Mobilization of fatty acids in genetically obese rats

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ABSTRACT
The mobilization of fatty acids has been studied in genetically obese rats of the Zucker strain and in control obese animals with bilateral destructive lesions in the hypothalamus. The body weight and size of adipose cells did not differ significantly between the genetically obese rats and the obese controls. Weight loss in control and genetically obese rats was identical during a 1 month fast. The release of glycerol and the rise in free fatty acids in adipose tissue incubated in vitro were similar in tissue from genetic and hypothalamic obese rats. Epinephrine, theophylline, and dibutyryl cyclic adenosine monophosphate all augmented lipolysis, and the effects were usually greater in the tissues from genetically obese rats.

SUPPLEMENTARY KEY WORDS hypothalamic obesity - epinephrine - theophylline - dibutyryl cyclic AMP

Obesity, as an inherited trait, is present in several strains of rodents (1–6). The strain of genetically obese rats described by Zucker and Zucker (5) have elevated triglycerides (7, 8), an increased rate of protein synthesis by the liver (9, 10), and an increased rate of glyceride-glycerol synthesis by isolated fat cells (11). Studies from our laboratory on this strain of rats demonstrated that adipose tissue from genetically obese rats converted greater quantities of radioactive glucose or pyruvate into glyceride-glycerol relative to the amount oxidized to CO2 than did tissue from control rats with obesity induced by hypothalamic lesions (11). The fatty acids for glyceride synthesis could come from one of two sources: either they could be synthesized de novo in the adipose tissue or they could arise from enhanced lipolysis. The experiments reported here were designed to explore this latter possibility.

Abbreviations: FFA, free fatty acids.

METHODS AND MATERIALS

Animals
The 10 genetically obese female rats and the 10 control rats were purchased from Dr. L. Zucker (Harriet G. Bird Memorial Laboratory, Stow, Mass.). Three of these lean rats (pairs 4–6, Table 1) were litter mates to the corresponding obese rats and may have heterozygous for the “fatty” gene. The remaining seven rats, however, did not carry the gene for obesity. These 10 control rats were made obese by introducing bilateral electrolytic lesions into the ventral medial hypothalamus (11). The locus of the electrodes (DeGroot Atlas [12]) was 6 mm anterior, 1.1 mm lateral to the midline and 0.5 mm above the cranium. All rats were fed Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) and had tap water ad lib.

Procedures
For in vitro incubation, pieces of subcutaneous fat weighing 5–10 g were removed from similar locations in each pair of rats, while the animals were under light ether anesthesia. Fat cells were prepared by the method of Rodbell (13), and their size assessed as previously described (14). Two types of experiments were performed. In the first, pieces of adipose tissue, weighing 60–80 mg or adipose cells equivalent to 180 mg of triglyceride, were incubated under an atmosphere of 95% O2–5% CO2 at 37°C for 1 hr in a medium of Krebs-Ringer bicarbonate buffer, pH 7.4, containing bovine albumin, 40 mg/ml (fraction V, Lot C-24503, Armour Pharmaceutical Co., Chicago, Ill.) and 2 mM glucose. At the end of the incubation, an aliquot of medium from incubations with tissue was removed for measurement of glycerol (15), and the remaining medium was separated from the tissue for determination of free
Body Weight and Fat Cell Volume

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Body Weight</th>
<th>Diameter</th>
<th>Volume</th>
<th>Body Weight</th>
<th>Diameter</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>535</td>
<td>125 µm</td>
<td>1.096</td>
<td>540</td>
<td>125 µm</td>
<td>1.086</td>
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<tr>
<td>2</td>
<td>735</td>
<td>138 µm</td>
<td>1.492</td>
<td>765</td>
<td>122 µm</td>
<td>1.014</td>
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<tr>
<td>3</td>
<td>535</td>
<td>159 µm</td>
<td>1.535</td>
<td>445</td>
<td>137 µm</td>
<td>1.435</td>
</tr>
<tr>
<td>4</td>
<td>560</td>
<td>132 µm</td>
<td>1.290</td>
<td>575</td>
<td>132 µm</td>
<td>1.290</td>
</tr>
<tr>
<td>5</td>
<td>590</td>
<td>125 µm</td>
<td>1.114</td>
<td>535</td>
<td>139 µm</td>
<td>1.426</td>
</tr>
<tr>
<td>6</td>
<td>610</td>
<td>116 µm</td>
<td>0.891</td>
<td>480</td>
<td>121 µm</td>
<td>1.066</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

Materials

N\(^6\), O\(^2\)-dibutyryl adenosine-3'-5'-cyclic monophosphate (DBC) was purchased from Boehringer, Mannheim Corp., New York; theophylline was from Matheson Co., Inc., East Rutherford, N. J.; epinephrine was from Parke, Davis, & Co., Detroit, Mich. The bovine albumin was obtained from Armour Pharmaceutical Co. Dexamethasone was purchased from Merck, Sharp & Dohme of Rahway, N. J.

RESULTS

Body Weight and Fat Cell Volume

Table 1 shows the body weights and volume of the fat cells in six pairs of rats used in these experiments. The repeated measurements of fat cell volume available on pair 3 were in good agreement. Pairs 4–6 were litter mates from each of three separate litters. The animals in the third pair differed in weight by 90 g, while the animals in pair 6 differed in weight by 130 g; the other pairs had similar weights. Since the cell volumes were close in all pairs, subsequent data on lipolysis have been compared using tissue weights or triglyceride content.

Lipolytic Response to Epinephrine, Dibutyryl Cyclic AMP, and Theophylline

In the first experiment (Fig. 1), pieces of subcutaneous adipose tissue from three genetically obese rats were incubated for up to 2 hr alone or with 3 mM dibutyryl cyclic AMP, 3 mM theophylline, or 10 µg/ml of epinephrine. Glycerol release was stimulated by all three drugs, but there was little lipolysis in their absence. The maximal effect was seen at 60 or 90 min. Dose response curves for each of these lipolytic agents were obtained during a 1 hr incubation of pieces of fat from four pairs of genetically obese rats and their "lesioned" litter mates (Figs. 2–4). The release of glycerol was stimulated by epinephrine in both groups (Fig. 2) to a similar degree. Since glucose was in the incubation medium, however, there was a high rate of reesterification with little accumulation of FFA in either medium or tissue. Theophylline (Fig. 3) and dibutyl cyclic AMP (Fig. 4), on the other hand, had a somewhat smaller effect as measured by the release of glycerol, but more fatty acids were released into the medium. In these latter experiments, the concentration of FFA in the tissues was significantly higher with fat from genetically obese rats. In a fourth experiment a dose response curve for dibutyryl cyclic AMP was determined using isolated fat cells (Fig. 5). The concentration of FFA in the combined cells and medium from genetically obese rats was again significantly higher.
FIG. 2. Effect of epinephrine on the lipolysis in adipose tissue from genetic and control obese animals. Segments of subcutaneous adipose tissue weighing 60–80 mg from four genetically obese and four paired control obese rats were incubated for 1 hr in Krebs-Ringer bicarbonate buffer with 4% albumin, alone or with various concentrations of epinephrine. Glycerol concentration (A) is on the left, and the concentrations of free fatty acids in the medium (B) and tissue (C) are shown in the middle and right, respectively. The open circles (○) are the mean ± SEM for observations on four genetically obese rats, and the solid circles (●) are the mean ± SEM for four paired control obese rats with data expressed per g of tissue.

FIG. 3. Effect of theophylline on the lipolysis in adipose tissue from genetic and control obese animals. Segments of subcutaneous adipose tissue weighing 60–80 mg from four genetically obese and four paired control obese rats were incubated for 1 hr in Krebs-Ringer bicarbonate buffer with 4% albumin, alone or with various concentrations of theophylline. Glycerol concentration (A) is on the left, and the concentrations of free fatty acids in the medium (B) and tissue (C) are shown in the middle and right, respectively. The open circles (○) are the mean ± SEM for observations on four genetically obese rats, and the solid circles (●) are the mean ± SEM for four paired control obese rats with data expressed per g of tissue.

Effect of Fasting
In the final experiment four pairs of obese rats (not shown in Table 1) were fasted for 28 days, and the loss of weight was recorded (Table 2). The loss in weight was more than one-third of total body weight. During the 4 wk fast the groups lost weight at the same rate, with almost identical total losses.

DISCUSSION
Our experiments show that the mobilization of fatty acids from the adipose tissue of genetically obese rats is normal or increased relative to that of obese, control rats and is in accord with the data of Zucker (17, 18). During a 1-month fast genetically obese rats and obese controls each lost about one-third of their body weight. On the basis of experiments by Zucker (17), and our unpublished data, these obese rats could be expected to survive more than 3 months on a total fast. Zucker (18) has also reported that fasting for 18 hr was followed by a significant increase in the concentration of FFA, further strengthening the concept of adequate mobiliza-

Fig. 4. Effect of dibutyryl cyclic AMP on the lipolysis in adipose tissue from genetic and control obese animals. Segments of subcutaneous adipose tissue weighing 60–80 mg from four genetically obese and four paired control obese rats were incubated for 1 hr in Krebs-Ringer bicarbonate buffer with 4% albumin, alone or with various concentrations of dibutyryl cyclic AMP. Glycerol concentration (A) is on the left, and the concentrations of free fatty acids in the medium (B) and tissue (C) are shown in the middle and right, respectively. The open circles (○) are the mean ± SEM for observations on four genetically obese rats, and the solid circles (●) are the mean ± SEM for four paired control obese rats with data expressed per g of tissue.

Fig. 5. Effect of dibutyryl cyclic AMP on the release of FFA by adipose cells of genetic and hypothalamic (control) obese rats. Fat cells for four genetic and four paired control rats were incubated in Krebs-Ringer bicarbonate buffer with 4% albumin for 1 hr with various concentrations of dibutyryl cyclic AMP. Each point is the mean ± SEM for the four animals with data expressed per g of triglyceride. The FFA, which were measured, were in the combined cells and medium.

Table 2. Effect of Fasting on the Weight Loss of Genetic and Hypothalamic Obese Rats

<table>
<thead>
<tr>
<th></th>
<th>Weight Loss During 4 wk Fast</th>
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<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Genetic</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>700 ± 12*</td>
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<tr>
<td>Hypothalamic</td>
<td>780 ± 55</td>
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</table>

* Mean ± SEM.
a reflection of the genetic abnormality in these animals. A small increase in intracellular FFA in adipose tissue may also explain our earlier observations on lipogenesis (11). In these experiments fat cells from genetically obese rats converted more glucose-14C or pyruvate-14C into glyceride-glycerol-14C relative to 14CO2 than did fat cells from the control obese rats. The increased rate of formation of glyceride-glycerol could have resulted from the higher intracellular levels of fatty acids.

Release of fatty acids from adipose tissue in vitro has been studied in the obese (Bar Harbor) mouse (22-26) and in the New Zealand obese mouse (27). In the obese mouse (Bar Harbor), lipolysis stimulated by epinephrine is impaired relative to that found in adipose tissue (22, 23) from lean mice or mice with obesity produced gold thioglucone. In these genetically obese mice, the FFA in the adipose tissue remained low with fasts of up to 6 days (24, 25). Since these mice survived fasting for more than 21 days (25), one must conclude, however, that the release of FFA from adipose tissue was adequate to provide for their energy needs. In contrast with the obese (Bar Harbor) mouse, adipose tissue from starved New Zealand obese mice (NZO) released FFA into the medium during incubation in vitro (27). Like the Bar Harbor mice these mice also survived a total fast of more than 21 days. Although differences exist in the pattern of lipolysis observed in vitro, these studies on obese mice and the data from obese rats support the concept that the free fatty acids can be mobilized from adipose tissue glycerides at an adequate rate to sustain prolonged fasting in rodents with metabolic obesity.

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