Weight cycling-induced alteration in fatty acid metabolism

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Sea, Man-Mei, Wing Ping Fong, Yu Huang, and Zhen-Yu Chen. Weight cycling-induced alteration in fatty acid metabolism. Am J Physiol Regulatory Integrative Comp Physiol 279: R1145–R1155, 2000.—Epidemiological studies have suggested that repeated weight cycling over time may increase the risk of coronary heart disease. The mechanism involved remains poorly understood, but the change in lipid metabolism during weight cycling has been offered as a possible explanation. The present study investigated the effect of weight cycling on the size and fatty acid composition of rat fat pads as well as serum cholesterol, triglyceride, glucose, insulin, and glucagon in rats. Two consecutive weight cycles were induced by 40% energy restriction followed by ad libitum refeeding of either a moderate-fat (MF; 22% energy) or a high-fat (HF; 45% energy) diet. The lipogenic enzymes, including fatty acid synthase, acetyl-CoA carboxylase, malic enzyme, pyruvate kinase, and lipoprotein lipase in the weight-cycled (WC) rats fed only the HF diet, yielded an overshoot of activities at the end of two weight cycles. These changes were accompanied by an 80% increase in the size of the adipocyte and a 40–50% increase in the size of perirenal and epididymal fat tissues in HF-WC rats. Regardless of whether the rats were fed the HF or MF diet, all WC rats showed a gradual reduction in linoleic and α-linolenic acid and an increase in palmitic, palmitoleic, and stearic acid in total body lipid. It is concluded that weight cycling in rats may promote body fatness if an HF diet is consumed and can significantly alter whole body fatty acid balance irrespective of whether they consumed an MF or HF diet. Most importantly, the weight cycling led to an overshoot or fluctuation of serum cholesterol, triglyceride, glucose, insulin, and glucagon. If weight cycling is associated with an increased risk of cardiovascular disease, then, part of the mechanism may involve the changes in these risk factors.

weight fluctuation; fasting; refeeding; linoleic acid; α-linolenic acid; adipose tissue

WEIGHT CYCLING IS PREVALENT when obese people are trying to lose weight. The health effect of weight cycling induced by repeated dieting is controversial. In rats, weight cycling has led to the increased metabolic efficiency, increased consumption of dietary fat, heavier fat pads, hyperinsulinemia, and hypertension (10, 12). In humans, several epidemiological studies show that an increase in mortality from all causes and from coronary heart disease may be associated with weight cycling (17, 29). In contrast, some studies demonstrate no correlation between weight cycling and heart disease (28, 38).

The underlying mechanisms contributing to the association between weight cycling and coronary heart disease are unknown. One of these may be related to the hyperinsulinemia and hypertension induced by weight cycling (12, 41). Another possibility is that repeated weight loss and subsequent regaining of weight may promote fat deposition and central obesity (11, 42). It is also possible that weight cycling could change the composition of tissue lipids and therefore induce an imbalance among saturated, monounsaturated, n–3 and n–6 polyunsaturated fatty acids. It has been known that dietary n–3 fatty acids are inversely related to risk in atherosclerosis (24, 36). Several investigations have also demonstrated that a lower level of linoleic acid (18:2n-6) in human adipose tissue is associated with a higher risk for coronary heart disease (30, 32, 34, 40). Previous reports showed that rapid weight loss was associated with accelerated depletion of α-linolenic acid [18:3(n–3)] from adipose tissue in humans (21, 27). Weight cycling may not change total body fat mass in rodents fed a low-fat diet, but it does modify whole body fatty acid composition. We have previously shown that four weight cycles induced by repeated fasting followed by refeeding significantly decreased both 18:2(n–6) and 18:3(n–3) in carcass and adipose tissue lipids (3, 5, 6). These changes occurred with or without significantly changing final total body weight of the weight-cycled rats compared with ad libitum-fed control animals. We have also used whole body fatty acid balance method to show that weight cycling significantly increased net whole body oxidation of 18:2(n–6) and 18:3(n–3) in the weight-cycled rats compared with ad libitum-fed control animals (2, 4). The present study was carried out to investigate the influence of weight cycling on several risk factors of cardiovascular disease, including serum cholesterol and triglyceride. In addition, the changes in two hormones, insulin and glucagon, were also followed during weight cycling.

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Several studies (3, 6, 18, 19) that used alternating caloric restriction and refeeding with a low- or moderate-fat diet were unable to confirm that weight cycling promotes obesity. Furthermore, several studies have shown that weight cycling with a constant low-fat diet did not affect food efficiency, energy expenditure, and body fat composition (18, 19, 39, 44). In contrast, Uhley and Jen (43) reported that weight cycling produced by alternating ad libitum consumption of a high- and low-fat diet led to an increase in food efficiency. The food efficiency is, however, consistently increased only during the early period of weight regain, but a permanent increase has not been found as a result of the cycle of repeated weight loss and regain. Lauer et al. (25) demonstrated that some rats within a strain were more susceptible than others to develop obesity when given caloric restriction and refeeding with a low- or moderate-fat diet. The present study examined the size of perirenal and epididymal fat pads in weight-cycled rats fed a constant high-fat diet compared with that of weight-cycled rats fed a constant moderate-fat diet. We have also sought to ascertain whether alternation of lipogenesis might occur during the weight cycle. The changes in lipogenesis were monitored by measuring the activity of the lipogenic enzymes including fatty acid synthase (FAS), malic enzyme (ME), pyruvate kinase (PK), acetyl-CoA carboxylase (ACC), and lipoprotein lipase (LPL).

MATERIALS AND METHODS

Animals and Diets

One hundred and forty male Sprague-Dawley rats were used in this study. They were housed with two rats per cage in an animal room at 23°C with 12:12-h light-dark cycles. The rats were randomly divided into two groups: 75 rats in a high-fat group (HF; 45% energy, Table 1) and 65 rats in a moderate-fat group (MF; 22% energy, Table 1). Before weight cycling, all rats were allowed ad libitum access to their corresponding diet for 4 wk. Their body weight and food intake were measured daily, and the baseline food intake was determined. The diet was placed in an open can inserted into a larger open can. The spillage could be recovered from the larger can.

After the 4-wk stabilizing period, each group of rats was further divided into an ad libitum-fed control group (CTL; 25 rats in the HF group and 15 rats in the MF group) and a weight-cycled group (WC; 50 rats in both HF and MF groups). All the CTL rats were allowed free access to the diet and water throughout the study period, whereas the WC rats were only allowed free access to water and were subjected to two energy restriction-refeeding cycles.

The WC rats in both the HF and MF groups were subjected to 40% food restriction, and the amount of food given during partial fasting was calculated according to the baseline food intake. In the HF group, two weight cycles were induced by partially fasting the rats (food intake = 12.5 g·rat⁻¹·day⁻¹) for a period of 7 days, followed by ad libitum refeeding for 10 days. The two weight cycles in the MF group were similarly induced, but the amount of food given to the rats was different during the energy restriction period (15.5 g·rat⁻¹·day⁻¹).

The HF-CTL rats were killed at days 0, 7, 17, 24, and 34, whereas MF-CTL rats were killed only at days 0, 17, and 34 (Fig. 1). The WC rats from both the HF and MF groups were killed at days 1, 7, 8, 12, 17, 18, 24, 25, 29, and 34 (n = 5).

All the rats were killed under nitrogen-induced anoxia and exsanguinated via the abdominal aorta by using a syringe. The serum was separated from the whole blood by centrifugation (2,000 g for 15 min) and stored in aliquots at −76°C. Liver, epididymal, and perirenal adipose tissue were removed from the abdomen, blotted dry, and then weighed. A portion of epididymal adipose tissue was used for adipocyte analyses. Liver and the remaining adipose tissue were washed with chilled 0.9% saline, freeze clamped in liquid nitrogen, and stored in aliquots at −76°C for the determination of fatty acid composition. The carcass [whole body perirenal adipose tissue (2 pads), epididymal adipose tissue (2 pads), liver, blood] was also retained for lipid analysis. This study was approved by the Animal Care Committee of the Chinese University of Hong Kong.

Analysis of Adipocytes

The number and size of adipocytes were determined according to Cheung et al. (7) with slight modifications. After removal, the epididymal fat pads were immediately rinsed with saline incubated at 37°C. After dissecting them free of blood vessels, the fat pads were minced. The minced adipose

Table 1. Percentage composition of the high-fat and moderate-fat diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>HF Diet</th>
<th>MF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>28.8</td>
<td>33.3</td>
</tr>
<tr>
<td>Canola oil</td>
<td>25.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Casein</td>
<td>23.5</td>
<td>23.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>14.3</td>
<td>24.3</td>
</tr>
<tr>
<td>Mineral mixture (AIN76)</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Alpharel</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Vitamin mixture (AIN76A)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Values are expressed in wt%. HF, high fat; MF, moderate fat.
tissues (1.5 g) were suspended in 4% BSA-Krebs-Ringerbicarbonate (KRB; 1 ml/g tissue) containing collagenase (1 mg/ml, from Clostridium histolyticum, Sigma, St. Louis, MO) in 50-ml Falcon tubes. They were then incubated at 37°C with continuous shaking (100 rpm) for 60 min.

Collagenase treatment was terminated by adding 2 vol of 1% BSA-KRB maintained in a water bath at 37°C. The isolated adipocytes were collected by filtering the mixture through a layer of cheesecloth that had been immersed in 1% BSA-KRB buffer in advance. The filtrate was allowed to stand for 3 min in a 37°C incubator. After the adipocytes had floated onto the surface, the buffer was removed with a plastic Pasteur pipette. Adipocytes were resuspended in 3 ml of BSA-KRB and swirled gently to disperse the cells. Finally, 0.1 ml of adipocyte solution was pipetted onto a hemacytometer. Adipocytes were taken and photographed under a Zeiss microscope at a magnification of ×50. The number and diameter of isolated adipocytes were measured on the photographs. To avoid any bias in sampling, four to five photographs were prepared for each adipocyte sample. All the cells in a ruled area were counted. Because the released adipocytes were spherical, the cell concentration (cells/g adipose tissue) could be calculated

\[
\text{cell concentration (cells/g adipose tissue)} = \frac{\text{cell number in the ruled area}}{(9 \times 10^{-4})(1.5/3)}
\]

The size of the adipocytes was determined by counting the cells distributed around the central horizontal line in photographs. Assuming the adipocytes were spherical, the cell volumes could be calculated

\[
\text{cell volume} = \frac{4}{3}\pi(3r^2 + d^2) \times d
\]

d is the mean cell diameter, and \(\sigma^2\) is the variance in the cell diameter (16).

**Fatty Acid Analysis**

Total lipids derived from adipose tissue, liver, and carcass were extracted by using chloroform-methanol (2:1, vol/vol). Heptadecanoic acid, triheptadecanoic acid, and L-α-phosphatidylcholine diheptadecanoyl (all from Sigma) were added as internal standards to quantitate the free fatty acids (FFA), triglycerides, and phospholipids (PL), respectively. The chloroform-methanol phase containing the lipid extracts was dried under a stream of nitrogen, then redissolved in chloroform, which was then applied to a thin-layer chromatography (TLC) plate (20 × 20 cm, precoated with 250-μm silica gel 60 Å; Macherey-Nagel, Duren, Germany) to separate different lipid classes. A solvent system of hexane-diethyl ether-acetic acid (80:20:10, vol/vol/vol) was used for development. The bands containing FFA, triglycerides, and PL were scratched off the plate, and the lipids extracted were converted to methyl esters by using a mixture of 14% BF₃ in methanol and toluene (1:1, vol/vol) under nitrogen at 90°C for 45 min. The fatty acid methyl esters in FFA, triglycerides, PL, and total lipids were analyzed by GLC as described previously (3).

**LPL (EC 3.1.1.34)**

The activity of LPL was determined as described by Nilsson-Ehle and Schotz (33) with some modifications. First, 10 mCi of [9,10-2H]tri-oleoylglycerol (1H)TO; 414 mCi/mmole, Amersham) was purified by using a column containing 2 g silica acid (Sigma) eluted with petroleum ether followed by diethyl ether. The labeled TO was mixed with 550 mg of unlabeled TO and 36 mg of lecithin in chloroform followed by evaporation of chloroform. The dried TO mixture was emulsified in 10 ml of glicerol and then diluted with 4 vol of Tris-HCl buffer (pH 8.0, 0.2 M) containing 3% (wt/vol) BSA (Sigma) and 1 vol of cold serum from rats fasted overnight. The TO mixture was then shaken vigorously in a Vortex mixer. Second, the adipose tissue (300 mg) was homogenized in 150 μl medium containing Tris-HCL (pH 7.4, 67 mM) and the cold serum (1.3, v/vol). After 3 ml of cold acetone were added, the tissue was rehomogenized and centrifuged (12,000 g for 2 min). The sediment was then homogenized and extracted three more times (twice with the same volume of cold acetone and once with cold ether; 12,000 g for 2 min). The powder containing LPL was dried under a stream of nitrogen gas. The extracted powder was mixed with 1.5 ml of buffer (50 mM Tris-HCl, pH 8.0; 1 M ethylene glycol). The supernate (in which all the LPL was dissolved) was saved after centrifugation (40,000 g for 10 min). Third, the reaction (0.2 ml) was initiated by adding 0.1 ml of freshly prepared TO mixture to 0.1 ml of the supernate. The reaction mixture was then incubated at 37°C for 30 min. The reaction was stopped by adding 3.25 ml of methanol-chloroform-heptane (1:1:25.1) and 1.05 ml of 0.1 M potassium carbonate-borate buffer (pH 10.5) followed by vortexing for 15 s and centrifugation (3,000 g for 2 min). Finally, 1 ml of methanol-water phase was counted for radioactivity by using 15 ml scintillation fluid (Triton-X 100-toluene, 1:1, vol/vol) containing 0.4% PPO (wt/vol, Sigma) and 0.04% POPOP (wt/vol, Sigma). A series of controls was also performed without addition of the enzyme solutions. LPL activity (mU) was expressed as the amount of enzyme needed to release 1 nmol of oleic acid/min at 37°C.

**FAS (EC 2.3.1.85)**

The activity of FAS was measured according to the method of Nepokroeff et al. (31). One gram of liver (or 2 g of adipose tissue) was homogenized in 1.5 vol of homogenizing buffer [in mM: 70 KHCO₃, 85 K₂HPO₄, 9 KH₂PO₄, and 1 dithiothreitol (DTT), pH 7.0; 33 nmol NADPH; 33 nmol acetyl-CoA; 100 nmol malonyl-CoA; 100 nmol NADPH; 1 μmol β-mercaptoethanol] was preincubated at 30°C for 5 min. The resulting supernatant was centrifuged at 105,000 g for 60 min. The supernatant was centrifuged again at 105,000 g for 60 min. The resulting supernatant obtained was immediately used for the enzyme assay, and the activity of FAS was measured spectrophotometrically by monitoring the rate of NADPH oxidation. All steps up to assay incubation were carried out on ice. The reaction mixture (500 μl potassium phosphate buffer, pH 7.0; 33 nmol NADPH; 33 nmol acetyl-CoA; 100 nmol malonyl-CoA; 100 nmol NADPH; 1 μmol β-mercaptoethanol) was preincubated at 30°C for 5 min. The reaction was initiated by the addition of 50–100 μg of the enzyme protein (supernatant after centrifugation at 105,000 g), which was preincubated in 40 μl of activating buffer (1 M potassium phosphate, pH 7.0; 10 mM DTT) at 37°C for 15 min, into 960 μl of the preincubated reaction mixture. The oxidation of NADPH was followed at 340 nm. A correction was made for the rate of NADPH oxidation in the absence of malonyl-CoA as a background. One unit of the enzyme activity represented the amount of enzyme needed to synthesize 1 nmol of palmitic acid/min (equivalent to the oxidation of 14 nmol of NADPH).

**ME (EC 1.1.1.40)**

ME activity was estimated from the rate of NADPH formation at 25°C (20). One gram of liver was first washed in 0.25 M sucrose and cut into small pieces. It was then homog-
enized in 3 vol of 0.25 M sucrose by using a glass Potter-Elvehjem homogenizer in an ice-water bath. The homogenate was centrifuged at 105,000 g for 1 h to obtain the supernatant. The reaction was initiated by mixing 990 μl of assay buffer (400 mM triethanolamine-HCl-30 mM L-malate-120 mM MnCl₂-4H₂O-3.4 mM NADP⁺, 10:1:2.4, vol/vol/vol, pH 7.4) with 10 μl enzyme (105,000 g supernatant). The reduction of NADP⁺ was followed at 340 nm. The background activity was measured in the absence of L-malate. One unit of enzyme activity was defined as the amount of enzyme needed to reduce 1 nmol of NADP⁺/min at 25°C.

PK (EC 2.7.1.40)

PK activity was measured by the rate of NADH reduction spectrophotometrically as described by Imamura and Tanaka (22). Liver (1 g) was homogenized in 3 vol of homogenization buffer (in mM: 20 Tris·Cl, pH 7.5, 100 KCl, 5 MgSO₄, 0.1 EDTA, 0.2 Fru-1,6-P₂, and 10 β-mercaptopoethanol) by using a Polytron for 1 min in an ice-water bath, and the supernatant was obtained by centrifugation at 20,000 g for 1 h. The enzymatic reaction was initiated by adding 10 μl supernatant into the assay buffer (50 mM Tris·Cl, pH 7.5, 0.1 M KCl, 5 mM MgSO₄, 2 mM phosphoenolpyruvate, 0.5 mM Fru-1,6-P₂, 0.18 mM NADH, and 8 U lactate dehydrogenase) that was preincubated at 37°C. The oxidation of NADH was followed at 340 nm by using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of pyruvate/min (or oxidizing 1 μmol of NADH in the coupled system) under the assay conditions. Background was measured by starting the assay without phosphoenolpyruvate.

ACC (EC 6.4.1.2)

The method of Inoue and Lowenstein (23) was used to determine the activity of ACC. In brief, 2 g of liver were homogenized in 2 vol of buffer (0.05 M Tris·Cl, pH 7.5, 20 mM sodium citrate, 0.5 mM EDTA, and 5 mM β-mercaptopoethanol) by using a Polytron for 10 s in an ice-water bath. It was centrifuged at 2,000 g for 10 min, and the residue was homogenized again with 1 ml of the same buffer by using a glass Potter-Elvehjem homogenizer. The homogenate was combined with the supernatant from the first centrifugation and centrifuged at 14,000 g for 45 min. The residue was then washed with 1 ml of buffer. The washing was combined with the supernatant from the second centrifugation and then centrifuged again at 105,000 g for 45 min to obtain the supernatant. Four milliliters of the supernatant were loaded to a Sephadex G-25 column (2.25 × 30 cm, Amersham) equilibrated with 20 mM Tris·Cl (pH 7.5) containing 1 mM DTT. Fractions of 1 ml were collected and those with the highest protein concentration were pooled.

The crude enzyme (supernate) was preactivated by mixing 0.5 ml of the fraction (crude enzyme) with 0.5 ml of activating buffer (in mM: 20 sodium citrate, 20 MgCl₂, 1 DTT, and 50 Tris·Cl, pH 7.5) containing 0.5 mg/ml of BSA (fatty acid poor or free; Sigma) at 37°C for 30 min. The activated enzyme was used for assay within 20 min. The assay was initiated by adding 30 μl of the activated enzyme into 370 μl of assay buffer (in mM: 100 Tris·Cl, pH 7.5, 1 DTT, 0.2 acetyl-CoA, 20 sodium bicarbonate (NaH₁₄CO₃; 0.25 μCi/μmol, Amersham), 5 ATP, 20 citrate, and 20 MgCl₂ as well as 0.5 mg/ml BSA), and the reaction mixture was incubated at 37°C for 5 min. The reaction was stopped by adding 0.1 ml of 4 N HCl, and the unreacted ¹⁴CO₂ was expelled by a gentle stream of air. The residue was then dissolved in 1 ml of H₂O, and its radioactivity was counted. One unit of ACC activity was equal to the amount of enzyme needed to form 1 μmol of malonyl-CoA at 37°C/min.

Determination of Serum Insulin and Glucagons

The level of serum insulin and glucagon was determined by using I-125 RIA kits (Amersham and Linco, respectively).

Determination of Serum Cholesterol, Triglycerides, and Glucose

Total serum cholesterol, triglycerides, and glucose were determined by using enzymatic kits (Sigma).

Statistics

Data were expressed as means ± SD. The fatty acid analysis, adipocytes analysis, serum glucose, cholesterol, and triglycerides were subjected to ANOVA followed by a least significant difference test for statistical evaluation of the significant difference between the CTL and WC rats, and only P < 0.01 was considered statistically significant. This was done by running the data on personal computer (PC) ANOVA software (PC ANOVA for the IBM PC, version 1.1; IBM, Armonk, NY).

RESULTS

Body Weight

The changes in body weight of the HF and MF groups over time are shown in Fig. 1. The body weight of all WC rats decreased gradually during the energy restriction periods of the two weight cycles. At the beginning of refeeding, the body weight of WC rats increased significantly, and then increased gradually until the end of the cycle. After the two consecutive weight cycles, the final body weight of the WC rats was not significantly different from that of their corresponding controls.

The HF-WC rats had a weight reduction of 12.9 and 22.4 g during the fasting periods of cycle 1 and cycle 2, respectively. During the refeeding period, the HF-WC rats had a weight gain of 74.2 and 74.8 g in cycle 1 and cycle 2, respectively (Fig. 1). In contrast, the MF-WC rat had a weight reduction of 31.4 g in cycle 1 and 20.2 g in cycle 2 during the food restriction period. The refeeding led MF-WC rats to gain 74.9 and 64.4 g in cycle 1 and cycle 2, respectively (Fig. 1).

Food Intake

The time course of food intake is graphically illustrated in Fig. 1. The food intake of the controls in HF and MF groups throughout the entire experiment was maintained at 20.9 ± 3.4 and 25.3 ± 2.8 g·rat⁻¹·day⁻¹, respectively. The weight reduction of WC rats was achieved by giving 40% food restriction for 7 days, i.e., 12.5 g·rat⁻¹·day⁻¹ for the HF group and 15.5 g·rat⁻¹·day⁻¹ for the MF group. Once the WC rats were allowed free access to the diet after food restriction, their food intake during the first 2 refeeding days was much higher than that of the CTL (Fig. 1). Thereafter, their food intake gradually decreased and became similar to that of the CTL rats.
Weight of Adipose Tissue

The weight of epididymal and perirenal adipose fat pads changed similarly during weight cycling in both the HF and MF groups (Fig. 2). Although the weight of the two fat pads of WC rats decreased and increased concurrently with the change of body weight in the two cycles in both groups, the HF-WC rats responded more prominently during the refeeding periods compared with the MF-WC rats. In HF-WC group, the weight of fat pads increased drastically in the first several days of refeeding. The HF-WC rats had significantly higher final fat pad weights than that of the HF-CTL at the end of the weight cycle. In contrast, the fat pads of MF-WC rats increased less prominently during refeeding, and their final weight was slightly lower than that of their MF-CTL.

Number and Size of Adipocytes

After the two weight cycles, the WC rats from both HF and MF groups had a similar number of adipocytes in epididymal adipose fat pad as their corresponding CTL rats (Fig. 3). In the HF-WC group, the number or density of adipocytes was 162.8 ± 39.8 × 10⁴ cells/g adipose tissue whereas that in the HF-CTL was 170.2 ± 35.6 × 10⁴ cells/g adipose tissue. In the MF WC group, the number of adipocytes in epididymal adipose tissue pad was 164.6 ± 32.0 × 10⁴ cells/g adipose tissue whereas that in the MF-CTL rats was 169.6 ± 32.8 × 10⁴ cells/g adipose tissue.

The size of adipocytes in epididymal adipose tissue of the HF-WC rats responded differently to weight cycling compared with the MF-WC and the HF-CTL rats (Fig. 3). In the HF-WC group, the size of adipocytes was considerably enlarged, being ~80% larger than that of the HF-CTL (P < 0.01). After two weight cycles, the average size of adipocytes of HF-WC rats was 677.5 ± 247.6 × 10³ μm² whereas that in the HF-CTL rats was 376.2 ± 88.9 × 10³ μm². In the MF-WC group, the average size of adipocytes was 366.5 ± 159.4 × 10³ μm² whereas that in the CTL was 377.3 ± 77.1 × 10³ μm², and there was no significant difference between MF-WC rats and MF-CTL rats.

Serum Triglycerides, Cholesterol, and Glucose

In the HF-WC group, serum total triglycerides decreased during the food restriction in both weight cycles and then rose to a significantly higher level than that of the HF-CTL rats during refeeding (Fig. 4). However, the triglyceride concentration of the HF-WC rats was not significantly different from that of the HF-CTL rats at the end of second weight cycle. Moreover, for both HF-WC rats and HF-CTL rats, their serum triglycerides levels at the end of the experiment were higher than that at the beginning. In MF-WC group, serum total triglycerides also fluctuated in a pattern similar to that in HF-WC group (Fig. 4). The final triglyceride concentration of MF-WC rats remained the same as that of MF-CTL rats.

Similar fluctuation of serum cholesterol level was observed in the HF-WC rats during weight cycling (Fig. 4). There was also no significant difference between the HF-WC and HF-CTL rats at the end of cycle 2. Though MF-WC group had a much higher serum cholesterol level than their MF-CTL during the refeeding, there was no difference between the two groups at the end of the experiment (Fig. 4).

Serum glucose of HF-WC rats fluctuated and differed significantly at several time points from that of HF-CTL rats (Fig. 4). Serum glucose concentration was not different between HF-WC and HF-CTL rats at the end of the experiment. In MF-WC group, serum glucose decreased to a greater extent than that of HF-WC group during the food restrictions (Fig. 4). The final serum glucose level in MF-WC rats was, however, slightly higher than that of the MF-CTL rats at the end of cycle 2 but the difference was not statistically significant.
Serum Insulin and Glucagon

In both HF and MF groups, there was no significant difference in plasma insulin and glucagon between the WC rats and CTL rats during fasting and refeeding (Fig. 5). The levels of serum insulin and glucagon fluctuated during the two weight cycles in WC rats compared with those in the CTL rats.

Carcass Total Lipids

The effects of two weight cycles on carcass total fatty acid composition in HF and MF groups were shown in Figs. 6 and 7. In the HF-WC group, 18:2(n–6) and 18:3(n–3) were gradually decreased during the two food restriction periods whereas 14:0, 16:0, 16:1n–7, and 18:0 acids were significantly increased in HF-WC rats compared with the HF-CTL group. At the end of the first and second cycles, the content of 18:2(n–6) and 20:5(n–3) was considerably decreased whereas 16:0 was significantly increased in the HF-WC rats compared with that of HF-CTL rats (data not shown).

Unlike the carcass PL, the fatty acid composition of carcass triglycerides changed in WC rats during the two weight cycles. 18:2(n–6) and 18:3(n–3) were gradually decreased in the WC rats fed both an HF and MF diet compared with CTL rats fed the same diet. During the two refeeding periods of each cycle, the content of 16:0 and 18:0 of carcass triglycerides was significantly higher than that in the CTL rats at the end of two cycles. Furthermore, the content of 16:1(n–7) in WC rats fed a MF diet was also increased after the two weight cycles (data not shown).

Adipose Tissue Fatty Acids

In HF-WC rats, 18:2(n–6), 18:3(n–3), eicosapentaenoic acid [20:5(n–3)], docosapentaenoic acid [22:5(n–3)], and docosahexaenoic acid [22:6(n–3)], in the two adipose tissues were significantly decreased during food restriction periods (data not shown). In the second food restriction period, 14:0, 16:0, and 18:0 were significantly increased in HF-WC rats compared with those in the MF-CTL group. At the end of the first and second cycles, the content of 18:2(n–6) and 20:5(n–3) was considerably decreased whereas 16:0 was significantly increased in the HF-WC rats compared with that of MF-CTL rats during food restrictions (data not shown). At the end of the weight cycles, 16:0 was significantly increased...
in MF-WC rats, whereas 18:2(n–6) was markedly re-
duced compared with MF-CTL group. At the end of the
second cycle, 18:3(n–3) and 20:4(n–6) were also reduced
in the MF-WC rats compared with those in MF-CTL
group (data not shown).

**LPL**

The function of LPL catalyzes the conversion
of plasma triglycerides to FFA, which enter adipocytes,
and are resynthesized into triglycerides. There was a
general increasing trend for the specific enzymatic
activity for LPL of all the rats in both the HF and MF
groups (Figs. 8 and 9). In the HF-WC group, the LPL
activity decreased during the fasting periods of the two
cycles compared with that of HF-CTL rats. During the
refeeding, the LPL activity in HF-WC rats increased
rapidly and remained significantly higher than that of
the HF-CTL rats (Fig. 8). Similar results were also ob-
tained for both epididymal adipose fat pads (data not shown).

**FAS**

This enzyme catalyzes the addition of acetyl-CoA to
the acyl end of a growing long-chain fatty acid in the
fatty acid synthesis pathway. In the HF-WC group, the
FAS activity of epididymal and perirenal adipose tis-
sues decreased considerably during the partial energy
restriction (only data for perirenal adipose tissue shown),
but with an overshoot in activities throughout the
refeeding periods compared with that of HF-CTL rats (Fig. 8). The FAS activity of the HF-WC rats at the
end of the two cycles was also significantly higher than
that of the HF-CTL rats (Fig. 8). For the hepatic FAS,
the specific activity of the HF-CTL rats remained un-
changed during refeeding (Figs. 8 and 9). Although the
final hepatic FAS activity of the HF-WC rats was
significantly different from that of the HF-CTL rats,
this difference was much smaller compared with the
difference observed in adipose tissues. In the MF-WC
group, the FAS activity of two adipose tissues (only
data for perirenal adipose tissue shown) and liver
showed similar responses to weight cycling (Fig. 9).
During the energy restriction, the FAS activities
dropped dramatically during energy restriction while
they only rose to a level similar to that of the MF-CTL
rats during the refeeding period. Moreover, the final
FAS activities at the end of the two weight cycles were
not significantly different between MF-WC rats and

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**Fig. 6.** Effect of weight cycling on fatty acid composition of carcass
total lipids in CTL (●) and WC (▲) rats fed an HF diet. Data are
expressed as means ± SD; n = 5 rats. 14:0, Myristic acid; 16:0,
palmitic acid; 16:1(n–7), palmitoleic acid; 18:0, stearic acid; 18:2(n–
6), linoleic acid; 18:3(n–3), α-linolenic acid. *P < 0.01 between CTL
and WC rats.

**Fig. 7.** Effect of weight cycling on fatty acid composition of carcass
total lipids in CTL (●) and WC (▲) rats fed an MF diet. Data are
expressed as means ± SD; n = 5 rats. *P < 0.01 between CTL and
WC rats.
MF-CTL rats. In fact, the former was slightly lower than the latter.

**ME**

This enzyme reduces NADP\(^+\) to NADPH, which is required as a cofactor for fatty acid synthesis. The activity of hepatic ME of the HF-CTL rats remained constant during 35 days of experimental observation. The ME activity in HF-WC rats, however, fluctuated coincidentally with the body weight loss and regain. It dropped during energy restriction and rose rapidly during the refeeding. At the end of the two weight cycles, the HF-WC rats had considerably higher activity than that in HF-CTL rats (Fig. 8). Furthermore, it was noteworthy that the difference in the activity between HF-CTL and HF-WC rats was remarkably larger at the end of the second cycle than that of the first cycle. In MF-WC group, the ME activity changed parallel to the body weight change. Distinct from the HF groups, there was no significant difference in the final ME activity between the MF-WC and MF-CTL rats (Fig. 9).

**PK**

The function of PK is to convert pyruvate to acetyl-CoA, which enters either the citric acid cycle or ME-citric lyase pathway for fatty acid synthesis. The hepatic PK activity of the HF-WC rats decreased significantly during fasting and then increased rapidly during the refeeding periods in both weight cycles. At the end of the cycles, the PK activity of the HF-WC rats was slightly higher than that of the HF-CTL rats (Fig. 8). In the MF WC group, the PK activity also decreased drastically during fasting, and with an overshoot in activity throughout the two refeeding periods. There was a significant difference in the final activity between the MF WC rats and the MF-CTL rats, with the MF-WC rats having a higher PK activity than the MF-CTL rats (Fig. 9).

**ACC**

This enzyme accelerates the first reaction in fatty acid synthesis, converting acetyl-CoA to malonyl-CoA. The hepatic ACC activity in HF-WC rats decreased considerably during the energy restriction in both cy-
cles (Fig. 8). During the refeeding periods, the ACC activity in HF-WC rats rose rapidly and was significantly higher than that of the HF-CTL rats. In the MF-WC group, the ACC activity also dropped when the rats were fasted, but there was no significant difference between the MF-WC and MF-CTL rats at the end of experiment (Fig. 9).

**DISCUSSION**

The present study clearly demonstrated that an HF diet and weight cycling were associated with a significant modification of both fatty acid composition and the size of fat depots in rats. This was in contrast to the report by Lauer et al. (25), who found that weight cycling did not promote whole body fatness in rats fed an HF diet. This is probably due to the varying susceptibility of different strains or individual differences within the same strain to develop obesity by HF feeding (25). The major observation in the present study was that weight cycling caused a substantial increase in the size of both epididymal and perirenal fat pads (the two most visible fat depots in rats) in HF-WC rats compared with HF-CTL after two consecutive weight cycles (Fig. 2). The increase in the size of epididymal adipose tissue pads in HF-WC rats was associated with an increase in the size of adipocytes but not associated with an increase in the number of adipocytes (Fig. 3). We hypothesize that the lipogenesis stimulated by two consecutive weight cycles lead to an enlarged size of adipose tissues in the HF-WC rats. To demonstrate this hypothesis, we measured all the key enzymes involved in fatty acid synthesis and LPL. The results clearly showed that FAS, ME, PK, ACC, and LPL were suppressed during the energy restriction period but they were activated to a higher level in HF-WC rats compared with HF-CTL during the refeeding phase. This higher activity in lipogenesis and LPL was sustained even when the body weight in HF-WC rats had returned to that of the HF-CTL rats. Thus the lipogenic enzymes were activated to induce hyperlipogenesis. Triglycerides endogenously synthesized or exogenously obtained from diet were then efficiently stored in the adipose tissue by the highly activated LPL. The results were consistent with previous reports (8, 9, 13, 14, 27), which showed that the lipogenic activity was higher when the rats were fasted and then refed. The response of HF-WC rats to weight cycling, however, was different from that of MF-WC rats. First, the increase of...
lipogenic enzyme activity during refeeding was more pronounced in HF-WC rats compared with MF-WC rats. Second, the duration of the overshoot of the enzymatic activities during refeeding was longer in HF-WC rats compared with MF-WC rats. There was no difference in the size of adipose tissue pads between MF-WC and MF-CTL rats at the end of two weight cycles. The present study emphasizes the role of dietary fat in weight maintenance and weight loss. If the data could be extrapolated to humans, the obesity promoted by weight cycling would be true only when a HF diet was consumed.

Several longitudinal epidemiological studies have documented an increased risk of cardiovascular disease with weight cycling, but relevant risk factors have not been identified (17, 18, 28, 38). To the best of our knowledge, the present study was the first to observe that weight cycling was associated with an overshoot of several risk factors including serum cholesterol and triglyceride in WC rats fed either an HF or an MF diet. Interestingly, the weight cycling also induced a fluctuation of serum glucose, insulin, and glucagon. These observations have clinical significance for several reasons. First, the present study was able to closely monitor these factors over periods of controlled weight cycling in rats, whereas most clinical studies failed to observe these phenomena because the time of sampling vs. the regain of lost weight is not able to be well controlled in human studies (21, 28, 29). Second, these factors were only measured at the end of the weight cycles in human studies, when the levels of serum cholesterol, triglyceride, glucose, insulin, and glucagon were back to the control level. If weight cycling in humans increases significantly the risk of cardiovascular disease, then the overshoot and fluctuation of these factors should not be overlooked.

Another major observation in the present study was that weight cycling caused a substantial decrease in 18:2(n–6) and 18:3(n–3) in both HF- and MF-WC rats. At the end of two consecutive weight cycles, 18:2(n–6) and 18:3(n–3) decreased by 43 and 32%, respectively, in the carcass lipid of HF-WC compared with that of the HF-CTL group (Fig. 6). Similarly, 18:2(n–6) decreased from 25.5 to 15.3% whereas 18:3(n–3) was reduced from 2.5 to 1.6% in the carcass lipid of MF-WC rats compared with that of the MF-CTL (Fig. 7). In contrast, 14:0, 18:0, 16:0, and 16:1(n–7) were proportionally increased in WC rats compared with those in CTL group (Figs. 6 and 7). Together with our previous reports (3–5), the present study clearly demonstrated that weight cycling does change the body fatty acid composition with or without changing the total body weight, total body fat, and size of fat pads. The reduction in 18:3(n–3) and 18:2(n–6) occurs regardless of the pattern of WC caused by either fasting (100% energy restriction) or partial energy restriction followed by ad libitum refeeding (3–5).

The mechanism by which 18:2(n–6) and 18:3(n–3) were substantially decreased whereas 14:0, 18:0, 16:0, and 16:1(n–7) were increased during WC remains poorly understood. It is possible that 18:2(n–6) and 18:3(n–3) were preferentially oxidized during the energy restriction (15, 26), and the lipogenesis of saturated and monounsaturated fatty acids during the refeeding was stimulated in liver and adipose tissue. This was reflected in the observation that the activity of FAS, ME, PK, ACC, and LPL was elevated during the refeeding phase (Figs. 8 and 9). Thus the repeated overshoot in lipogenesis during weight cycling may lead to the higher concentrations of 14:0, 18:0, 16:0, and 16:1(n–7), all of which can be synthesized by mammals, and concomitantly, to the lower concentration of those fatty acids that cannot be synthesized by mammals, such as 18:2(n–6) and 18:3(n–3), in adipose tissue and carcass. It has been reported that starved rats have a two- to eight-fold increase in the rate of 16:0 and 16:1(n–7) synthesis during refeeding (1). It should also be pointed out that alteration of the fatty acid profile of carcass total lipids was mainly due to the change in the fatty acid composition of carcass triglycerides. There was no or little change in the carcass PL and carcass FFA.

**Perspectives**

The relevance of weight cycling in normal-weight rats fed an MF or HF diet as a model for risks associated with weight cycling in humans is unclear (17, 21, 28, 29). The present study clearly demonstrated that two weight cycles remodeled the whole body fatty acid composition. This change was specific and irrespective of the level of dietary fat. This weight cycling-induced alteration markedly lowered the ratio of polyunsaturated to saturated fatty acids in tissue (3, 4, 6). The present study emphasizes that weight cycling may promote body fatness if an HF diet was given. Most important was that weight cycling repeatedly led to an overshoot or fluctuation of lipogenic enzymes, serum cholesterol, triglycerides, glucose, insulin, and glucagon. If weight cycling increases cardiovascular risk, part of the mechanisms may be associated with the disturbance of whole body fatty acid balance, overshoot of risk factors including serum cholesterol and triglyceride, and changes in metabolic and hormonal profile.

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