+2.06 ± 0.37</td>
<td>3.32 ± 2.07</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the difference between the control and experimental results in seven replications.
† Difference between dexamethasone plus ACTH versus ACTH alone.

Aspects of Delayed Response to Dexamethasone—The time of onset of dexamethasone effects was studied in tissues incubated with radioglucose (Fig. 3). At the end of 2 hours, there was no effect of dexamethasone on fatty acid release, glucose uptake, or glucose utilization (p > 0.05 by paired t test). At 4 hours, however, the total amount of fatty acid released was 2-fold greater and the amount of glucose taken up was 40% less in the treated tissue (p < 0.001). Conversion of uniformly labeled glucose-14C to total lipid and carbon dioxide was also significantly decreased (p < 0.05). Since fatty acid formation was very low in these experiments, the recovery of glucose-14C as total lipid represents primarily glyceride-glycerol formation. In this study, the effects of dexamethasone did not appear until during the last half of the incubation period.

The nature of the delayed action of dexamethasone was examined in more detail in the following way. Adipose tissue from each rat was distributed among five flasks and incubated for 2 hours. The tissues were then rinsed three times, blotted, and transferred to new media containing glucose-14C and incubated for another 2 hours. One flask served as the initial control, another as final control, and the other three contained 0.016 μg of dexamethasone per ml of media during either the first, the second, or both 2-hour periods.

During the first 2 hours, there was no difference between control and dexamethasone-treated tissues in fatty acid release or glucose uptake. The observations made during the second period of incubation are presented in Fig. 4.

Adipose tissue exposed to dexamethasone only during the first period (0 to 2 hours) released more fatty acid (p < 0.001 by paired t test) and converted less labeled glucose to carbon dioxide (p < 0.001), total lipid (p < 0.001), and fatty acid (p < 0.001) than did the control tissues (Fig. 4). The decreased glucose uptake in treated tissues was not statistically significant (p > 0.05 < 0.1).

Addition of dexamethasone only during the second period (2 to 4 hours) increased the release of fatty acid (p < 0.05) but had no significant effect on glucose uptake or its conversion to carbon dioxide, total lipid, and fatty acid.

The greatest effects of dexamethasone were obtained when the steroid was present during both incubation periods (0 to 4 hours); all parameters of metabolism studied were affected (p < 0.001).

Less than 0.2% of the radioactivity was found in the defatted residue of adipose tissue (glycogen, protein, and other substances) in these experiments.

Comparison of α-Methylcorticoid and β-Methylcortisone—It has been postulated (14, 15) that only 11-hydroxysteroids possess glucocorticoid activity and that before steroids such as cortisone or prednisone are active the 11-keto group must be reduced to a hydroxy group. This was investigated in adipose tissue by

![Fig. 3. The delayed action of dexamethasone in vitro. The parametrial adipose tissue obtained from each rat was distributed among five flasks. After 15 minutes, the reaction in one flask was stopped to furnish initial values. Control and experimental pairs were stopped 2 and 4 hours later. The values are shown as the means of 10 experimental replications. Percentage uptake of glucose was calculated as the fraction of added glucose taken up from the medium per 100 mg of tissue. Conversion of labeled glucose was expressed as the fraction of the added radioactivity converted to the various products per 100 mg of tissue.](http://www.jbc.org/)

Preparation of the dimedon derivative of formaldehyde after periodate oxidation of the aqueous fraction obtained by hydrolysis of the total lipid extract confirmed that the radioactivity of that fraction was in the form of glycerol.
comparing the effects of 2α-methylcortisone with those of 2α-methylcortisol, because the presence of the 2α-methyl group prevents the interconversion of 11-keto and 11-hydroxy groups (16).

Table III shows that 2α-methylcortisol augmented fatty acid release and reduced glucose uptake and its conversion to carbon dioxide. The concentration of steroid needed for increased fatty acid release, 0.16 \mu g per ml, was the same as that found for corticosterone (Fig. 1). 2α-Methylcortisone had no effect at concentrations of 0.3 and 1.6 \mu g per ml.

**DISCUSSION**

These experiments demonstrate that glucocorticoids increase fatty acid release and decrease the uptake and utilization of glucose in incubated adipose tissue from normal female rats. Dexamethasone, one of the most potent glucocorticoids, had the greatest effect on the adipose tissue. The above effects were seen when the concentration of dexamethasone was only 0.004 \mu g per ml (10⁻⁴ M). Betamethasone and triamcinolone, analogues in which 16β-methyl and 16α-hydroxy groups, respectively, replace the 16α-methyl group, were approximately as effective as dexamethasone.⁴ Corticosterone also increased fatty acid release if a greater concentration, 0.02 to 0.16 \mu g per ml, was used. The latter is approximately equal to the plasma corticosterone concentration in the rat (17). Deoxycorticosterone had no effect except at a concentration of 1.6 \mu g per ml; it was ineffective at 0.32 and 3.2 \mu g per ml. 2α-Methylcortisol stimulated fatty acid release and decreased glucose uptake when present at a concentration of 0.16 \mu g per ml, whereas 2α-methylcortisone was ineffective at concentrations up to 1.6 \mu g per ml. The inactivity of 2α-methylcortisone and deoxycorticosterone support the view that there is a close relationship between glucocorticoid activity in vivo and ability to affect adipose tissue metabolism.

The above findings are in agreement with observations of other workers. Jeanneraud and Renold (18) found that cortisol, corticosterone, and, to a lesser degree, deoxycorticosterone increased fatty acid release in incubated adipose tissue if the concentration of steroid was 30 \mu g per ml; they found no effect on glucose utilization. More recently Leboeuf, Renold, and Cahill (19) reported that cortisol at a high concentration, 30 \mu g per ml, not only stimulated fatty acid release but reduced conversion of radioglucone to carbon dioxide, glyceride-glycerol, fatty acid, and glycogen. They also observed that the cortisol effects on metabolism of labeled glucose did not occur in the presence of insulin. In their studies, cortisol suppressed the stimulatory effect of epinephrine on glucose uptake. Munck (20) observed that cortisol, corticosterone, and, to a lesser extent, deoxycorticosterone reduced glucose uptake in epididymal adipose tissue taken from adrenalectomized rats.

The action in vitro of glucocorticoids on fatty acid release by (isolated) adipose tissue differs in several ways from that of ACTH and the catecholamines. (a) Glucocorticoids decrease glucose uptake when they stimulate fatty acid release, whereas the other (lipolytic) hormones accelerate both processes. (b) The maximal rate of fatty acid release is less and the onset of action is much slower in tissues treated with glucocorticoids than with the other hormones (21). (c) The lipolytic action of glucocorticoids is prevented by small amounts of insulin, whereas the stimulatory action of ACTH and of catecholamines on fatty acid release is unaffected (12) or slightly reduced (21) by insulin.

These experiments show clearly that glucocorticoids in the absence of insulin have an inhibitory effect on glucose uptake and utilization. It is very likely, then, that the increased release of fatty acid in the glucocorticoid-stimulated adipose tissue is secondary to decreased glucose metabolism. Whether

⁴ J. N. Fain, unpublished experiments.
glucocorticoids block glucose transport, phosphorylation, or other processes is not known at present. Perhaps as the result of decreased glucose uptake, formation of glycero-phosphate may be reduced, which would retard re-esterification of fatty acid and make more fatty acid available for diffusion to the media. This is supported by the finding that incorporation of radioglucose into glyceride-glycerol was actually decreased by glucocorticoids (12).

The increased uptake of glucose and its incorporation into media. This is supported by the finding that incorporation of decreased glucose uptake, formation of cr-glycerophosphate release and glucose uptake in parametrial adipose tissue. Deoxycorticosterone had little effect on fatty acid release, 21.

SUMMARY

1. Dexamethasone at very low concentrations, $10^{-8}$ to $10^{-7}$ M, increased the release of fatty acid by incubated parametral and mesenteric adipose tissue. Corticosterone at 10 times the above concentrations produced similar effects. 2a-Methylcortisol was as potent as corticosterone, whereas 2a-methylcortisone was ineffective. Deoxycorticosterone had little effect on fatty acid release.

2. Glucose uptake and its conversion to carbon dioxide, total lipid, and fatty acid was decreased by glucocorticoids.

3. Addition of small amounts of insulin (4 milliunits per ml) to the media reversed the effects of dexamethasone on fatty acid release and glucose uptake in parametral adipose tissue.

4. The onset of action of glucocorticoids was slow; at least 2 hours of incubation were required before dexamethasone had any effect on adipose tissue.

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