Effects of meal frequency on energy utilization in rats

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MEAL FREQUENCY has been suggested to be an important factor in the regulation of energy balance in rats and humans (see Ref. 1 for review). A key question is whether the pattern with which food is ingested can affect energy balance independently of the caloric value of the food eaten. Rats are usually "nibblers," ingesting their food in 10–15 small meals during each 24-h period. When they are made into "gorgers," by limiting their access to food to 1–2 h/day (18) or by subjecting them to intermittent food restriction (13), metabolic changes such as increased body fat (3, 4, 19), increased lipogenesis (18), and more rapid absorption of glucose and fat (13) have been reported. However, some of these effects, such as changes in body composition, have not been found by other investigators (16, 17). One problem with these studies is that limiting access to food and producing intermittent food restriction does not always result in total compensation by the rats (8, 14), so that food intake of gorgers may be different from that of nibblers.

The most likely way that meal frequency could affect energy balance is by altering total energy expenditure. The component of energy expenditure most likely to be altered by changing meal frequency is the thermic effect of food (TEF), which is the increase in energy expenditure produced by ingesting food. In humans, TEF increases with meal size, but the increase may be nonlinear, suggesting that differences in meal frequency could affect total energy expenditure (11). Additionally, it has been shown in man that continuous enteral administration of a formula diet at very low rates produces little or no TEF (10), suggesting that ingesting energy in amounts close to rates of fuel oxidation may be more efficient than ingesting food in discrete meals that represent, at the time, an excess above energy requirements. Thus it is possible that changes in meal frequency could affect energy balance by altering total energy expenditure, even if total food intake remains unchanged.

The intent of the present study was to determine whether energy utilization (as body energy and energy expenditure) was altered by changing the meal pattern of rats. Gorging was produced in such a way as to avoid changes in the total amount of food eaten, allowing a direct comparison between two groups of rats eating the same amount and composition of food, but differing in meal frequency.

METHODS

Animals

Thirty-two male Wistar rats were purchased from Harlan (Madison, WI). All rats were given ad libitum access to a stock diet (Purina rodent chow, Ralston-Purina, St. Louis, MO) and water until they reached an average body weight of 215 g. Base-line measures of food intake were taken for 2 wk before that time. When the rats reached an average body weight of 215 g, 10 rats were given 80% of control food intake. Control rats (n = 6) continued to receive ad libitum access to the stock diet, but the other two groups were given 80% of control food intake. This amount was adjusted upward as the food intake of controls increased slightly throughout the study so that the two food-restricted groups always received ~80% of control food intake. One food-restricted group (nibblers; n = 10) was given its daily allotment of food in 10–12 equal-sized meals, and the other food-restricted group (gorgers, n = 10) was given its daily allotment of food in two equal-sized meals. The number of meals varied between 10 and 12 in the nibblers as slight modifications were made in total intake to keep it at ~80% of control intake.

Food Intake

The gorgers were given one meal in the morning (9:00–10:00 A.M.) and one meal in the late afternoon (3:00–4:00 P.M.). This meal was carefully weighed and delivered by hand into the animal's cage. The nibblers were fed using

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automated food dispensers, which were similar in design to those developed by DiGirolamo et al. (5), except that the solenoids were driven by air pressure rather than electricity. The feeders allowed delivery of the animal’s daily food in 10–12 meals, most of which were given at night. All food was a stock diet (Teklad, Madison WI), which was delivered in powdered form.

Deaths of Animals

Six animals from the control group were killed at the beginning of the study, and the remaining six animals were killed at the end of the study (131 days later). Half of the rats in each food-restricted group were killed after 47 days of food restriction, and the remainder were killed at the end of the study.

Dependent Variables

Body weight. Body weights were recorded at least three times weekly for each group. Weights were recorded to the nearest gram.

Food intake. Food intake was measured throughout the study for all rats. Food intake of controls and gorgers was measured by weighing food provided minus food not eaten or food spilled. Food intake of nibblers was measured by weighing the amount of food put into the feeders and periodically measuring the amount of food remaining after the animals’ daily allotments of food were delivered. Because there was a slight variation in the delivery rate of individual feeders, the amount of food given the nibblers varied slightly, but nonsignificantly. In general, all food-restricted rats ate all food given.

Body composition. Carcass energy was determined for each animal killed. After decapitation, the intestinal tract (with its contents) was trimmed of visible fat, weighed, and discarded. The carcass was autoclaved for 90 min and then homogenized with water in a polytron homogenizer. Aliquots of the homogenate were analyzed for lipid and water content. Lipid was determined gravimetrically after extraction in chloroform-methanol and evaporation to constant weight under a fume hood. Water content was determined by the difference in weight of the homogenate before and after drying at 60°C in a vacuum oven. Fat-free dry weight (FFDW) was estimated by subtracting the weight of lipid and water from that of the homogenate. Corrections were made, by appropriate analysis of lipid and FFDW content, for tissues taken out of the animal for use in other determinations. Lipid, FFDW, and water content of those tissues were determined by the method of Folch et al. (9). Total energy content of the carcass was determined by bomb calorimetry.

Food efficiency. We measured efficiency of food utilization during the study as change in carcass energy divided by food intake.

Energy expenditure. Twenty-four-hour energy expenditure was measured in rats from each group, using indirect calorimetry. The indirect calorimeter consisted of a flow-through system where a constant flow of air is drawn, via a diaphragm vacuum pump (Thomas Industries, Sheboygan, WI), through two metabolic chambers.
RESULTS

Body weight. Figure 1 shows the body weights of all rats throughout the study. Both food-restricted groups grew more slowly than controls but were not different from each other in body weight at any time during the study.

Body composition. Figure 2 shows the body composition of all groups in the study. Body fat content did not differ between nibblers and gorgers at any time during the study (Fig. 2A). Body fat content was significantly higher ($P < 0.05$) in controls than in either food-restricted group at 131 days. Figure 2B shows FFDW of each group throughout the study. There was no difference between the two food-restricted groups at either 47 or 131 days. Controls had significantly more ($P < 0.05$) FFDW at 131 days than either food-restricted group. Figure 2C shows the average total carcass energy content of all rats killed. Again, the two food-restricted groups did not differ carcass energy content at either 47 or 131 days. Carcasses of controls contained significantly more energy than either food-restricted group at 131 days.

Food efficiency. Table 1 shows weight gain, food intake, and food efficiency in all groups during the study. There was not a difference in total food intake or in total weight gain at any time during the study between the two food-restricted groups, although intake was more variable in the nibblers because of variations in feeder performance. Control rats ate more food and gained more weight during all phases of the study than did either food-restricted group. Control rats were more efficient during days 0–47 than the food-restricted groups ($P < 0.05$) but not during days 48–131 or when the entire 131-day period is considered.

Energy expenditure. Figure 3 shows the daily rates of energy expenditure in both food-restricted groups at 38–68 days and again at 94–124 days. Rates of energy expenditure for controls are shown at 94–124 days. There was no difference in daily energy expenditure between the nibblers and gorgers at either measurement period. When energy expenditure was calculated as kilocalories per kilogram body weight per day, there were also no differences between nibblers (143 ± 3) and gorgers (146 ± 7) at 38–48 days. Absolute energy expenditure of control rats was significantly higher ($P < 0.05$) at 94–128 days than that of either food-restricted group. However, when expressed per kilogram of body weight, there was no significant difference among groups (154 ± 4, 156 ± 7, 153 ± 3 for controls, nibblers, and gorgers, respectively). Absolute rates of energy expenditure did increase from the first measurement period to the second one in both food-restricted groups. When energy expenditure was expressed as kilocalories per kilogram body weight per day, the increase in energy expenditure from the first to the second measurement period was signifi-
TABLE 1. Weight gain, food intake, and food efficiency during study

<table>
<thead>
<tr>
<th></th>
<th>Days 0–47</th>
<th>Days 48–131</th>
<th>Days 0–131</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight gain, g</td>
<td>Food intake, g</td>
<td>FE</td>
</tr>
<tr>
<td>Controls</td>
<td>237±7*</td>
<td>1,229±22*</td>
<td>78±1*</td>
</tr>
<tr>
<td>Nibblers</td>
<td>121±12</td>
<td>805±23</td>
<td>40±2</td>
</tr>
<tr>
<td>Gorgers</td>
<td>125±6</td>
<td>815±0</td>
<td>42±3</td>
</tr>
</tbody>
</table>

Values are means ± SE. For analysis of days 0–47, there were 6 rats in control group and 10 rats in other 2 groups. For analysis of days 48–131 and 0–131, there were 6 rats in control group and 5 rats in other 2 groups. FE, food efficiency (change in carcass energy per food intake).

Different from nibblers and gorgers, P < 0.05.

Adipose tissue depots. Figure 4 shows the weights of the epididymal and retroperitoneal white adipose depots in each group. Although depot weights tended to be slightly higher in gorgers, there were no significant differences between the two food-restricted groups in the weights of either depot. Controls, as expected, have heavier depots than either food-restricted group. The weight of the epididymal adipose depot comprised the same percentage of total body fat in nibblers and gorgers at 47 days (10.9 ± 0.7 vs. 10.7 ± 0.9%) and 131 days (13.4 ± 0.8 vs. 14.6 ± 0.9%). Epididymal depots of control rats comprised 16.4 ± 0.7% of total body fat at 131 days. Similarly, the retroperitoneal depot accounted for similar proportions of total body fat in nibblers and gorgers at 47 (12.4 ± 0.8 vs. 12.5 ± 0.6%) and 131 (17.7 ± 1.4 vs. 17.9 ± 0.7%) days. Retroperitoneal adipose depots comprised 16.5 ± 1.5% of total adipose tissue in control rats at 131 days.

Figure 5 shows the volume of cells in the epididymal white adipose tissue depot for each group. At 47 days, cells were significantly larger in gorgers than nibblers (P < 0.05). However, at 131 days there was no significant difference in cell size between the two food-restricted groups. At 131 days, control cells were larger than nibblers (P < 0.05) but not gorgers.

DISCUSSION

No component of energy balance differed between nibblers and gorgers. These groups had the same food intake, body composition, carcass energy content, food efficiency, and energy expenditure rates. The amount of food eaten, rather than the pattern with which it is ingested, appears to be the major influence on energy balance during food restriction.

Body composition and energy expenditure were assessed after 47 and again after 131 days of the experimental diets. There was no indication, at either time, that meal frequency had an effect on any component of body composition or on total daily energy expenditure. Energy expenditure increased from 47 to 131 days in both groups of food-restricted rats, but the increase was similar in both groups and was accompanied by an increase in body weight and FFDW. Additionally, there were no differences between the food-restricted groups in calculated food efficiency at either 47 or 131 days.

![Figure 3](http://ajpregu.physiology.org/)

**FIG. 3.** Average daily energy expenditure is shown for food-restricted rats at 2 times during study and for controls at 1 time during study. Each rat was measured on 3 separate days during each measurement period, and average of 3 measures was taken as rate of energy expenditure for that rat. Values are means ± SE for 5 rats per group.

![Figure 4](http://ajpregu.physiology.org/)

**FIG. 4.** Weights of epididymal (A) and retroperitoneal (B) adipose depots are shown for each group during study. Values are means ± SE for 6 control rats and 5 rats in each experimental group.
Others (7, 8, 19) have reported increased lipogenesis in rats made into gorgers. Lipogenesis was not measured in the present study, but the weights of both the epididymal and retroperitoneal adipose depots did not differ between the food-restricted groups at either 47 or 131 days.

In contrast to meal frequency, the total amount of food eaten did affect energy balance. Body composition and energy expenditure were not measured in the control group at 47 days, so that comparisons with these animals and with the two food-restricted groups on those variables were made only at the end of the study (131 days). At that time, controls had more body fat and FFIDW and a higher rate of 24-h energy expenditure than either food-restricted group. Food intake was higher for controls than for food-restricted rats throughout the study. Food efficiency was higher in controls than in food-restricted rats during the first 47 days of the study but was lower than that of food-restricted groups during days 48–131. When the entire 131-day period was considered, there were no differences among the three groups in food efficiency. Thus the food restriction produced a reduction in total energy expenditure that allowed food efficiency, at least during the latter part of the study, to be greater than that of controls.

This is the first demonstration that total energy expenditure in food-restricted rats is not affected by the size and number of meals in which daily food intake is ingested. It must be noted, however, that energy expenditure in both food-restricted groups was reduced compared with control rats. It is possible that the changes in energy expenditure due to food restriction may have masked any effects due to differences in meal frequency. Cohn and Joseph (4) have suggested that this may be the case with severe food restriction. The food restriction in the present study was very mild, and the fact that the animals continued to grow demonstrates that food intake was in excess of energy requirements.

It is unlikely that the present study was too short to observe differences that might have occurred because of meal frequency. This study lasted ~19 wk, a substantial period in the life of a rat, and a much longer period than most previous studies. There was no indication at any time during the study that meal frequency affected any component of energy balance. Previous metabolic differences due to meal frequency have been observed with studies of much shorter duration (2–4 wk; see Ref. 1).

It is important to realize that animals in this study were food restricted, and thus results may not be directly comparable to other studies evaluating the role of meal frequency in energy balance. However, these results may have important implications for dieting humans. They suggest that dieters should be more concerned with the amount (and possibly composition) of food eaten rather than with whether they eat large vs small meals. In summary, these results suggest that previously reported differences in metabolism between gorgers and nibblers (7, 8, 19) may have been due to other factors related to the method of producing gorging rather than to the actual meal frequency itself. At least during caloric restriction, the pattern with which food is ingested does not appear to affect energy balance.

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