Effects of Intermittent Food Restriction and Refeeding on Energy Efficiency and Body Fat Deposition in Sedentary and Exercised Rats

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Summary The effects of body weight cycling on energy metabolism and body fat accumulation were examined in sedentary and exercised rats. Ten rats were sacrificed before the experiment to obtain basal data, and then 90 rats were divided into three groups; control (CN), food restricted (FR) and weight cycling (WC). Food intake in rats of the FR group was restricted constantly to 70% of the intake of the CN group. The rats of WC group were subjected to four bouts of weight cycling consisting of 7-days food restriction followed by 7-days refeeding, but were fed the same total amount of dietary energy as that of the FR group throughout the experimental period. The rats of all groups were meal-fed twice a day. Half of the rats in each group were exercised by running on a treadmill (30 min/day) throughout the experimental period. The body weight, abdominal adipose tissue weight, body fat, body protein and energy restoration for the study in both sedentary and exercised groups were greater in the WC group than in the FR group. The resting metabolic rate of the WC group after four bouts of weight cycling was lower than that of the FR group in the sedentary rats, but this difference was not observed in the exercised rats. Also, the thermic effect of food (TEF) in the sedentary rats for 6h after a meal was significantly less in the WC group as compared to that of the FR group. However, the TEF for the exercised rats was not different between the two groups. The serum insulin level,

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activities of lipogenic enzymes and lipoprotein lipase in adipose tissue for the sedentary rats of the WC group were higher than those of the FR group, but did not differ in the exercised rats. These results suggest that weight cycling increases body fat deposition and energy efficiency by decreasing energy expenditure, particularly the TEF, and that exercise training can alleviate the effects of weight cycling on the energy metabolism.

**Key Words** weight cycling, food restriction, refeeding, exercise, resting metabolic rate, thermic effect of food, lipogenic enzymes

Weight cycling, defined as repeated periods of weight loss followed by weight regain, has been reported to have significant effects on energy balance in humans (1, 2) and experimental animals (3–8). In particular, weight cycling in rats has been reported to make subsequent weight loss more difficult, make subsequent weight regain easier, alter body composition, decrease total daily energy expenditure (9) and increase food efficiency (3, 4, 7, 9, 10). Similar results have been reported for athletes undergoing weight cycling (2).

In rodents, greater food efficiency is demonstrated by a lesser loss and more rapid regain of body weight during successive weight cycling (4–6, 9, 11, 12). It has been suggested that the composition of body weight regained may be different from that of lost in that more protein is lost than is regained (1). Because body protein, mainly skeletal muscle mass, is thought to contribute to total energy expenditure (1), body protein loss might lead to greater food efficiency (1). Indeed, there is a study which suggests that weight cycling lowers the total daily metabolic rate in humans (2). However, whether weight cycling lowers energy expenditure by diminishing the thermic effect of food (TEF) and/or the resting metabolic rate (RMR) is unknown.

Hill et al. (7) reported that abdominal adipose tissue weight and body composition were not different between weight-cycled rats and control rats after four bouts of weight cycling. However, when the rats were allowed *ad libitum* access to an experimental chow diet for an additional 18 days after four bouts of weight cycling, carcass fat and fat-free dry weight were greater in the weight-cycled rats than in the control rats, although food intake did not differ significantly between the two groups during the *ad libitum* feeding period. They also reported that body composition was not different between the weight-cycled and control rats when the rats were allowed *ad libitum* access to a high-fat diet for an additional 52 days after three bouts of weight cycling (9). Although the rats with a history of weight cycling had a lower daily metabolic rate than the control group, this was a consequence of their lower food intake. In that study, it was concluded that weight cycling may have a greater effect on food intake than on energy expenditure. As mentioned above, the effects of weight cycling on body fat accumulation and energy efficiency are not clear. However, this is an important issue, since obese humans

often go through cycles of weight loss followed by weight regain. On the other hand, exercise training is generally thought to increase total energy expenditure. Hill et al. (13) trained female rats (swimming 2 h/day; 148–168 days) and noted an elevated RMR expressed/kg body weight in fasted animals measured 24 h after the last exercise period. Gleeson et al. (14) reported that a 56-day running exercise program in male rats increased total energy expenditure. This was due to a higher meal-induced thermogenesis and an anticipatory increase in energy expenditure preceding exercise. The cause of increased energy expenditure induced by exercise training may be due to 1) the direct energy cost of the exercise itself and energy expenditure during the immediate postexercise period (15,16) and, 2) an elevation in resting metabolic rate (16,17), although this effect has not been observed in all studies (18). Therefore, there is a possibility that exercise training may suppress the increased body fat accumulation and energy efficiency caused by weight cycling. However, the interaction between exercise training and weight cycling is unknown.

The primary purposes of this paper are to provide new information on physiological changes in energy metabolism and body fat accumulation in weight-cycled rats, and to determine whether or not exercise training can alleviate the effects of weight cycling.

METHODS

Animal care and experimental design

One hundred Sprague-Dawley female rats 10 wk of age (body weight; about 200 g) were obtained from CLEA-Japan. After three weeks of prefeeding with a powdered chow diet (CE-2, Tokyo, CLEA-Japan), 10 rats were sacrificed before beginning the experiment to obtain basal data. The remaining 90 rats were divided into three groups; control (CN), food restriction (FR) and weight cycling (WC). The rats of the CN group continued to receive the average amount of chow diet in the case of ad libitum feeding. The rats of the FR group were restricted to 70% of the CN group intake. The rats of the WC group were subjected to four bouts of weight cycling consisting of 7-days food restriction (40% of the CN group) followed by 7-days refeeding (100% of the CN group) except for the first cycle. For the first weight cycle, the food intake of the WC group was restricted to 50% that of the CN group for the initial five days of the restriction period, and the restriction and refeeding periods were 10 days, respectively, because we did not know the reduction rate of body weight by food restriction. From the second cycle, the rats of the WC group were restricted to 40% of the CN group intake. The amount of food offered to the rats of the WC group during the weight cycling period was exactly the same amount of food offered to the FR rats. A summary of groups and treatment is shown in Fig. 1.

The rats of all groups were meal-fed the corresponding amount of the powdered chow diet twice a day (1100 and 2200): for example, 10 g/meal for the
Fig. 1. Experimental design.

CN group; 7 g for the FR group; and 4 g in the restricted period or 10 g in the refeeding period for the WC group. The rats in all groups consumed the corresponding amount within 2 h after receiving the meal. Moreover, the meal boxes used in this study involved an inner cap for prevention of meal spillage. Therefore, all rats in each group were fed the same weighed amount of food. The sedentary and exercised rats in the CN, FR and WC groups were given the same amount of dietary energy. All rats had free access to water and were kept in rooms maintained on a 12-h light (2200-1000)/12-h dark cycle (1000-2200) at 23°C.

Half of the rats in each group were subjected to running exercise on a treadmill designed for the rats (model III, Autome-Kogyo, Tokyo (19, 20)) before the meal during the dark cycle. The intensity and duration of the exercise was gradually increased during the first 10 days of the exercise programme (including pre-exercise training for adaptation; 10 min/day and 10 m/min for three days, and 20 min/day and 20 m/min for two days), with the final speed and duration of 28 m/min (7° incline) and 30 min/day, respectively. The rats were exercised five days per week throughout the experimental period.

On completion of the fourth period of weight cycling, using 4–5 rats from each group, the resting metabolic rate and thermic effect of food for 6 h after a meal were measured. The remaining rats were sacrificed by decapitation 3 h after the final meal.

Measurements and analysis

Body weight: The body weights of the rats were recorded everyday for each group.

Body composition: Carcass fat and protein contents were determined for each animal sacrificed. The carcass was autoclaved for at least 90 min at the pressure of 1 kg/cm² and then homogenized with a Waring Blender (20, 21). The homogenates were analyzed for lipid and protein contents. Carcass lipid content was determined by extraction with ether using the Soxhlet lipid extraction method after drying at
Carcass protein content was determined using the Micro-Kjeldhal method (a factor of 6.25 was used for protein conversion from nitrogen (22)). Carcass energy was calculated from body composition data using a value of 9 kcal/g for lipid content and a value of 4 kcal/g for protein content (21).

Abdominal adipose tissue depots: The parametrial, perirenal and mesenteric adipose tissues were removed and weighed. The perirenal adipose tissues were used for assay of the malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PD) and lipoprotein lipase (LPL) activities.

Blood sampling: Blood was collected after decapitation for the measurement of serum glucose, free fatty acid and insulin (21).

Oxygen consumption before and after meals: On completion of the fourth period of weight cycling, pre- and postprandial O$_2$ consumption were measured for 4–5 rats in each group using an open-circuit system (20, 21, 23). The oxygen consumption of the exercised rats was measured 48 h after the last exercise period. All rats were adapted to the chamber overnight before being tested. Each rat was put in a cylinder-type chamber (10.3 cm internal diameter × 28 cm long) at 1700 the day before the measurement, at which time the appropriate amount of food was given to each group of rats. Oxygen consumption before the meal (preprandial) was measured for 1 h as the value of resting metabolic rate (RMR). All rats were fed the same amount of food (21 kcal), and then O$_2$ consumption was measured for 6 h as the value of thermic effect of food (TEF). The measurements for six rats, consisting of one rat from each group, were done in one day. The room temperature was 23°C, and the room air was constantly suctioned from the chamber by a pump at a flow of 700 ml/min through a nozzle (0.3 cm internal diameter). All of the air was collected in Douglas bags (Fukuda Sangyo, Tokyo), which were changed every 30 min. The concentration of oxygen in the air collected was immediately analyzed by a gas mass analyzer (MGA 1100, Perkin-Elmer, St. Louis). All gas volumes were converted to standard temperature and pressure (STP).

Assays of enzymes activities

The activities of ME and G6PD in the perirenal adipose tissue were assayed by the methods reported previously (21).

The LPL activities in the heart, soleus muscle and perirenal adipose tissue were measured as follows (20, 21). The acetone-ether-extracted tissues were prepared by the method of Nilsson-Ehle et al. (24), except that the heart and soleus muscle were homogenized with a glass homogenizer. The preparations were dried at room temperature under nitrogen gas. The lipoprotein lipase in the preparations was extracted with 50 mM of Tris-HCl (pH 8.0) containing 1 M of ethylene glycol and used for the assay of LPL activity (25). The substrate for LPL was prepared according to the method of Nilsson-Ehle and Schotz (26), but using unlabeled triolein. The LPL activity assay was performed by incubation of the extract with the substrate at 37°C for 30 min (26). The free fatty acids released during incubation were measured using a NEFA-Kit (Wako Pure Chemical Industries,
Data analysis

A $3 \times 2$ (food intake pattern $\times$ exercise) analysis of variance was used to quantify the effects of the diet and exercise training treatment. When F-values were significant ($p < 0.05$), homogeneous subgroups were identified using Tukey's multiple-range test (27). The significant differences between the sedentary and exercised rats for each dietary pattern group were analyzed using Student's $t$-test (27). A probability of $p < 0.05$ was taken as the level of significance for all comparisons. All data are presented as $M \pm SEM$.

RESULTS

Body weight

The body weight curves for each group during the experimental period are shown in Fig. 2. The body weight of the WC group showed approximately the same reduction during each of the three weight cycling periods, except for the first period in both the sedentary rats (27.3, 26.7 and 26.9 g, respectively) and exercised rats (31.4, 28.0 and 28.9 g, respectively). The body weight loss and regain of the exercised rats during the second weight cycling period were significantly lower than those of the sedentary rats (Table 1).

At the end of the fourth weight cycling period, body weight was not different between the sedentary and exercised rats of the CN group, but body weights were significantly greater for the sedentary rats as compared to the exercised rats in the

![Body weight](chart.png)

Fig. 2. Body weights are shown for the control (□), FR (△) and WC (○) groups during the study. The open symbols for each group mean the values for sedentary rats, and the dark symbols the values for exercised rats.

Table 1. The body mass changes in rats after four bouts of weight cycling

<table>
<thead>
<tr>
<th>Cycle</th>
<th>WC-SED (g)</th>
<th>WC-EX (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restricted period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>−17.4±0.9</td>
<td>−19.7±1.1</td>
</tr>
<tr>
<td>2nd</td>
<td>−27.3±0.5</td>
<td>−31.4±0.8*</td>
</tr>
<tr>
<td>3rd</td>
<td>−26.7±0.6</td>
<td>−28.0±0.6</td>
</tr>
<tr>
<td>4th</td>
<td>−26.9±0.7</td>
<td>−28.0±0.9</td>
</tr>
<tr>
<td>Refed period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>35.5±1.1</td>
<td>35.7±0.9</td>
</tr>
<tr>
<td>2nd</td>
<td>32.3±1.1</td>
<td>28.7±0.8*</td>
</tr>
<tr>
<td>3rd</td>
<td>34.8±1.0</td>
<td>32.1±0.9</td>
</tr>
<tr>
<td>4th</td>
<td>27.3±0.9</td>
<td>29.8±1.0</td>
</tr>
</tbody>
</table>

Values are M±SEM. *Statistically significant difference from the sedentary rats (Student’s t-test, p<0.05).

FR and WC groups (261±2 vs. 247±2 g and 275±3 vs. 265±2 g for the FR and WC groups, respectively). The body weight of the WC group was significantly greater than that of the FR group for both the sedentary and exercised rats, despite the fact that food intake in the WC and FR groups was exactly same throughout the experimental period.

Oxygen consumption before and after the meal

At the end of the fourth weight cycling period, oxygen consumption before the meal and for 6 h after the meal was measured to assess the effect of weight cycling on the thermic effect of food. As shown in Fig. 3, the O₂ consumption in all groups peaked at 30 min after the meal. The O₂ consumption appeared to be lower at all time points for the sedentary rats in the WC group as compared with the other two groups, but this was not statistically apparent in the exercised rats.

Figure 4 shows the preprandial and the sum of O₂ consumption for 6 h after the meal. In the sedentary rats, the preprandial O₂ consumption of the WC group was less than that of the other two groups (Fig. 4; top). For the exercised rats, the preprandial O₂ consumption of the CN group was greater than that of the FR and WC groups, however, a difference between the FR and WC groups was not apparent. Exercise training did not affect preprandial O₂ consumption in any group in this study.

For the sedentary rats, the sum of postprandial O₂ consumption for 6 h was significantly lower in the FR and WC groups as compared to the CN group (Fig. 4; bottom). For the exercise rats, the postprandial O₂ consumption of the WC group was less than that of the other two groups. The postprandial O₂ consumption was increased by exercise training in the FR and WC groups, but not in the CN group (Fig. 4; bottom).
Fig. 3. Oxygen consumption for 6 h after the meal is shown for 4–5 rats in each group at the end of the fourth weight cycling. The open circles are values for the sedentary rats, and the dark circles the values for exercised rats. The values with different superscripts mean significant difference within the sedentary or exercised rats (ANOVA, p<0.05). All values are M±SEM.

Adipose tissue weights
The weights of parametrial, perirenal and mesenteric adipose tissues and interscapular brown adipose tissue (IBAT) were greater in the WC group than in the FR group for both the sedentary and exercised rats (Table 2). The abdominal adipose tissue weights were significantly decreased by exercise training in all groups, however, IBAT weight was not reduced.

Glycogen contents
The glycogen content in liver was significantly higher in the WC group than in the FR group for both the sedentary and exercised rats (Table 3). However, the glycogen content in gastrocnemius muscle was not different between the WC and FR groups. Exercise training significantly increased the glycogen content in the liver,
but not in the gastrocnemius muscle.

**Carcass composition**

Table 4 shows the contents of carcass fat and protein for each group. The carcass protein content (%) was not different between the FR and WC groups, however, the carcass protein mass (g) was significantly greater in the WC group as compared to the FR group for both the sedentary and exercised rats. The body protein mass was not affected by exercise training.

For both the sedentary and exercised rats, the percentage and mass of carcass fat content were significantly greater in the WC group than in the FR group. Exercise training decreased the carcass fat mass in the FR and WC groups, but not in the CN group.

**Changes in body protein, fat and energy restoration**

The changes in body protein, fat and energy restoration during the four bouts
Table 2. The weights of abdominal adipose tissue and interscapular brown adipose tissue of rats for each group after four bouts of weight cycling.

<table>
<thead>
<tr>
<th></th>
<th>Adipose tissue (g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CN</td>
</tr>
<tr>
<td>Sedentary</td>
<td></td>
</tr>
<tr>
<td>Parametrial</td>
<td>7.4 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Perirenal</td>
<td>6.0 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>4.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IBAT</td>
<td>0.30 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Exercised</td>
<td></td>
</tr>
<tr>
<td>Parametrial</td>
<td>5.8 ± 0.3&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Perirenal</td>
<td>4.3 ± 0.3&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>3.3 ± 0.2&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>IBAT</td>
<td>0.31 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are M ± SEM. IBAT; interscapular brown adipose tissue. The values with different superscripts mean statistically significant difference within horizontal columns (ANOVA, p < 0.05). *Statistically significant difference from the sedentary rats (Student’s t-test, p < 0.05).

Table 3. The glycogen contents of liver and gastrocnemius muscle in rats for each group after four bouts of weight cycling.

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Gastrocnemius muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CN</td>
<td>FR</td>
</tr>
<tr>
<td>Sedentary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight (g)</td>
<td>8.28 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.21 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen content (mg/g)</td>
<td>17.6 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.5 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Exercised</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight (g)</td>
<td>9.02 ± 0.20&lt;sup&gt;**&lt;/sup&gt;</td>
<td>7.37 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen content (mg/g)</td>
<td>28.7 ± 2.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>29.3 ± 1.7&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sedentary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight (g)</td>
<td>3.66 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.40 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen content (mg/g)</td>
<td>3.58 ± 0.28</td>
<td>3.88 ± 0.36</td>
</tr>
<tr>
<td>Exercised</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight (g)</td>
<td>3.81 ± 0.08&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.26 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen content (mg/g)</td>
<td>3.63 ± 0.23</td>
<td>3.73 ± 0.15</td>
</tr>
</tbody>
</table>

Values are M ± SEM. The values with different superscripts mean statistically significant difference within horizontal columns (ANOVA, p < 0.05). *Statistically significant difference from the sedentary rats (Student’s t-test, p < 0.05).
Table 4. The composition of carcass in rats for each group after four bouts of weight cycling.

<table>
<thead>
<tr>
<th>Group</th>
<th>CN</th>
<th>FR</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Exercised</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>%</td>
<td>g</td>
<td>%</td>
</tr>
<tr>
<td>%</td>
<td>25.1 ± 0.4ª</td>
<td>25.3 ± 0.2ª</td>
<td>25.8 ± 0.1ª</td>
</tr>
<tr>
<td>g</td>
<td>60.1 ± 1.2ª</td>
<td>62.3 ± 1.4ª</td>
<td>59.6 ± 0.6ª</td>
</tr>
<tr>
<td>Fat</td>
<td>%</td>
<td>g</td>
<td>%</td>
</tr>
<tr>
<td>%</td>
<td>13.3 ± 0.7ª</td>
<td>12.7 ± 0.7ª</td>
<td>10.8 ± 0.3ª</td>
</tr>
<tr>
<td>g</td>
<td>31.9 ± 1.5ª</td>
<td>31.1 ± 1.4ª</td>
<td>24.9 ± 0.7ª</td>
</tr>
</tbody>
</table>

Values are M±SEM. The values with different superscripts mean statistically significant difference within horizontal columns (ANOVA, p<0.05). *Statistically significant difference from the sedentary rats (Student’s t-test, p<0.05).

The body protein mass markedly changed in all groups; for the sedentary rats, the value of the WC group was significantly greater than that of the FR group (Fig. 5; upper). Exercise training did not affect the change in body protein in any group.

For both the sedentary and exercised rats, the change in body fat mass was significantly greater in the WC group than in the FR group (Fig. 5; middle). Exercise training made the changes in body fat smaller in all groups.

The change in body energy restoration of the WC group was significantly greater than that of the FR group for both the sedentary and exercised rats (65±12 vs. 175±14 kcal and -1.9±12.9 vs. 65±11 kcal for sedentary and exercised rats, respectively, Fig. 5; bottom). Energy restoration was significantly decreased by exercise training in the FR and WC groups, but not in the CN group.

**Serum measurements**

Figure 6 shows the concentrations of glucose, free fatty acid (FFA) and insulin in the serum obtained after decapitation 3 h after the final meal. The glucose concentration was similar in all groups (Fig. 6; upper).

For the sedentary rats, the serum concentration of FFA was significantly lower in the WC group than in the CN group but was not significantly different from the FR group (Fig. 6; middle). For the exercised rats, the serum FFA concentration of the WC group was significantly lower as compared to that of the FR group.
Fig. 5. The changes of body protein (upper) and fat (middle), and energy restoration (bottom) in rats for each group during the four bouts of weight cycling. Each value (M±SEM) was calculated by the differences between the data in Table 4 and the data for carcass composition before beginning the study (data not shown). Body fat was calculated by carcass fat (g) + 85% wet weight of abdominal adipose tissue (g). The values with different superscripts mean significant difference within the sedentary or exercised rats (ANOVA, p<0.05). *Statistically significant difference from the sedentary rats (p<0.05, Student's t-test).

For the sedentary rats, the serum insulin level was significantly higher in the WC group as compared to the CN and FR groups. However, the difference was not apparent in the exercised rats, which was due to a significantly lower insulin level in the exercised rats of the WC group.

**Activities of lipogenic enzymes in adipose tissue**

The activities of ME and G6PD in perirenal adipose tissue are shown Fig. 7.
Weight Cycling in Sedentary and Exercised Rats

Fig. 6. Concentrations of glucose (upper), free fatty acids (middle) and insulin (bottom) in the serum obtained after sacrificing following four bouts of weight cycling (M±SEM). The values with different superscripts mean significant difference within the sedentary or exercised rats (ANOVA, p<0.05). * Statistically significant difference from the sedentary rats (p<0.05, Student’s t-test).

For both the sedentary and exercised rats, the ME activity was markedly higher in the WC group than in the CN and FR groups (Fig. 7; top). The activity of G6PD was also higher in the WC group than in the FR group (Fig. 7; bottom). The activities of these enzymes were elevated by exercise training in all groups, most markedly in the FR and WC groups.

Lipoprotein lipase activities

Figure 8 shows values for the LPL activity determined in the heart, soleus muscle and perirenal adipose tissue. The activity of LPL in the heart was not different between the FR and WC groups for both the sedentary and exercised rats (Fig. 8; upper). The activity of this enzyme in soleus muscle was not affected by exercise training or dietary pattern (Fig. 8; middle).

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Fig. 7. Activities of malic enzyme (ME, top) and glucose-6-phosphate dehydrogenase (G6PD, bottom) in perirenal adipose tissue after sacrificing following four bouts of weight cycling (M±SEM). One unit (U) of both enzymes catalyzed the formation of 1 mmol of NADH/min. The values with different superscripts mean significant difference within the sedentary or exercised rats (ANOVA, p<0.05). *Statistically significant difference from the sedentary rats (p<0.05, Student’s t-test).

On the other hand, the activity of LPL in the adipose tissue of the sedentary rats was much higher in the WC group than in the FR group, but this difference was not apparent in the exercised rats (Fig. 8; bottom).

DISCUSSION

The study provides more information on the possible mechanisms that give rise to the increasing accumulation of body fat caused by weight cycling, and whether or not exercise training can alleviate the effect of weight cycling. We have shown that body fat accumulation in the sedentary rats of the WC group was greater than that of the FR group. As the rats of both groups were offered the same amount of experimental chow diet throughout the experimental period (four bouts of weight cycling), the difference in body fat accumulation between the two groups can be ascribed to the different feeding patterns (weight cycling vs. constant food restriction). We have also shown that exercise training can, in part, alleviate the effects of weight cycling on body fat deposition, possibly due to a reversal of the lowering of the thermic effect of food caused by weight cycling.
Weight Cycling in Sedentary and Exercised Rats

Fig. 8. Activities of lipoprotein lipase in the heart (upper) and soleus muscle (middle), and the perirenal adipose tissue (bottom) obtained after sacrificing following four bouts of weight cycling (M±SEM). One unit (U) of LPL catalyzed the release of 1 mmol FFA/h. The values with different superscripts mean significant difference within the sedentary or exercised rats (ANOVA, p<0.05). * Statistically significant difference from the sedentary rats (p<0.05, Student's t-test).

Effects of weight cycling in sedentary rats

For the sedentary rats, the body weight of the WC group after four bouts of weight cycling was greater than that of the FR group fed the same amount of food throughout the experimental period. The difference in body weight between the two groups was due to greater body protein and fat contents as well as the abdominal adipose tissue weight of the WC group. Our observations may not be directly comparable to others who have examined weight cycling in rats because of methodological differences. Our findings for the effect of weight cycling on body fat accumulation, including abdominal adipose tissue weight, are consistent with other reports (3–6, 8). However, the results in this study are not in agreement with those...
of Hill et al. (7), who found that male Wistar rats, after four bouts of weight cycling, did not show greater body fat deposition as compared to the control group subjected to the same food restriction without weight cycling. However, when the rats subjected to weight cycling were allowed ad libitum access to a stock diet for 18 days, body fat accumulation was greater in the rats with a history of weight cycling than in the control group. Therefore, they concluded that weight cycling has the potential to affect the restoration of carcass energy during subsequent refeeding. We recognize that the difference of results in body fat accumulation between the study by Hill et al. (7) and the present study might be due to the differences in animal sex and strain as well as the feeding method during the food restriction-weight loss period. The weight-cycled rats in their report were fasted for three days during the weight loss period, and then refed ad libitum for seven days. It may also explain the reason for not seeing a difference in body fat at the end of weight cycling if the body weight reduction during the 3-day fasting period was not be recovered during the following refeeding period of seven days. Recently, Reed et al. (10) reported that weight cycling does not promote body weight or body fat gain in female Wistar rats allowed a choice of diet. They concluded that the effects of weight cycling on body fat gain could be changed by methodological differences: choosing subjects, method of producing weight cycling and choosing appropriate controls. However, our study concludes that weight cycling increases more body fat accumulation as compared to constant food restriction controls, given the same amount of dietary energy as the WC group energy intake over the experimental period, because the design of the feeding regimen was tightly controlled: the rats of the WC and FR groups were fed the same diet at the same time (meal-fed twice a day, see “METHODS”).

It is well established that weight cycling increases body fat accumulation (3, 8, 28), but the effect on body protein mass is unknown. Our results of body composition provide evidence that weight cycling might enhance body protein mass.

The effect of weight cycling on body energy restoration in the sedentary rats is shown in Fig. 9. The energy restoration was 2.7-fold greater in the WC group than in the FR group during the experimental period (Fig. 9). We did not present the food efficiency (energy increment in body/energy intake), however, the data in Fig. 9 indicates that food efficiency was higher in the WC group than in the FR group because the food consumption of the two groups was exactly same throughout the experimental period. Additionally, this result suggests that the total daily energy expenditure is decreased by weight cycling. We examined whether this could be seen as a difference in RMR or TEF, and found that the rats of the WC group had a lower RMR and TEF for 6 h after a meal as compared to the rats of the FR group. Therefore, the differences in body composition and body energy restoration between the two groups, in part, might be explained by altered thermogenesis. Although some investigators have reported such a trend (2, 9), it is interesting that weight cycling decreased both RMR and TEF despite a greater body protein
Fig. 9. The effects of weight cycling and exercise training on the change in body energy during the experimental period. The values were calculated on the basis of the mean value from the sedentary FR rats (100%). A: The difference due to weight cycling of the sedentary rats. B: The difference due to exercise training in the FR group. C: The difference due to exercise training in the WC group.

content in the WC group than in the FR group. However, the reason why the rats of the WC group showed lower RMR and TEF is unclear.

The activities of ME and G6PD in adipose tissue were higher in the rats of the WC group than in the rats of the FR group, suggesting that the capacity for lipogenesis in adipose tissue was higher in the rats of the WC group than in the rats of the FR group. This view coincides with the greater abdominal adipose tissue weight observed in the former as compared to the latter.

Lipoprotein lipase plays a critical role in the supply of circulating triacylglycerol fatty acids to various tissues (29). The adipose tissue of the rats in the WC group appeared to have a higher LPL activity than that of the FR group, suggesting that blood triacylglycerol was taken into adipose tissue at a higher rate in the rats of the WC group than in the rats of the FR group. This might result in a greater adipose tissue weight in the WC rats. The rats of the WC group also had a higher serum insulin level as compared to the FR rats, which could be responsible for the enhanced activities of these enzymes in the adipose tissue of the WC group. Insulin is one of the main factors known to increase these enzyme activities (20,21,23). This result is in agreement with that of Reed et al. (8), who reported that weight-cycled female Sprague-Dawley rats, given a diet with a higher percentage of fat, had larger adipose depots and higher plasma insulin values.

Effects of exercise on weight-cycled rats

Body weights and body fat accumulation were significantly less in the exercised rats than in the sedentary rats for both the WC and FR groups, but this trend was not found in the CN group. The RMR in the CN, FR and WC groups was not affected by exercise training in this study, however, the TEF was enhanced by exercise training in the FR and WC groups. The effects of exercise training on energy efficiency are not consistent with studies supporting decreased energy
efficiency after exercise training (15,16,30), and this has not been seen in other studies (18). Ballor (31) reported that exercise training (treadmill running at a 15° incline; 24 m/min for 60 min per day; 5 day/wk; 9 wk) increased the RMR and daily energy expenditure (for 23 h) in female rats fed ad libitum or a moderately restricted diet (about 20% restricted), but this effect was not apparent in animals with severely restricted diets (about 40% restricted). He suggested that severe dietary restriction may interfere with the ability of exercise training to elicit increases in RMR. The reasons for discordance between his results and our results are the following: 1) the timing of indirect calorimetry measurement relative to the last exercise bout; 2) differences in intensity and duration of exercise training; and 3) differences in food intake. Our data on energy restoration suggest that the effect of exercise training on energy efficiency might be changed by food intake, because the difference of energy efficiency in exercised rats was greater when food intake was restricted than when food intake was not restricted.

The exercise training in this study did not completely suppress the increase in energy efficiency by weight cycling, since greater energy restoration was observed in exercised rats in the WC group than in those of the FR group. However, the difference in energy restoration between the sedentary and exercised rats of the WC group was 1.6-fold greater than that of the FR group (110 vs. 67 kcal, respectively, Fig. 5; bottom). This difference is apparent in Fig. 9 (C value was greater than B value). Moreover, the TEF in the WC group for 6 h after the meal was significantly higher in the exercised rats than in the sedentary rats. It is possible from these results to suggest that exercise training can alleviate, though not completely, the effects of increasing energy efficiency due to weight cycling.

It has been reported that exercise training increases insulin sensitivity in the whole body (32,33). In this study, the exercised rats had a lower serum insulin concentration than the sedentary rats in the WC group, but not in the CN and FR groups. It is possible that a lower serum insulin level resulted in no difference in the LPL activities of the adipose tissue from the FR and WC groups. However, in both the FR and WC groups, the activities of the lipogenic enzymes of the adipose tissue were higher in exercised rats than in sedentary rats, leading to a higher turnover of fat storage in the adipose tissue in exercised rats as compared to that in sedentary rats. This might be the reason the exercised rats in the FR and WC groups had lower adipose tissue weights.

In conclusion: 1) TEF was lower in the sedentary weight-cycled rats than in the control rats fed the same diet without weight cycling; 2) it is assumed that a decrease in TEF causes an increase in energy efficiency and body fat restoration during weight cycling in sedentary weight-cycled rats; 3) sedentary weight-cycled rats had higher serum insulin levels and more active lipogenic enzymes and LPL in the adipose tissue (i.e., higher lipogenesis and uptake of blood triacylglycerol into adipose tissue, which might explain the increased body fat accumulation in the sedentary weight-cycled rats); 4) exercise training, in part, prevented the lowering of TEF and the elevation of LPL activity caused by weight cycling. We conclude
that weight cycling may increase energy efficiency and body fat accumulation by
decreasing thermogenesis, and that exercise training can, partly, alleviate the effects
of weight cycling on energy efficiency.

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