The Regulation of Food Intake and Correlated Energy Balance in Mice

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ABSTRACT. In order to study the regulation of food intake and correlated body energy metabolism, the effect of restricted feeding during the light period in female IVCS mice was investigated. Access to food and water was restricted (RF group) for 3 weeks only from 10:00 hr to 17:00 hr, and that in the control group remained ad libitum. After starting food restriction (day 1), mean food intake decreased to 10% of the control value, then rose sharply, over the next 3 days, to reach 70% of the control value. Then, it decreased gradually to about 50% of the control value and remained at this low level thereafter. There was no significant difference between mean body weights for the two groups. Feed-efficiency was considered, therefore, to be higher in the RF than in the control group. RF-treatment increased plasma corticosterone levels and decreased locomotor activity. However, the diurnal patterns of plasma corticosterone levels and locomotor activity observed suggest that the circadian rhythm, synchronized with the light-dark cycle, persisted during RF-treatment. These findings suggest that restricted feeding during the naturally inactive phase (light period) induces a decrease in food intake. Animals seem to adapt to underfeeding, at least partly, by increasing feed-efficiency and plasma corticosterone levels and decreasing locomotor activity. — KEY WORDS: body weight, energy requirement, feed efficiency, plasma corticosterone level, restricted feeding.


Several studies have demonstrated that food intake control is a vital component in the regulation of energy balance in animals [1, 13, 16]. It is generally accepted that the ventromedial nucleus of the hypothalamus (VMH) regulates the feeding center, located in the lateral hypothalamic, in order to maintain energy stability [2]. Since the glucose-sensitive neurons reside in the VMH [12, 14–15], it has been proposed that blood glucose level is a signal indicative of nutritional status, which controls food intake (glucostat theory: [16]). However, although this theory explains energy metabolism regulation in well fed animals, it does not address the underfed condition. Furthermore, it seems unlikely that such a transient signal could maintain adequately the long-term, such as lifelong, energy balance stability, which is observed commonly in animals.

The circadian mechanism is another factor involved in the regulation of food intake. Several studies in rodents have suggested that the hypothalamic suprachiasmatic nucleus (SCN) may be an oscillator for circadian rhythm, as destruction of this nucleus eliminates many circadian rhythms, such as locomotor activity, corticosterone level and body temperature rhythm [7, 10, 18]. The fact that animals eat most food during their active phase (diurnal animals eat food during the day while nocturnal animals do so during the night) has given rise to a generally accepted hypothesis that feeding behavior is under the control of a master clock.

Feeding behavior may also be a factor which regulates the circadian rhythm. Krieger hypothesized that feeding behavior exerts an entraining effect on the circadian rhythm and that the VMH is the oscillator responsible for the corticosterone rhythm in SCN-lesioned rats [8]. It has been demonstrated, however, that corticosterone secretion from the adrenal gland exhibits circadian rhythmicity in vitro [4], indicating that corticosterone rhythm does not always reflect the rhythm of the master clock or VHM, and that the oscillator under periodic feeding does not necessarily manifest the rhythm under ad libitum feeding [3, 6]. These results cast doubt upon the hypothesis of feeding behavior acting as an entraining influence on the circadian rhythm.

This study, therefore, was undertaken to examine regulation of food intake and correlated body energy metabolism. The effects of restricted feeding during the light period on basal metabolism, corticosterone levels and locomotor activity were investigated in mice. The relationship between these
parameters and food intake is discussed.

MATERIALS AND METHODS

Animals and experiments: Three-week-old female IVCS mice were obtained from the Imamichi Institute of Animal Reproduction (Ohmiya, Japan) and were housed in groups consisting of 6–7 mice. They were acclimatized to standard laboratory condition (lights on 05:00–19:00 hr; room temperature 23±2°C) and given laboratory chow (LABO MR BREEDER, Nihon Nousan Co., Japan) and water ad libitum. Food and water containers were replenished at random time intervals for 2 weeks. Then, the animals were removed to single-housing cages with wood shavings on the floor. Access to food and water was restricted (RF group) to 7-hr period (10:00–17:00), for 3 weeks, in 4 groups (n=6 or 7 per group), and remained ad libitum in the other 4 (control) groups (n=6 or 7). Animals were handled daily (between 10:00 and 10:30 hr) and changes in body weight (BW) and total food consumption were recorded. At the end of the study period, blood samples were taken by decapitation at 5 different times of day (05:00, 11:00, 17:00, 19:00 and 23:00 hr) and frozen at −20°C until hormone assays were performed.

Hormone assay: Each plasma sample was diluted to 1 ml with distilled water and extracted twice with 2 ml diethyl ether. The extracts were radioimmunoassayed using a specific antibody, raised in rabbits in our laboratory.

Locomotor activity: Locomotor activity was recorded by apparatus consisting of mouse cages, a microcomputer (PC-8001, NEC), a monitor and a printer. Each cage was equipped with a newly devised switch [20] which detected each movement the mice made. The on and off signals of the switch were transmitted via the interface and fed to and integrated by the microcomputer (PC-8001). The total number of counts in each 30 min period was printed out and double-plotted graphically.

Data analysis: The body weight (BW) gains of each mouse during the 1st, 2nd and 3rd weeks were calculated as the differences in body weight between days 6 and 1, days 13 and 6, and days 20 and 13, respectively. Feed-efficiency was calculated according to the following formula:

\[
\text{Feed-efficiency} = \frac{\text{gained BW(g)}}{\text{total food consumption(g)}}
\]

Since each gram of food pellet supplies 3.38 Kcal of metabolizable energy (ME), the estimated ME was calculated as follows:

\[
\text{ME (Kcal)} = 3.38 \times \text{total food consumption (g)}
\]

Metabolic body size (MBS) was calculated according to the equation of Heusner [5];

\[
\text{MBS (kg)} = \frac{\text{BW (kg)}}{\text{K (kg/2.3)}}
\]

The relationship between ME and MBS is defined as

\[
\text{ME (Kcal)} = K \times \text{MBS (kg/2.3)}
\]

and the K value represents the metabolizable energy requirement.

RESULTS

Food intake: Essentially, food consumption in the control group did not change throughout the experimental period. Each mouse consumed about 4.0 g of food pellets every day. More than 90% of the food was consumed during the dark period. Immediately after starting food restriction (day 1) the mean food intake decreased to less than 10% of the control value. There was a sharp rise in food consumption over the next 3 days, but it did not reach that of control mice. The mean food intake then decreased gradually to about 50% of the control intake by day 11 and remained low thereafter (Fig. 1). The total quantity of food consumed in the control group during the experiment was 2,104 g.

![Fig. 1. Changes in food consumption (square symbols) and body weight (triangular symbols) in mice under ad libitum (control) and restricted feeding (RF) conditions. The value for each experimental group is expressed as the mean ± SEM of results for 26 mice.](image-url)
and 1.146 g in the RF group (n=26, for 20 days for both groups).

**Body weight:** Mice of both groups gained weight daily throughout the experimental period with the exception of day 4 in the RF group. There was no significant difference between mean body weights of the two groups (Fig. 1). More detailed analysis, however, showed that some mice in the RF group lost weight during the 1st week and the overall increase in body weight was lower during the 1st week than during the 2nd and 3rd weeks. The difference in body weight gain between the control and RF group was evident only during the 1st week and disappeared by the 2nd and 3rd weeks (Fig. 2).

**Feed-efficiency and energy metabolism:** As a consequence of changes in body weight and food intake, feed-efficiency was estimated to be higher in the RF than in the control group during both the 2nd and 3rd weeks. Data for energy requirement (K values) are shown in Table 1. The K value for the 1st week was significantly lower in the RF than in the control group. The K value for the 1st and 2nd weeks was essentially the same in both groups, so the value for the 2nd week was also significantly lower in the RF than in the control group. For the 3rd week the difference in the K value between the RF and the control groups became more marked than for the 1st and 2nd weeks.

**Changes in corticosterone levels:** Both groups showed a clear 24-hr rhythm of hormone level fluctuations. The pattern, however, differed between the two groups in the following respects: first, the highest corticosterone level occurred at 19:00 hr in the control group but at 11:00 hr in the RF group; second, feed restriction affected the absolute concentration of plasma corticosterone. The highest peak value in the RF group was more than twice that of the control group and the concentration was significantly higher in the RF than in the control group at all times measured except 19:00 hr. The corticosterone level in the control group increased after the onset of the light period and just before feeding time (dark period), producing two peaks in the rhythm, while the RF group exhibited just one peak (Fig. 3). This can be attributed to the increases before feeding time and after the onset of the light period being superimposed.

**Locomotor activity:** Figure 4 shows a typical pattern of locomotor activity before and during RF treatment. Before feed restriction the mouse showed a clear nocturnal pattern of locomotor rhythm; locomotor activity increased during the dark period and decreased during the light period. During RF-treatment a similar waveform rhythm pattern was observed, which implies that the circadian rhythm, driven by the light-dark rhythm, persisted during RF-treatment. On the other hand, locomotor activity during the RF-period decreased, compared with that under ad libitum feeding conditions.

<table>
<thead>
<tr>
<th>Week</th>
<th>Control (Kcal/kg)</th>
<th>RF (Kcal/kg)</th>
</tr>
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<tbody>
<tr>
<td>1st</td>
<td>182.3 ± 8.9</td>
<td>111.8 ± 9.7</td>
</tr>
<tr>
<td>2nd</td>
<td>185.8 ± 9.9</td>
<td>117.5 ± 4.5</td>
</tr>
<tr>
<td>3rd</td>
<td>170.0 ± 7.1</td>
<td>80.8 ± 3.4</td>
</tr>
</tbody>
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Table 1. K values of mice during RF period

K value (Kcal/kg) is an index for the requirement of metabolizable energy. Each experimental group is expressed as mean ± SEM of average values of four separate trials (n=6 or 7 each).

a) P<0.002 vs. control
b) P<0.001 vs. control
c) P<0.001 vs. control
Fig. 4. Typical pattern of locomotor activity of mice under ad libitum (control) and restricted feeding (RF) conditions. Locomotor activity is shown in histogram of counts/30 min.

DISCUSSION

This study clearly shows that restricted feeding of mice during the light period reduces total food consumption. This might be expected that reducing the feeding time would cause a decrease in food consumption. However, detailed analysis of the data revealed that this is not necessarily the case. In the RF group mice were fed for 7 hr a day, while those in the control group fed mainly during the 10 hr dark period. This means that feeding time in the RF group was 30% less than in the control group. Thus, if the mice in the RF group had a strong appetite during their feeding time, their decrease in food consumption would be expected to be 30% at most. However, food consumption in the RF group was only about half that of the control group. These results indicate that the decrease in food consumption induced by RF-treatment was not solely attributable to the passive effect of reducing feeding time.

As well as changing the length of the feeding period, the time of the day feeding took place was also changed by RF-treatment. It is likely that the latter change caused the decrease in food intake. Feeding behavior is regulated by several factors, such as gastrointestinal factors, blood glucose levels, hormonal levels and a body nutrient depletion-repletion signal. Some of these factors are governed by the circadian process [16], thus showing a daily rhythm, and consequently, nocturnal animals eat most food during the night. The decrease in food consumption in the RF group can be explained if we assume that the mice are sated by less food in the light than in the dark period due to nocturnal rhythmicity.

Although this assumption appears valid for the first few days of RF-treatment, when abrupt decreases in consumption were observed, the observations that food consumption changed over the next 10 days and body weight was maintained during restricted feeding indicate that mice adapt to this condition in a more complex manner. Since corticosterone levels, the vaginal cytological cycle and feeding behavior are controlled by the circadian clock [8, 19], one possibility is that the mice adapted to the new feeding time with a phase shift of the circadian rhythm responsible for body metabolism and reproductive function. However, a detailed analysis of the corticosterone rhythm showed that corticosterone levels increased just after the onset of the light period in the RF group, as it did in the control group. The lack of a peak of corticosterone levels just before the onset of the dark period in RF group can be attributed to the increases before the dark period and after the onset of the light period being superimposed. Thus, it is indicated that the circadian rhythm, driven by the light-dark cycle, persisted during RF-treatment. A similar waveform rhythm pattern of locomotor activity was also observed. These findings suggest that restricted feeding did not exert its effect on the master clock responsible for circadian rhythmicity, but rather masked the rhythm, i.e. certain effects due to RF
were superimposed on the circadian rhythm. This provides indirect evidence in support of the concept of multiple systems with oscillatory capacity, such as the circadian system which is driven by a master clock and not affected by RF, and the feeding-dependent system which can be uncoupled from the circadian system and can be entrained by feeding behavior [6].

If the nocturnal rhythm does persist during RF-treatment, then mice would be expected to adapt to underfeeding by changing their body metabolism and/or decreasing locomotor activity. This seems likely as the total locomotor activity decreased markedly and feed-efficiency increased during restricted feeding. It has been reported that a marked caloric restriction, using a wide range of dietary regimens, suppresses reproductive cyclicity and fertility in female animals [9, 11]. Thus, the disruption of normal cyclicity observed during the first two weeks of RF-treatment (determined by vaginal cytological changes; data not shown) may be attributable to nutrient deficiency. If this is the case, then mice in the RF group overcame the nutrient deficiency by the 3rd week as cyclicity had returned to normal (data not shown). This assumption is in good agreement with the marked decrease in energy requirement (K value) determined for the 3rd week of RF-treatment. A signal for body nutrient deficiency, therefore, may change body metabolism and locomotor activity to adapt to the underfed condition, which in turn decreases food consumption during RF-treatment.

In order to maintain body weight during a period of insufficient nutrition, mice appear to perceive a long-term body nutrient depletion-repletion signal and regulate their body metabolism. A likely candidate for this long-term signal and/or regulator is corticosterone, as our results suggest that corticosterone levels increased during long-term energy depletion, and because corticosterone increases gluconeogenesis, which will affect food intake [1, 17].

To summarise, we have shown that the circadian rhythm, synchronized with the light-dark cycle, persists during RF-treatment. We propose that a restricted feeding of animals during the inactive (light) phase induces a decrease in food intake by a phase difference between feeding behavior rhythms and the actual time of day feeding takes place. This, in turn, produces an increase in plasma glucocorticoid levels and a decrease in locomotor activity, which may enable animals to adapt, at least partly, to underfeeding, by decreasing energy requirements and, consequently, increasing feed-efficiency.

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