Effect of Factors besides Hyperphagia on Cellularity of Adipose Tissue in Gold Thioglucose-Induced Obese Mice

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Summary Whether or not factors besides hyperphagia influence cellularity of adipose tissue in gold thioglucose (GTG)-treated mice was examined. The animals were treated with GTG (800 mg/kg body weight) or saline as control. Control and one of the GTG-treated groups were given a diet ad libitum and another of the GTG-treated groups was pair-fed to control. In GTG-treated group fed ad libitum, hyperphagia and obesity were observed and an enlargement in the parametrial adipose tissue was mainly due to increases both in size and number of adipocytes. The GTG-treated group became obese compared to control even if food intake was comparable. The pair-feeding failed to inhibit completely the increase of both size and number of adipocytes in the fat pads. These results suggest that other factors besides hyperphagia might influence changes of cellularity of the parametrial adipose tissue in the GTG-induced obesity.

Key Words obesity, cellularity, adipose tissue, hyperphagia, gold thioglucose

An obesity induced by gold thioglucose (GTG) has been concluded by Hollifield and Parson (1) to be solely the result of the hyperphagia. They found that GTG-induced obese mice have the same total fat content at control when reduced to the same weight as control by limited feeding, and their conclusion has been generally accepted. This obesity has also been known as the syndrome of hypothalamic obesity (2, 3), which has been reported as a type of obesity with both hypertrophy and hyperplasia of adipocytes (4). It appears that the hyperphagia influences the cellularity of adipose tissues and contributes to the development of GTG-induced obesity. However, because of the proven importance of the hypothalamus as a regulatory center for many bodily functions, it is possible that

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hypothalamic lesion by GTG-treatment directly causes major changes in the cellularity of adipose tissue.

The present study, therefore, was designed to examine whether or not factors besides the hyperphagia influence the cellularity in adipose tissue of the obesity induced by GTG-treatment. To eliminate contributions of the hyperphagia, pair-fed animals were used in the present study.

**MATERIALS AND METHODS**

**Diet.** The diet was of the following composition: casein, 20%; corn oil, 5%; salt mixture, 4%; vitamin mixture, 1%; choline chloride, 2%; sucrose, 23%; and corn starch to make 100%. The salt and the vitamin mixture was identical with Haper's mixtures (5). All dietary sources were purchased from Oriental Yeast Co., Tokyo.

**Animal and care.** Female ICR mice (Japan Charles River Co., Kanagawa, Japan) weighing about 19 g were used. They were given the diet for 3 days for acclimation and then 30 animals received GTG (gold thioglucose; aurothioglucose, Sigma Chemical Co., USA) treatment (800 mg/kg body weight, i.p). The concentration of GTG solution was 5%. After the GTG-treatment, mice were given the diet *ad libitum*. Animals which did not eat more than control mice within 1 week after the GTG-treatment were discarded as not being hyperphagic. The hyperphagic GTG-treated mice were then divided into two groups. Nine control mice were prepared by i.p injection of a saline as control (C). Group C and 9 mice of hyperphagic GTG-treated group (GO) were given the diet *ad libitum* and 9 mice of another hyperphagic GTG-treated group (GP) were pair-fed to provide the same amount of the diet as that consumed one day before by control mice. Animals were housed in individual cages with a screen bottom of stainless steel and kept in a room maintained at 23 ± 1°C in a 12-h light (8:00 to 20:00) and 12-h dark (20:00 to 8:00) cycle for 9 weeks. Food intake was recorded daily at 10:00 in the morning before replenishing the diet. Body weight was recorded weekly in the morning.

**Cellularity.** At the end of the experimental ninth week after the GTG-treatment, animals were killed by vertebrate dislocation. The liver and the parametrical adipose tissue were removed, blotted with a filter paper, and weighed. The adipose tissue was provided for measurements of cellularity. Small fragments from three portions of the adipose tissue (total about 100 mg) were fixed for 48 h in 2% osmium tetroxide in collidine-HCl buffer (pH 7.4) at 37°C as described by Hirsch and Gallian (6). The fixed cells were freed by washing with distilled water through a 250-μm steel mesh filter and collected on a 25-μm steel mesh filter. With a projection microscope, 200 to 300 cells were sized by an image analysis system equipped with a classification program (TAS, Leitz, West Germany). This instrument directly provides frequency distribution of cell diameters, with class intervals of 10 μm. The total numbers of fat cells in this adipose tissue were estimated by dividing the total triglyceride content of the depot by the mean triglyceride content.
of fat cells. After calculation of the average volume of adipocytes by the formula of Goldrick (7), the triglyceride content of adipocytes was obtained by assuming that the density of adipocytes is that of triolein (0.915 g/ml) as proposed by Lemonnier (8). Triglyceride content in the adipose tissue was measured according to the method of Soloni (9).

Estimation of lipogenesis from $[^{14}C]$glucose into triglyceride in vitro in adipose tissue. Lipogenesis was measured as the rate of incorporation from $[^{14}C]$glucose to triglyceride in adipose tissue fragments as described by Masuno et al. (10). Adipose tissue fragments were used here in order to avoid the complicating effects of cell damage because larger adipocytes are more fragile than smaller ones during isolation procedures. The parametrial adipose tissues were minced with sharp scissors in Krebs-Ringer bicarbonate buffer (pH 7.4) and washed with this buffer. In a glass-stoppered test-tube, 80 mg of tissue fragments ($d < 1.0$ mm) were incubated at $37^\circ C$ for 30 min with 1 ml of this buffer (pH 7.4) containing 2.5% bovine serum albumin, 1.25 mM CaCl$_2$, 1 mM glucose, and 0.5 $\mu$Ci of $[^{14}C]$glucose. The tubes were gassed with 95% O$_2$: 5% CO$_2$ mixture. At the end of the incubation period, triglyceride in tissue fragments was extracted by the method of Dole (11). After washing with 0.05 N NaOH in 50% (w/w) ethanol to remove free fatty acids, an aliquot of the extracts containing triglyceride was used for the estimation of radioactivity.

Statistical analysis. All data were analyzed by analysis of variance and significant differences among means were identified by the Student's $t$-test. All statements of significant differences refer to the 5% level of probability. Values were expressed as mean ± SEM.

RESULTS AND DISCUSSION

In the present study, we examined the effect of factors besides hyperphagia on the cellularity of adipose tissue in GTG-induced obese mice.

Changes in food intake after GTG-treatment are shown in Fig. 1. The food intake was constant in group C and there were no significant differences in intake between group C and group GP throughout the experiment. In contrast, the food intake in group GO rapidly increased for the first 4 weeks after the GTG-treatment and then remained constant. The hyperphagia was observed only in group GO.

Changes in body weight after GTG-treatment are shown in Fig. 2. The growth rate in group GO was remarkably increased when compared with that in group C. The body weight gain was significantly greater in group GP than in group C. Patterns of changes in body weight and food intake during the experimental period in group C and group GO were similar to those in CBA/Ki mice with this obesity type reported by Gray and Liebelt (12). Although the food intake was the same, the body weight gain was significantly greater in group GP than in group C. The feed efficiency (total body weight gain/total food intake) was the greatest in group GO and was also significantly greater in group GP than in group C (feed efficiency:
Fig. 1. Changes in food intake after GTG-treatment. ●, control group; ○, GTG-induced obese group; and ▲, GTG-treated group pair-fed to control. Total food intake was 270 ± 7 g in group C, 351 ± 3 g in group GO, 263 ± 5 g in group GP, respectively. Vertical bars represent mean ± SEM (n=9); values not sharing common superscript letters are significantly different (p<0.05).

These results demonstrate that the GTG-induced obesity is due not only to an increase in the food intake but also to an increase in the efficiency of feed utilization. In this study, apparent digestibilities of dietary protein, fat, ash, and carbohydrate were comparable among the three groups and the diet was highly digested by all mice (data not shown). The elevated feed efficiency in group GP, therefore, was not due to differences in the digestibilities of nutrients. The liver weight increased significantly in group GO, 2.42 ± 0.20 g, when compared to group C, 1.51 ± 0.09 g, or group GP, 1.49 ± 0.09 g. There was no significant difference in the liver weight between group C and group GP. In addition, we observed that also the prevention of hyperphagia normalized the hypertrophy of organs except for adipose tissue in GTG-treated mice (data not shown). Probably, the increase in the body weight in group GP was mainly accompanied by an increase in the percentage of body fat. In VMH-lesioned obese rats, pair-feeding did not prevent the rise in body fat (13).

Cellularity of the parametrial adipose tissue is shown in Table 1. In group GO, changes in the rate of increase in the parametrial adipose tissue corresponded well with that of increase in body weight. The enlargement in this depot was mainly due to increases both in size and number of adipocytes. As shown in Fig. 3, distributions of adipocyte diameters were of peakedness and large adipocytes more than 180 μm in diameter were remarkably increased when compared with group C. Our results also are in agreement with the results that both hypertrophy and hyperplasia of adipocytes occurred in GTG-induced obese mice reported by Wise (4). On the other hand, the pair-feeding to control did not completely block the development of the
Fig. 2. Changes in body weight after GTG treatment. ●, control group; ○, GTG-induced obese group; and ▲, GTG-treated group pair-fed to control. Average initial body weight was 23 g (range: 20 to 25 g). Final body weight was 24 ± 1 g in group C, 58 ± 3 g in group GO, and 39 ± 2 g in group GP, respectively. Vertical bars represent mean ± SEM (n=9); values not sharing common superscript letters are significantly different (p<0.05).

Table 1. Comparison of cellularity in parametrial adipose tissues.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group C&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Group GO&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Group GP&lt;sup&gt;3&lt;/sup&gt;</th>
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<tr>
<td>Tissue weight (g)</td>
<td>1.45 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.65 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.52 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cell volume (TG&lt;sup&gt;2&lt;/sup&gt;, μg/cell)</td>
<td>1.69 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.63 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cell number (× 10&lt;sup&gt;9&lt;/sup&gt;/tissue)</td>
<td>5.22 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.65 ± 0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.18 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>1</sup>Group C, control group.  <sup>2</sup>Group GO, gold thioglucose induced obese group.  <sup>3</sup>Group GP, gold thioglucose treated group pair-fed to control mice.  <sup>4</sup>Mean ± SEM (n=9), values not sharing a common superscript letter are significantly different at p < 0.05.  <sup>5</sup>TG, triglyceride.

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obesity as measured by increased weight of the parametrial adipose tissue in the GTG-treated mice. Although the pattern of distribution of cell size in group GP was similar to that in group C, the population of adipocytes in group GP shifted slightly toward increasing cell size when compared with group C. Strikingly, the pair-feeding to control did not inhibit completely the hyperplasia as well as the hypertrophy of adipocytes (Table 1, Fig. 3). These results suggest that other factors besides the hyperphagia might also contribute to the development of this obesity and be involved in the control of adipose cellularity. Our observations are in agreement with the reports in which central nervous system lesion produces weight gain and excess fat without a change in food intake (13, 14). The GTG-induced obesity has also been reported as one of hypothalamic obesity (2, 3). Because of the proven importance of the hypothalamus as a regulatory center for many bodily functions, it is possible that hypothalamic lesion by GTG-treatment directly causes major changes in the cellularity of adipose tissue.

Rate of lipogenesis from [14C]glucose in vitro in the parametrial adipose tissue are shown in Fig. 4. The rate of lipogenesis per cell significantly decreased in the obese mice and the pair-feeding did not completely prevent the decrease of the cellular lipogenesis. The rate of lipogenesis per adipose tissues was comparable among the three groups. Decreased lipogenesis per cell in the obese animals may be...
related to the time at which the measurements were made, relative to the static phase of obesity. It seems likely that the decrease of lipogenesis per cell in the obese mice might indicate a protective response in adipocytes to the development of the obesity. Martin and Lamprey (15) reported that the rate of in vivo lipogenesis and some lipogenic enzyme activities in adipose tissue were increased during the onset of this obesity.

Generally, excessive intake of energy, defined by the difference between energy intake and expenditure is accepted to be a cause of obesity. Although the level of energy intake is the same, an animal with a low expenditure of energy gains excess weight and fat. Recently, hypoactivity of thermogenesis has been reported in VMH-lesioned rats (16). Possibly, the thermogenic activity also decreased in GTG-treated mice.

Our results have discrepancies against the results that GTG-induced obese mice have the same total fat content as controls when reduced to the same weights as controls by limited feeding reported by Hollifield and Parson (1). One problem with single meal pair-feeding in this study might be alterations of feeding patterns. Presumably, pair-fed animals might eat the food rapidly and then be starved longer. If so, this may produce changes in the cellularity of adipose tissue.

The present results suggest that other factors besides the hyperphagia might contribute to changes of the cellularity in adipose tissue in the GTG-induced obese mice. The factors responsible for changes of the cellularity, particularly the number of adipocytes, with respect to the GTG-treatment were not clear at the present time. Further studies are needed to clarify the relationship among the GTG-treatment, the factors and the cellularity of adipose tissue in the obesity induced by GTG-treatment.
REFERENCES


