Weight cycling-induced alteration in fatty acid metabolism

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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 fol-
lowed by ad libitum refeeding of either a moderate-fat (MF;
22% energy) or a high-fat (HF; 45% energy) diet. The lipo-
genic 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. Re-
gardless of whether the rats were fed the HF or MF diet, all
WC rats showed a gradual reduction in linoleic and α-linole-
ic 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 irrespec-
tive of whether they consumed an MF or HF diet. Most
importantly, the weight cycling led to an overshoot or fluc-
tuation of serum cholesterol, triglyceride, glucose, insulin,
and glucagon. If weight cycling is associated with an in-
creased risk of cardiovascular disease, then, part of the
mechanism may involve the changes in these risk factors.

WEIGHT CYCLING IS PREVALENT when obese people are
trying to lose weight. The health effect of weight cy-
cling 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 dem-
onstrate no correlation between weight cycling and
heart disease (28, 38).

The underlying mechanisms contributing to the as-
sociation 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, monounsatu-
rated, 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 in-
vestigations 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 de-
creased 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 oxida-
tion 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 hor-
mones, insulin and glucagon, were also followed during
weight cycling.

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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 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 was used for adipocyte analysis.

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 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.

MATERIALS AND METHODS

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 tissue was then pooled and minced further. The minced adipose tissue was then minced further.

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 was used for adipocyte analysis.

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 tissue was then pooled and minced further. The minced adipose tissue was then minced further.
tissues (1.5 g) were suspended in 4% BSA-Krebs-Ringer bicarbonate (KR; 1 mg/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 from 1.5 g of adipose tissue were resuspended in 3 ml of buffer, and the whole ruled area was 3 × 3 mm with a depth of 0.1 mm, the total volume of ruled area was 9 × 10⁻⁴ ml. Thus

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

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

\[
\text{cell volume} = \frac{(\pi/6)(3\pi^2 + d^2) \times d}{d^3}
\]

where \(d\) 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 \(\alpha\)-phosphatidylcholine diheptadecanoyl (all from Sigma) were added as internal standards to quantify the free fatty acids (FFA), triglycerides, and phospholipids (PL), respectively. The chloroform-methanol phase containing the lipid extracts was dried under a gentle stream of nitrogen and 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. The labeled 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, vt/vol). After 3 ml of cold acetic acid were added, the tissue was homogenized 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 (1:41:125) 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 25 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 Nepokroef 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; 10 mM DTT] at 37°C for 15 min, into 960 µl of activating buffer (1 M potassium phosphate, pH 7.0; 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 µl of the enzyme protein (supernatant after centrifugation at 105,000 g for 60 min). The resulting supernatant was centrifuged again at 105,000 g for 60 min. The resulting supernatant 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) contained 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-
enzized in 3 vol of 0.25 M sucrose by using a glass PotterElvehjem 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/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, 1 EDTA, 0.2 Fruct-1,6-P2, and 10 β-mercaptoethanol) 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 Fruct-1,6-P2, 0.18 mM NADH, and 8 μl 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-mercaptoethanol) 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 recentrifuged 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 DT T. 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 MglCl2, 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 Glucagon

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 rats had a weight reduction of 31.4 g in 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 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 pronouncedly 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 $162.8 \pm 39.8 \times 10^4$ cells/g adipose tissue whereas that in the HF-CTL was $170.2 \pm 35.6 \times 10^4$ cells/g adipose tissue. In the MF WC group, the number of adipocytes in epididymal adipose tissue pad was $164.6 \pm 32.0 \times 10^4$ cells/g adipose tissue whereas that in the MF-CTL was $169.6 \pm 32.8 \times 10^4$ 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 $\sim 80\%$ larger than that of the HF-CTL ($P < 0.01$). After two weight cycles, the average size of adipocytes of HF-WC rats was $677.5 \pm 247.6 \times 10^3 \mu m^3$ whereas that in the HF-CTL rats was $376.2 \pm 88.9 \times 10^3 \mu m^3$. In the MF-WC group, the average size of adipocytes was $366.5 \pm 159.4 \times 10^3 \mu m^3$ whereas that in the CTL was $377.3 \pm 77.1 \times 10^3 \mu m^3$, 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 (Fig. 6). In the MF-WC group, 18:2(n–6), 18:3(n–3), eicosatrienoic acid [20:3(n–6)], arachidonic acid [20:4(n–6)], docosatraenoic acid [22:4(n–6)], 20:5(n–3), and 22:5(n–3) were significantly lower than in MF-CTL rats during food restrictions (data not shown). At the end of the weight cycles, 16:0 was significantly increased in the HF-WC rats compared with that of HF-CTL rats (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 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).

In the MF-WC group, 18:2(n–6), 18:3(n–3), eicosatrienoic acid [20:3(n–6)], arachidonic acid [20:4(n–6)], docosatraenoic acid [22:4(n–6)], 20:5(n–3), and 22:5(n–3) were significantly lower than in 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 compared with that of MF-CTL rats (data not shown).
in MF-WC rats, whereas 18:2(n–6) was markedly reduced 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. At the end of the two cycles, the LPL activity of the HF-WC rats was essentially higher than that of the HF-CTL rats (Fig. 8). Similar results were obtained for 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 tissues 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 unchanged 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 MF-CTL rats during the refeeding period.
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 always 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, 18:3(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, and concomitantly, to the lower concentration of those fatty acids that cannot be synthesized by mammals.

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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REFERENCES

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