Feeding in Response to Insulin and 2-Deoxy-D-Glucose in Zucker Rats on Dietary Self-Selection

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Summary Adult male fatty and lean rats of Zucker strain were given access ad libitum to either a single nutritionally complete diet, or a self-selection regime with separate sources of three macronutrients, protein (casein), fat (hydrogenated coconut oil), and carbohydrate (sucrose). Animals on the single diet were fed on a powdered stock diet, and then switched to the self-selection regime. Energy intake on the self-selection regime was the same as that for the single diet condition in both fatty and lean rats. Fatty rats consumed 45% more energy than did their lean littermates. Further, fatty rats selected 47.0% of their total calories as protein, 30.1% as fat, and 22.9% as carbohydrate. The respective percentages for lean rats were 56.1, 13.0 and 30.9. In lean rats, the injection of insulin (10 U/kg) or 2-deoxy-D-glucose (500 mg/kg, 2DG) failed to increase energy intake, but increased carbohydrate intake 2 times by attenuating protein intake. Also in fatty rats, insulin did not increase energy intake, but it did increase carbohydrate intake by 50% by attenuating fat intake. 2DG decreased energy intake by attenuating carbohydrate and fat intakes in fatty rats. Fatty rats were slightly less hypoglycemic to insulin, but more hyperglycemic to 2DG than lean rats. These different self-selection patterns of fatty rats seemed to be associated with their endocrine, metabolic, and behavioral abnormalities.

Key Words diet selection, glucoprivation, obesity

Hyperphagia is a common factor in obesity (1). However, in most experiments documenting hyperphagia in obese animals, the animals were provided with access to only a single nutritionally complete diet. One problem with experiments of this nature is that it is impossible to determine if the increases in food intake reflect modifications in energy requirements or in the need for specific nutrients. One way to overcome this problem is to provide animals with separate sources of three
macronutrients, protein, fat, and carbohydrate.

In the preceding report, we employed this method of diet presentation to show that, in Sprague-Dawley rats, dietary self-selection was governed by body energy status (2). The feeding patterns of Zucker fatty rats are different from those of their lean littermates in several ways (3–6). We reported that fatty rats showed impaired responses of food intake to brain glucoprivation, loss of body storage of energy, or decreased assimilation of body energy (7). Furthermore, their food consumption was very sensitive to changes in circulating insulin levels caused by streptozotocin or insulin administration (7).

The present study was undertaken to determine if Zucker fatty rats were hyperphagic when given a dietary self-selection regime, and if dietary self-selection patterns of the rats could be modified by glucoprivation.

METHODS

Animals and diets. Male Zucker fatty rats and their lean littermates (bred and supplied from the Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., Takatsuki, Japan) were used. They were weaned at 28 days of age, and fed on a pelleted stock diet (CE-2, Clea Japan, Inc., Tokyo). At 25 weeks of age, the animals were given a powdered stock diet (CE-2) for 10 days, and then placed on dietary self-selection for 21 days. Body weight at the beginning of the self-selection regime was 406±8 g for lean and 641±18 g for fatty rats. Animals on the self-selection regime were presented with three separate dietary rations: a protein ration, a carbohydrate ration, and a fat ration as described in the preceding paper (2). They were housed individually in wire-mesh hanging cages in a room controlled for temperature (23±1°C), humidity (55±5%), and light (08:00–20:00 h). On the self-selection regime, three food containers equipped with metal discs (CL-0920, Clea Japan, Inc., Tokyo), each filled with different nutrients, were given to each animal. The containers were placed at random in the cages and could be moved around by the rats, thereby precluding place conditioning. Every day at 09:00 h, each rat was weighed, and its containers were weighed and refilled in most cases; however, the measurements were omitted on weekends in certain cases. Correction for spillage was occasionally needed for a fat source. Food and water were available at all times.

Feeding response to insulin and 2-deoxy-D-glucose. The rats were injected subcutaneously with saline on days 10 and 15, 500 mg/kg body weight of 2-deoxy-D-glucose (2DG) (Wako Pure Chemical Industries, Ltd., Osaka) on day 11, or 10 U/kg body weight of regular insulin (Novo Industri, Copenhagen) on day 16 after the self-selection regime was begun. Energy intake and distribution were similar for rats injected with saline on days 10 and 15, so we used data obtained on day 15.

On the injection day, each animal was removed from its cage, weighed, injected, and returned to the cage with refilled diet containers; food intake was measured to an accuracy of 0.1 g at 1, 2, 4, and 6 h after injection.

Response of blood glucose to insulin and 2DG. The animals were injected
subcutaneously with saline on day 20, and divided into two groups. One group received a subcutaneous injection of insulin (10 U/kg) and the other received a subcutaneous injection of 2DG (500 mg/kg) on day 21 after the self-selection regime. Rats were given no diet for 3 h after injection, and blood samples were taken by cutting the tips of tails at 0, 1, 2, and 3 h after injection. Blood glucose was determined by the glucose oxidase method (8).

Statistical analysis. The data reported are the means with their standard errors, and statistical significance was determined between means by use of Student's unpaired t-test (9).

RESULTS

Energy intake on dietary self-selection

Body weight gains during the last 7-day period on stock diet and the first 7-day period on the dietary self-selection regime were $2.7 \pm 0.8$ g and $1.8 \pm 1.2$ g for lean rats, and $9.6 \pm 1.6$ g and $12.8 \pm 2.6$ g for fatty rats, respectively. The energy intake from the three sources and the stock diet was similar in both fatty and lean rats (Table 1). Fat rats were hyperphagic on the dietary self-selection regime; their fat intake was 3.3 times that of lean rats. The protein intake of fatty rats was slightly, but significantly higher than that of lean rats. On the other hand, carbohydrate intake was similar in both types of rats. Fatty rats chose a diet that provided 47.0% of the total energy intake as protein, 22.9% as carbohydrate, and 30.1% as fat. Lean rats chose a diet that provided 1.3 times as many carbohydrate calories (30.9% of the total energy intake), and less than one-half of the proportion of fat (13.0% of the total energy intake).

Dietary self-selection after insulin and 2DG

Insulin failed to increase the energy intake of either fatty or lean rats on the self-selection regime (Fig. 1). 2DG decreased the energy intake of fatty rats during the experimental period of 6 h. The energy intake of lean rats injected with 2DG decreased during the first 2 h, but thereafter increased to reach saline-injected control levels (Fig. 1). Both insulin and 2DG injection in lean rats increased carbohydrate intake, but decreased protein intake without affecting total energy intake (Fig. 2). Increased fat intake was observed in saline-injected fatty rats; the value was 4 times that for the corresponding lean rats. Insulin injection increased carbohydrate intake by attenuating fat intake, whereas 2DG injection decreased both carbohydrate and fat intakes in fatty rats. As a result of changes observed in absolute nutrient intakes, percent nutrient intakes were also modified by insulin or 2DG injection (Fig. 2). Both insulin and 2DG increased percent carbohydrate intake, but decreased percent protein intake in lean rats; percent fat intake tended to decrease. In fatty rats, insulin increased percent carbohydrate intake by attenuating percent fat intake, whereas 2DG increased percent protein intake by attenuating percent fat intake.
Table 1. Energy intake of the last and the first 7 days before and after dietary self-selection.1

<table>
<thead>
<tr>
<th>Rats</th>
<th>Energy intake</th>
<th>Stock diet</th>
<th>Three separate sources</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kcal/7 days</td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Lean</td>
<td>470.4±26.6</td>
<td>451.5±9.8</td>
<td>139.3±8.4</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td>30.9±1.6</td>
</tr>
<tr>
<td>Fatty</td>
<td>642.6±28.0a</td>
<td>653.1±28.0a</td>
<td>149.8±1.4</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td>22.9±2.2</td>
</tr>
</tbody>
</table>

1 Means±SEM (n=10). Data for the last and the first 7 days before and after dietary self-selection were collected from animals given powdered stock diet for 10 days and then placed on dietary self-selection for 21 days. Significantly different from the corresponding lean rats (*p<0.001, †p<0.01, ‡p<0.05).

Response of blood glucose to insulin and 2DG

Insulin produced a marked hypoglycemia and 2DG a marked hyperglycemia in both lean and fatty rats (Table 2). However, fatty rats, compared to lean rats, showed a less hypoglycemic response to insulin, but a more hyperglycemic response to 2DG.

DISCUSSION

The results of this study demonstrate that both lean and fatty rats given a dietary self-selection regime with separate sources of the three macronutrients — protein, carbohydrate, and fat — consume the same amounts of energy as rats fed
on a nutritionally complete single diet. However, there was a distinct difference between lean and fatty rats in dietary self-selection patterns. Fatty rats preferentially consumed fat. Their absolute and percent fat intake was, respectively, 3.3 and 2.3 times greater than that of lean rats. These results are consistent with the recent report of a dietary self-selection experiment in which Zucker fatty rats were hyperphagic and consumed excessive fat (10). These data suggest that the enhanced appetite for fat may reflect a behavioral mechanism that is related to the lipid-

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Table 2. Changes in blood glucose levels after subcutaneous injection of insulin or 2DG.  

<table>
<thead>
<tr>
<th>Rats</th>
<th>Injection</th>
<th>N</th>
<th>0</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>Saline</td>
<td>10</td>
<td>79 ± 2</td>
<td>81 ± 2</td>
<td>68 ± 3</td>
<td>61 ± 2</td>
</tr>
<tr>
<td></td>
<td>Insulin (10 U/kg)</td>
<td>5</td>
<td>80 ± 1</td>
<td>21 ± 1*</td>
<td>16 ± 2*</td>
<td>12 ± 2*</td>
</tr>
<tr>
<td></td>
<td>2DG (500 mg/kg)</td>
<td>5</td>
<td>80 ± 3</td>
<td>213 ± 5*</td>
<td>172 ± 4*</td>
<td>112 ± 5*</td>
</tr>
<tr>
<td>Fatty</td>
<td>Saline</td>
<td>10</td>
<td>116 ± 5</td>
<td>97 ± 3</td>
<td>83 ± 2</td>
<td>77 ± 2</td>
</tr>
<tr>
<td></td>
<td>Insulin (10 U/kg)</td>
<td>5</td>
<td>105 ± 6</td>
<td>35 ± 3*</td>
<td>26 ± 3*</td>
<td>24 ± 1*</td>
</tr>
<tr>
<td></td>
<td>2DG (500 mg/kg)</td>
<td>5</td>
<td>110 ± 5</td>
<td>278 ± 10*</td>
<td>303 ± 12*</td>
<td>251 ± 11*</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM. Significantly different from the corresponding saline injection (*p < 0.001).

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Fig. 2. Energy intake from carbohydrate ( ), protein ( ), and fat ( ) for 6 h after subcutaneous injection of saline, insulin, or 2DG. Means ± SEM for 10 rats. Significantly different from saline (*p<0.05, **p<0.02, ***p<0.01, ****p<0.001).
storing predisposition of these rats (11–13).

In the preceding paper (2), we observed an inverse relation between fat intake and body energy storage in Sprague-Dawley rats. Although excessive fat accumulates in fatty rats (11–13), such a relation was not observed in the present experiment. Furthermore, fatty rats fail to increase their food intake when fed an energy-diluted diet (3), and when large amounts of energy are lost as urinary glucose in diabetes induced by streptozotocin (17). These results suggest that a negative feedback mechanism is not responsible for the regulation of energy intake in fatty rats.

A large number of investigators have observed that rats in the single diet condition increase their food intake in response to a hypoglycemic emergency produced by insulin injection (7, 14–18) or to decreased glucose utilization produced by injection of 2DG (7, 18–21). However, when maintained on the self-selection regime, neither lean nor fatty rats were hyperphagic, but selectively consumed carbohydrate in response to insulin. In contrast to carbohydrate intake, protein intake in lean rats and fat intake in fatty rats decreased as a function of insulin administration. A similar modification in selection pattern was observed in lean rats injected with 2DG.

The alterations found in dietary self-selection patterns following the administration of insulin or 2DG reflect the animal’s attempt to restore homeostatic balance to its internal milieu. Both insulin and 2DG lead to a decrease in glucose availability at glucoreceptors within the central nervous system (CNS). Feeding during glucoprivation provides exogenous glucose and thereby increases glucose availability to the CNS. Among the three macronutrients, it may be presumed that ingestion of carbohydrate following insulin or 2DG administration would result in the most rapid elevations in glucose availability. The increase in food intake typically observed following insulin or 2DG administration, thus may be a consequence of a specific need for carbohydrate rather than a general requirement for energy. The fact that total energy intake was not elevated for animals maintained on the self-selection regime supports this conclusion. In fatty rats 2DG did not produce hyperphagia, but a marked hyperglycemia, indicating that fatty rats detect glucoprivation induced by 2DG injection. Therefore, it appears that in these rats feeding and hyperglycemia are dissociable; hyperglycemia occurs, but feeding does not. Fatty rats, even when fed on a single diet, fail to increase their food intake to 2DG-injection in spite of a marked hyperglycemia (7). Therefore, the glucosensitive site that is responsive for feeding elicited by 2DG appears to be impaired in fatty rats.

Numerous investigators have established that obesity is not a unitary disorder; it can be associated with a variety of different endocrine, metabolic and behavioral parameters (1, 22–25). The present results, in conjunction with data from our preceding experiments (2), demonstrate that patterns of diet selection also differ among animals with different forms of experimental obesity. These differences may have important implications for the study of obesity, as patterns of diet selection

may reflect underlying metabolic regulation mechanisms.

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REFERENCES


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