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Weight cycling-induced reduction of linoleic acid in carcass and adipose tissue in rats

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Recent epidemiological studies have proposed the hypothesis that large fluctuations in body weight over time may increase the risk of cardiovascular disease. Several studies have demonstrated that there is an inverse correlation between adipose tissue linoleic acid content and the risk of coronary heart disease. This led us to consider whether several weight cyclings induced by food restriction followed by regain could affect metabolism of linoleic acid and α-linolenic acid and their longer-chain polyunsaturated fatty acids. Fifty rats were fed a semipurified diet containing 15% fat (30.5% kcal) in which linoleic acid and α-linolenic acid accounted for 7.2 and 3.5% of total fatty acids, respectively. They were randomly divided in two groups. The control (CTL) group was allowed free access to diet whereas the weight-cycled (W-CYC) rats were subjected to four cycles of partial food restriction (24% intake of ad libitum controls, about 5 g/day) for 3 or 4 days to reduce body weight followed by ad libitum refeeding until the weight reached the corresponding average of the CTL group. Four rats were killed as baseline values before weight cycle. The rest of the rats from each group were killed at the beginning (n = 5), the end of partial food restriction (n = 9), and the end of ad libitum refeeding (n = 10) in weight cycle 4. The fatty acid compositions of the liver, epididymal, and perirenal adipose tissue and carcass were determined by gas liquid chromatography. The W-CYC rats, compared with the CTL rats, increased significantly their food intake immediately after each partial food restriction but thereafter gradually reached that of the CTL. No differences in the weights of liver, epididymal, and perirenal adipose tissue were observed between the W-CYC and CTL group after four weight cycles. Contrary to ad libitum feeding, four weight cyclings decreased the content of linoleic acid in the carcass, epididymal and perirenal adipose tissue. α-linolenic acid was also lower in the W-CYC rats than in the CTL group, but a significant difference was observed only at the end of partial food restriction. Compared with those in the CTL, the liver fatty acids in the W-CYC rats were remarkably modified during the partial food restriction, showing an increase in linoleic, α-linolenic, and arachidonic acids but a decrease in oleic and palmitoleic acids. The food restriction-induced changes in liver fatty acids were reversed after the ad libitum refeeding. We conclude that weight cycling alters body fatty acid composition characterized by a selective decrease in the linoleic acid content of carcass and adipose tissues without significantly changing the total body fat content and the weight of perirenal and epididymal fat pads. (J. Nutr. Biochem. 6:653–660, 1995.)

Keywords: weight cycling; yo-yo dieting; linoleic acid; α-linolenic acid; cholesterol

Introduction

Weight cycling (WC) is commonly practiced by many people, especially overweight individuals. There is growing concern that fluctuations in body weight may have negative health consequences.[1] For example weight fluctuation during young adulthood has been shown to be associated with a higher risk of coronary heart disease and premature death.[2,3] However, some studies do not support the WC hypothesis relative to heart disease.[4–6]

WC may alter lipid metabolism in both humans and animals. In rats, WC has been shown to result in increased consumption of dietary fat, increased metabolic efficiency,

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heavier fat pads, hyperinsulinemia,[7] and in some cases hypertension.[8] In humans, the response of adipose tissue to weight reduction induced by very low caloric diet is characterized by a selective decrease in its content of α-linolenic acid (18:3n-3) despite providing adequate 18:3n-3 in the diet.[9–11] No data are yet available to demonstrate whether this very low caloric diet-induced reduction in human adipose 18:3n-3 is reversible during weight regain.

The content of linoleic acid (18:2n-6) in adipose tissue reflects its long-term intake. Several studies have demonstrated that 18:2n-6 in adipose tissue is inversely related to the incidence of coronary heart disease.[12–15] We have previously used the whole body balance method to show that a single WC (2 days of fasting followed by 5 days of refeeding) increases the apparent whole body oxidation of 18:3n-3 and 18:2n-6.[16] It was further demonstrated that four WCs caused by 100% food restriction for 1 day followed by 3 days of ad libitum refeeding depleted 18:2n-6 and 18:3n-3 in the carcass and adipose tissue of young rats.[17] However, this occurs with significantly changing total body weight of the WC rats compared with the ad libitum feeding control. In addition, these rats were fed a rodent chow diet containing relatively higher 18:2n-6 (30% of total fatty acids or 3.0% of total energy).[17] This led us to consider whether several WCs would still decrease the content of 18:2n-6 and 18:3n-3 of carcass and adipose tissue in adult rats fed a semipurified diet containing relatively lower 18:2n-6 content (7.2% of total fatty acids or 2.1% total energy) without significantly changing the final body weight. The diet used in the present study also contained 6% energy as *trans* fatty acids because intake of these fatty acids is not avoidable in the diet of weight-cycled individuals and could be 0.2 to 11.3% of energy according to our recent estimation.[18] It was, therefore, of interest to study how *trans* fatty acids respond to WC.

Methods and Materials

Animals and diets

Male Wistar rats (452 g, n = 52) were obtained from Charles River Canada (St. Constant, Quebec Canada) and were housed individually in an animal room at 23°C with 12 hr light/dark cycles. They consumed a semipurified diet containing (g/kg): 45 canola oil, 105 partially hydrogenated canola oil, 235 casein, 288 cornstarch, 243 sucrose, 32 Solha-Flock, 35 AIN-76 mineral mix, 10 AIN-76A vitamin mix, 4 choline bitartrate, and 3 DL-methionine. The diet contained 15% fat (30.5% kcal) with 18:2n-6, 18:3n-3, and 18:1 *trans* contributing 2.1, 1.1, and 6.0% to total energy, respectively. The fatty acid composition of diet is shown in *Table 1*.

Food intakes were measured three times weekly, and body weights were measured daily. The rats were stabilized on the diet for 2 weeks. Four rats were then killed as baseline values (initial) before WC. The rest of the rats were randomly divided into two groups: ad libitum fed controls (CTL) and weight-cycled (W-CYC). The CTL rats were allowed free access to the diet and tap water whereas the W-CYC rats were partially energy-restricted (5 g of diet/day, approximately 24% of CTL value) for 3 to 4 days followed by ad libitum refeeding until the average body weight reached that of the CTL. This was repeated for four cycles (*Figure 1*). In brief, WC 1 was induced by a period of 3 days of partial food restriction followed by a period of 4 days of ad libitum refeeding. WC 2 consisted of a period of partial food restriction for 4 days followed by ad libitum refeeding for 10 days. WC 3 was induced by a period of 3 days of partial food restriction followed by a period of 6 days of ad libitum refeeding. WC 4 consisted of a period of partial food restriction for 4 days followed by ad libitum refeeding for 10 days. Five rats from the CTL and W-CYC groups were killed at the end of WC 3, i.e., at the beginning of WC 4. Nine rats from each group were killed at the end of partial food restriction of WC 4. The rest of the rats in each group were killed at the end of WC 4. All rats were euthanized under carbon dioxide and exsanguinated with a syringe from the abdominal aorta. After clotting, serum was separated from whole blood. Liver, perirenal, and epididymal adipose tissue (one pad) were removed, washed with saline, frozen at −20°C, and lipids were extracted within 1 week. Carcass (whole body − perirenal adipose tissue [one pad] − epididymal adipose tissue [one pad] − liver − blood) was also retained for analysis as previously described.[16,17] The study was approved by the Animal Care Committee of Health Canada.

Table 1 Fatty acid composition (wt% of total fatty acids) of diet

14:0	0.1
16:0	4.6
18:0	3.9
16:1n-7	0.2
18:1n-9	52.2
18:1 *trans*	21.2
18:2n-6	7.2
18:2 isomer	3.3
18:3n-3	3.5

Lipid analysis

After tissue homogenization (Polytron, Brinkmann, Rexdale, Ontario Canada), total lipids of liver, adipose tissues, and carcass were extracted using chloroform:methanol (2:1, vol/vol) containing 0.02% butylate hydroxytoluene (Sigma Chemical Co., St. Louis, MO USA) as an antioxidant. For the carcass samples, three

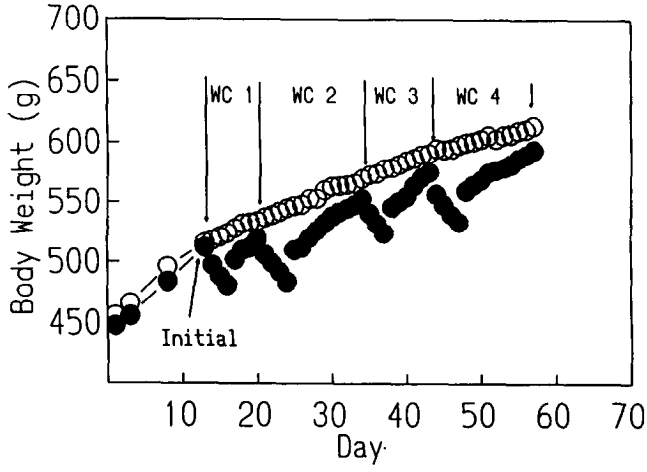

Figure 1 Changes in body weights during weight cycling (WC) in rats. Each value was the mean ± SD of n = 4 to 9 rats. Each WC consisted of a 3 to 4 day food restriction (5.0 ± 0.2 g/day) followed by ad libitum refeeding. The solid circles represent the WC and the open circles represent the controls.

aliquots of homogenized carcass were extracted.[16,17] Triheptadecanoin (Sigma) were added as an internal standard to quantitate total lipids.

The extracted lipids were converted to fatty acid methyl esters (FAME) using 14% BF_3-MeOH reagent (Sigma). FAMEs were analyzed by gas liquid chromatography (GLC) using a SP-2560 flexible fused silica capillary column (100 m × 0.25 mm I.D., 20 μm film thickness; Supelco, Inc. Bellefonte, PA USA) in a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (Palo Alto, CA USA). The column temperature was programmed from 150 to 180°C at a rate of 0.5°C/min and then to 210°C at the rate of 3°C/min. Injector and detector temperatures were set at 250 and 300°C, respectively.

Other assays

Total serum triacylglycerol and serum cholesterol were determined using enzymatic kits (Abbott Laboratories, North Chicago, IL USA) and an ABA-200 Abbott Biochromatic Analyzer (Abbott Laboratories).

Statistics

Data are expressed as mean ± SD. Analysis of variance (ANOVA) followed by a least significance difference test were used for statistical evaluation of the significant difference between the CTL and W-CYC groups.

Results

Body, liver, and adipose tissue weights

The experiment lasted a total of 57 days. The body weight increased from a mean of 456 ± 8 to 610 ± 21 g in the CTL, whereas in the W-CYC rats it increased from 448 ± 25 to 591 ± 36 g (*Figure 1*). The initial and final body weights were not significantly different between the two groups (*Figure 1*). In WC 1, compared with that of the CTL, the weight of W-CYC rats was reduced by 10% during 3 days of partial food restriction and required 4 days of ad libitum refeeding to reach the body weight of CTL group. In WC 2, compared with that of the CTL, the weight of W-CYC rats was decreased by 12% during 4 days of partial food restriction and needed 10 days to reach the value of CTL rats. In WC 3, compared with that of the CTL, the body weight of W-CYC rats was reduced by 10% during 3 days of partial food restriction and took 6 days to reach the value of the CTL group. In WC 4, compared with that of the CTL, the body weight of W-CYC rats was reduced by 10% during 4 days of partial food restriction and took 10 days to reach the body weight of CTL rats.

The final weights of epididymal and perirenal adipose tissue pads were not significantly different between the CTL and W-CYC rats (*Figure 2*). However, the perirenal adipose tissue weighed less in the W-CYC rats than that in the CTL rats at the end of partial food food restriction in WC 4 ($P < 0.01$). Both the CTL and W-CYC rats had similar final liver weights (*Figure 2*). As expected, the liver weight of the W-CYC rats was less than that of the CTL rats at the end of partial food restriction in WC 4 ($P < 0.01$).

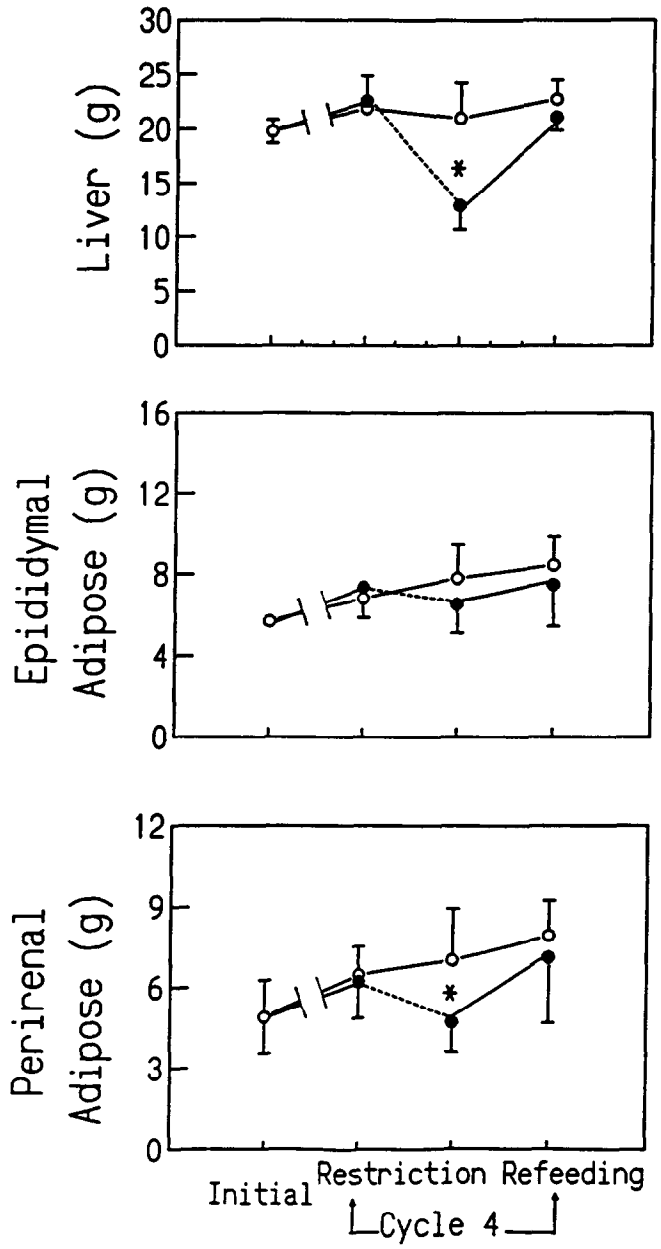

Figure 2 Changes in liver (top panel) and epididymal (middle panel) and perirenal (bottom panel) adipose tissue weights during weight cycling (WC) in rats. Each value was the mean ± SD of n = 4 to 9 rats. Each WC consisted of a 3 to 4 day food restriction (●---●; 5.0 ± 0.2 g/day) followed by ad libitum refeeding (●—●). *$P < 0.01$ versus ad libitum controls (○—○) at the same time point.

Food intake

The time-course food intake pattern is graphically illustrated in *Figure 3*. The food intake of the CTL group during the entire experimental period was maintained at a constant level of 23 ± 2 g/day. The food intake of the W-CYC rats was also maintained at this level prior to the weight cycling process. When the W-CYC rats were given free access to diet following each partial food restriction phase, their food intake sharply increased compared with that of the CTL

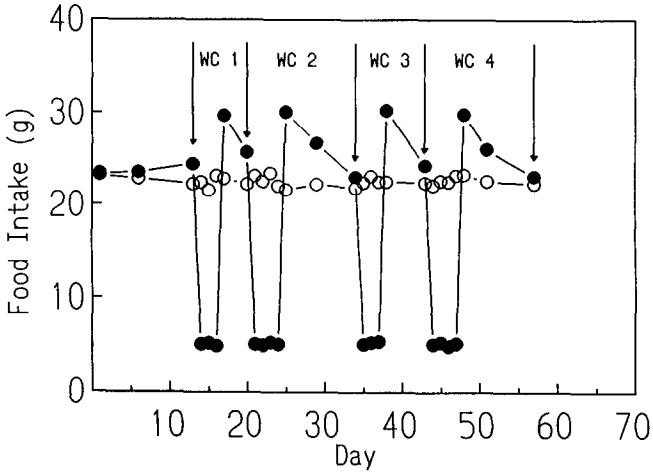

Figure 3 Changes in food intake during the weight cycling (WC) in rats. Each value was the mean ± SD of n = 4 to 9 rats. Each WC consisted of a 3 to 4 day food restriction (5.0 ± 0.2 g/day) followed by ad libitum refeeding. The solid circles represent the WC and the open circles represent the controls.

group, particularly in the first few days ($P < 0.01$; *Figure 3*). Thereafter, the intakes gradually decreased and reached that of the CTL group.

Serum total triacylglycerols and total cholesterol

Serum total triacylglycerols and cholesterol were not significantly different between the CTL and W-CYC rats at the end of WC 4 (*Figure 4*). However, the partial food restriction of WC 4 decreased significantly the serum total cholesterol and triacylglycerols in the W-CYC group compared with the CTL rats ($P < 0.01$; *Figure 4*).

Carcass fatty acids

As the consequence of three WCs (at the beginning of cycle 4), carcass palmitic (16:0) and palmitoleic (16:1n-7) acids were higher whereas arachidonic acid (20:4n-6) were lower in the W-CYC rats than those in the CTL group (*Table 2*). However, a difference in carcass 20:4n-6, 16:0, and 16:1n-7 between the CTL and W-CYC rats disappeared at the end of the partial food restriction and the end of ad libitum refeeding in WC 4. The W-CYC rats had a significantly lower content of 18:2n-6 and total n-6 fatty acids than the CTL group at the beginning, the end of partial food restriction, and the end of ad libitum refeeding in WC 4 (*Table 2*). Similarly, 18:3n-3 and total n-3 fatty acids in the W-CYC rats were lower than in the CTL group at all three time points of WC 4, but they were only significantly different at the end of partial food restriction. Compared with the CTL value, carcass 18:2n-6 in the W-CYC rats was reduced by 13, 12, and 11% at the beginning, the end of partial food restriction, and the end of ad libitum refeeding in WC 4, respectively. Contrary to the CTL value, carcass 18:3n-3 in the W-CYC rats was reduced by 6, 17, and 8% at the beginning, the end of partial food restriction, and the end of ad libitum refeeding in WC 4, respectively. However, the total carcass fatty acid content (mg/g of tissue) did not differ between the W-CYC and CTL rats except at the end of partial food restriction phase in WC 4 ($P < 0.05$; *Table 2*).

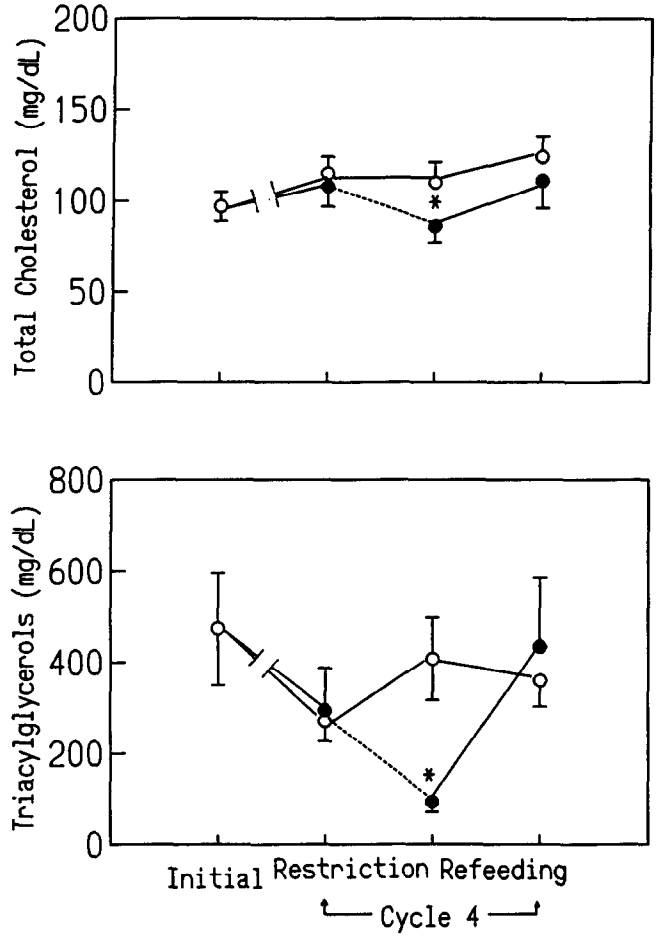

Figure 4 Changes in serum total cholesterol (top panel) and total triacylglycerols (bottom panel) during the weight cycling (WC). Each value was the mean ± SD of n = 4 to 9 rats. Each WC consisted of a 3 to 4 day food restriction (●----●; 5.0 ± 0.2 g/day) followed by ad libitum refeeding (●—●). *$P < 0.01$ versus ad libitum controls (○—○) at same time point.

Adipose tissue fatty acids

The fatty acids in epididymal and perirenal adipose tissue responded similarly to WC. To illustrate this, only data for the epididymal adipose tissue are presented. At the beginning of WC 4, adipose tissue 16:1 *trans* was lower whereas 18:1 *trans* was higher in the W-CYC than in the CTL rats. However, a difference in these two fatty acids between the W-CYC and CTL rats disappeared at the end of WC 4 (*Table 3*). In WC 4, the W-CYC rats had a lower adipose tissue content of 18:2n-6 and total n-6 fatty acids than the CTL rats (*Table 3*). Although 18:3n-3 and total n-3 fatty acids were lower in the W-CYC rats than in the CTL rats but they were only significantly different at the end of partial food restriction phase (*Table 3*). Compared with the CTL value, adipose tissue 18:2n-6 in the W-CYC rats was

Table 2 Effect of weight cycling on fatty acid composition (wt% of total fatty acids) of carcass total lipids

		Cycle 4					
		Beginning		Food restriction		End	
Fatty acids	Initial (n = 4)	CTL (n = 5)	W-CYC (n = 5)	CTL (n = 9)	W-CYC (n = 9)	CTL (n = 10)	W-CYC (n = 10)
C14:0	0.92 (0.06)	0.79 (0.06)	0.79 (0.20)	0.80 (0.09)	0.87 (0.06)	0.82 (0.09)	0.93 (0.12)
C16:0	16.32 (1.09)	13.51 (0.58)	14.58 (0.70)*	13.21 (1.12)	14.16 (0.61)	13.63 (0.77)	14.82 (1.23)*
C16:1 *tr*	0.46 (0.03)	0.76 (0.03)	0.58 (0.03)†	0.76 (0.03)	0.62 (0.04)	0.84 (0.10)	0.68 (0.06)
C16:1n-9	0.52 (0.03)	0.57 (0.04)	0.52 (0.04)	0.59 (0.04)	0.58 (0.03)	0.58 (0.06)	0.58 (0.05)
C16:1n-7	2.66 (0.40)	2.18 (0.31)	2.85 (0.25)†	2.21 (0.38)	2.66 (0.27)	2.42 (0.44)	3.01 (0.39)†
C18:0	4.10 (0.34)	3.71 (0.22)	3.52 (0.14)	3.70 (0.26)	3.61 (0.15)	3.43 (0.27)	3.43 (0.23)
C18:1 *tr*	4.50 (0.55)	6.57 (0.77)	7.23 (0.56)	7.15 (0.45)	7.66 (0.24)	7.77 (1.60)	7.79 (0.79)
C18:1n-9	41.55 (0.53)	48.58 (1.21)	48.20 (0.68)	47.60 (0.71)	47.24 (0.98)	47.38 (1.98)	47.06 (1.81)
C18:1n-7	3.11 (0.12)	2.93 (0.26)	3.02 (0.10)	3.22 (0.15)	3.39 (0.11)	3.37 (0.12)	3.34 (0.25)
C18:2n-6	17.35 (1.05)	11.81 (0.31)	10.26 (0.84)†	12.14 (0.46)	10.71 (1.00)*	11.40 (1.08)	10.10 (0.58)†
C20:4n-6	1.21 (0.30)	0.90 (0.07)	0.74 (0.07)†	0.87 (0.03)	0.84 (0.06)	0.72 (0.11)	0.61 (0.09)*
C22:4n-6	0.13 (0.03)	0.09 (0.01)	0.05 (0.02)	0.06 (0.02)	0.05 (0.02)	0.05 (0.01)	0.05 (0.02)
C22:5n-6	0.04 (0.01)	0.04 (0.02)	0.04 (0.02)	0.04 (0.03)	0.02 (0.02)	0.03 (0.03)	0.03 (0.01)
C18:3n-3	1.89 (0.15)	1.60 (0.12)	1.51 (0.07)	1.58 (0.08)	1.31 (0.13)†	1.43 (0.21)	1.31 (0.12)
C22:5n-3	0.22 (0.17)	0.13 (0.02)	0.11 (0.04)	0.19 (0.12)	0.14 (0.01)	0.14 (0.03)	0.13 (0.02)
C22:6n-3	0.73 (0.17)	0.53 (0.08)	0.46 (0.06)	0.52 (0.05)	0.53 (0.04)	0.45 (0.07)	0.43 (0.05)
Total sat	21.71 (0.79)	18.31 (0.52)	19.20 (0.71)†	18.01 (1.20)	18.98 (0.77)	18.17 (0.93)	19.46 (1.21)
Total mono	49.77 (0.80)	56.71 (0.94)	57.26 (0.76)	55.98 (0.87)	56.58 (1.21)	56.26 (2.02)	56.56 (1.68)
Total *trans*	6.11 (0.70)	9.06 (0.83)	9.63 (0.62)	9.70 (0.53)	9.92 (0.30)	10.55 (1.77)	10.55 (0.88)
Total n-6	19.05 (1.37)	13.10 (0.28)	11.32 (0.85)†	13.38 (0.47)	11.83 (1.05)†	12.44 (1.07)	10.80 (0.60)*
Total n-3	3.00 (0.29)	2.35 (0.15)	2.17 (0.15)	2.37 (0.09)	2.09 (0.16)	2.13 (0.27)	1.94 (0.13)
Total fatty acid (mg/g)	41.03 (6.90)	48.33 (3.50)	46.82 (6.90)	46.28 (4.99)	41.11 (3.61)*	46.24 (3.96)	46.95 (9.37)

Data are expressed as mean ± SD.
*$P < 0.05$; †$P < 0.01$, between the control (CTL) and the weight cycled (W-CYC) animals.

reduced 24, 17, and 14% at the beginning, the end of partial food restriction, and the end of ad libitum refeeding in WC 4, respectively. Contrary to the CTL value, adipose tissue 18:3n-3 in the W-CYC rats was reduced by 13, 16, and 3% at the beginning, the end of partial food restriction, and the end of ad libitum refeeding in WC 4, respectively (*Table 3*).

Liver fatty acids

Total liver fatty acid content (mg/g of liver) did not differ between the W-CYC and the CTL rats at the end of WC 4 although livers in the W-CYC group weighed less than in the CTL rats at the end of partial food restriction phase (*Table 4* and *Figure 2*). Compared with those in the CTL rats, the partial food restriction modified appreciably the profile of liver fatty acids in the W-CYC group; notably, the proportions of 16:1 *trans*, 16:1n-7, 16:1n-9, and oleic acid (18:1n-9) decreased whereas the proportions of 18:2n-6, 20:4n-6, and 18:3n-3 increased (*Table 4*). After the ad libitum refeeding phase (end of WC 4), all the individual liver fatty acids in the W-CYC rats returned to the values of CTL group, but the liver total n-6 and n-3 fatty acids still remained at a significantly higher level in the W-CYC rats than in the CTL at the end of WC 4.

Discussion

The most notable observation in the present study was a 14 to 24% reduction of 18:2n-6 in adipose tissue and a 11 to 13% reduction of this fatty acid (equivalent to 0.2 to 0.3 g/rat) in the carcass of W-CYC rats after three or four WCs compared with the CTL rats. The WC-induced change in adipose tissue and carcass contents of 18:2n-6 was relatively small but it occurred with the final body weight of W-CYC rats similar to that of CTL group. In a recent review, Prentice et al.[4] stated that WC does not alter body fatty acid composition in animals and humans based on their own and other evidence from the literature. This is more pertinent to total fat mass than to total lean body mass. To our knowledge, the present study is the first report that demonstrates that WC changes whole body fatty acid composition without changing total body fat and total body weight in the rats. Based on the results of our previous study using young rats in which 3.1% dietary energy (30% total fatty acids) was derived from 18:2n-6[17] together with the present study in which 18:2n-6 contributed 2.2% to total energy (7.2% total fatty acids), it appears that WCs selectively reduce the 18:2n-6 content in carcass and adipose tissue.

No data are yet available to show how 18:2n-6 in human adipose tissue and in whole body responds to weight cycling. However, an inverse correlation between the proportion of 18:2n-6 in human adipose tissue and risk of the coronary heart disease have been shown in the Edinburgh-Stockholm and Finland studies.[12–15] If WC in humans is associated with a significant increase in cardiovascular risk,[1–3] part of the possible mechanism may involve selective depletion of 18:2n-6 from adipose tissue and the subsequent changes in the efficacy of insulin action resulting

Table 3 Effect of weight cycling on fatty acid composition (wt% of total fatty acids) of epididymal adipose tissue

		Cycle 4					
		Beginning		Food restriction		End	
Fatty acids	Initial (n = 4)	CTL (n = 5)	W-CYC (n = 5)	CTL (n = 9)	W-CYC (n = 9)	CTL (n = 10)	W-CYC (n = 10)
C14:0	0.85 (0.43)	0.85 (0.09)	0.87 (0.06)	0.83 (0.12)	0.85 (0.09)	0.81 (0.13)	0.83 (0.09)
C16:0	16.61 (1.24)	13.96 (0.42)	13.94 (0.91)	14.05 (1.32)	13.84 (0.96)	13.44 (1.10)	13.96 (1.18)
C16:1 *tr*	0.39 (0.06)	0.70 (0.03)	0.60 (0.06)†	0.75 (0.08)	0.64 (0.07)	0.79 (0.08)	0.68 (0.09)
C16:1n-9	0.62 (0.03)	0.64 (0.03)	0.58 (0.04)†	0.57 (0.15)	0.63 (0.05)	0.60 (0.05)	0.57 (0.05)
C16:1n-7	3.12 (0.63)	2.50 (0.69)	2.36 (0.67)	2.08 (0.46)	2.04 (0.48)	2.26 (0.63)	1.85 (0.54)
18:0	3.36 (0.12)	3.07 (0.30)	3.11 (0.22)	3.22 (0.14)	3.30 (0.21)	3.30 (0.23)	3.27 (0.16)
C18:1 *tr*	3.12 (0.94)	5.94 (0.40)	7.45 (0.85)†	6.40 (0.99)	7.08 (0.89)	7.18 (0.72)	7.66 (0.60)
C18:1n-9	37.41 (0.21)	44.65 (2.80)	47.78 (2.34)	45.88 (3.86)	48.53 (1.95)	46.91 (3.21)	48.50 (1.56)
C18:1n-7	0.47 (0.13)	3.25 (0.27)	3.26 (0.16)	3.07 (0.19)	3.14 (0.27)	3.33 (0.27)	3.12 (0.17)
C18:2n-6	22.23 (1.96)	16.14 (1.74)	12.28 (1.76)†	15.50 (1.63)	12.81 (1.35)*	13.74 (1.43)	11.78 (0.94)†
C20:4n-6	0.66 (0.18)	0.47 (0.21)	0.21 (0.13)	0.34 (0.24)	0.14 (0.03)†	0.24 (0.17)	0.14 (0.02)
C22:4n-6	0.13 (0.04)	0.10 (0.05)	0.04 (0.04)	0.06 (0.06)	0.03 (0.01)	0.04 (0.04)	0.02 (0.01)
C22:5n-6	0.06 (0.03)	0.05 (0.04)	0.03 (0.03)	0.02 (0.03)	0.01 (0.01)	0.03 (0.04)	0.01 (0.01)
C18:3n-3	2.13 (0.16)	1.82 (0.21)	1.59 (0.14)	1.81 (0.25)	1.52 (0.14)*	1.55 (0.51)	1.51 (0.11)
C22:5n-3	0.28 (0.09)	0.19 (0.12)	0.08 (0.06)	0.15 (0.13)	0.06 (0.03)	0.14 (0.15)	0.08 (0.03)
C22:6n-3	0.57 (0.21)	0.41 (0.25)	0.17 (0.17)	0.28 (0.26)	0.10 (0.04)	0.27 (0.30)	0.12 (0.04)
Total sat	22.40 (1.62)	18.16 (0.47)	18.20 (0.85)	18.40 (1.51)	18.29 (0.94)	17.51 (1.22)	18.32 (1.24)
Total mono	46.13 (2.04)	53.38 (2.10)	56.65 (1.83)*	53.93 (3.67)	56.77 (1.81)*	55.57 (2.53)	56.62 (1.40)
Total *trans*	4.45 (1.20)	8.10 (0.49)	9.71 (0.97)	8.71 (1.27)	9.31 (1.02)	9.78 (0.86)	10.24 (0.70)
Total n-6	23.16 (2.12)	16.95 (1.98)	12.57 (1.94)†	15.82 (1.83)	13.14 (1.37)†	14.46 (1.47)	12.12 (0.99)†
Total n-3	3.20 (0.35)	2.56 (0.62)	1.89 (0.35)	2.34 (0.66)	1.72 (0.22)*	2.07 (0.78)	1.77 (0.16)

Data are expressed as mean ± SD.
*$P < 0.05$; †$P < 0.01$, between the control (CTL) and the weight cycled (W-CYC) animals.

from remodeling of the adipose tissue and muscle membrane composition.[19,20]

Compared with that in the CTL rats, carcass 18:3n-3 decreased in the W-CYC rats by 6 to 8% at the beginning and the end of ad libitum refeeding but this reduction in 18:3n-3 was maximum (by 17%) at the end of partial food restriction in WC 4, suggesting that energy restriction may accelerate oxidation of 18:3n-3 (*Table 2*). In obese humans, weight loss induced by dieting was accompanied by a small but specific decrease in 18:3n-3 in adipose tissue.[9,11] Preferential reduction in adipose tissue 18:3n-3 during very low caloric dieting was independent from moderate levels of supplementation with this fatty acid.[10] There are no data yet available to show whether this reduction in human adipose tissue 18:3n-3 is reversible during weight regain following weight reduction as demonstrated in rats.

Our data suggest that 18:2n-6 and 18:3n-3 respond to WC differently. Compared with the CTL value, 18:2n-6 was reduced by 11% in the carcasses of the W-CYC rats whereas 18:3n-3 was decreased by 8% during the ad libitum refeeding in WC 4. However, the partial food restriction in WC 4 accelerated reduction in carcass 18:3n-3 (17%) but it did not affect the reduction in carcass 18:2n-6 (12%). This phenomenon was also demonstrated in our previous study.[17] Because 18:3n-3 usually accounts for <0.8% of total adipose tissue fatty acids in humans and <2.0% in rodents, it appears that the major fatty acids are probably oxidized in proportion to their composition in adipose tissue and carcass but the net mobilization of 18:3n-3 may be disproportionably high during food restriction.

Our result was in agreement with that of Leyton et al.[21] who showed that 18:3n-3 was oxidized preferentially to 18:2n-6. In fact, 18:2n-6 and 18:3n-3 are known to have a higher susceptibility to oxidation than other fatty acids.[4,19] In the present study, 18:2n-6 contributed 2.1% to the total energy intake (7.2% total fatty acids) and its concentration in carcass total lipids accounted for 11.4% of total fatty acids in the CTL rats and 10.1% in the W-CYC rats (*Table 2*). In contrast, 18:3n-3 contributed 1.1% to the total energy intake (half of that for 18:2n-6) but its incorporation in carcass total lipids (CTL, 1.4%; W-CYC, 1.3%) was only one ninth of that for 18:2n-6 (*Table 2*). In addition, these two fatty acids were not diverted more toward chain-elongation and desaturation since the content of 20:4n-6 and docosahexaenoic acid (22:6n-3) in liver, adipose tissues, and carcass did not increase throughout the entire experiment in either the CTL or the W-CYC rats (*Table 2*). This suggests that 18:3n-3 is more susceptible to oxidation than 18:2n-6 in both the W-CYC and CTL rats.

In a separate study, we used the whole body technique to assess the apparent oxidation of 18:2n-6 and 18:3n-3 in the ad libitum feeding controls and WC rats for 4 cycles induced by 1 day of fasting followed by 3 days of ad libitum refeeding (apparent oxidation = {intake − [body content at the end of study − body content at the beginning of study] − fecal excretion}/intake). In the controls, 66% of total dietary 18:2n-6 but 78% of total dietary 18:3n-3 was apparently oxidized. In the WC rats, the apparent oxidation of total dietary 18:2n-6 increased to 82% whereas that of total dietary 18:3n-3 increased to 88% (Chen et al., *Brit. J. Nutr.*, in press). To simplify this, the effect of WC on the apparent oxidation of these two fatty acids was characterized by: 18:3n-3 was oxidized preferentially to 18:2n-6 in the ad libitum feeding controls or weight-cycled rats; the

Table 4 Effect of weight cycling on fatty acid composition (wt% of total fatty acids) of liver total lipids

		Cycle 4					
		Beginning		Food restriction		End	
Fatty acids	Initial (n = 4)	CTL (n = 5)	W-CYC (n = 5)	CTL (n = 9)	W-CYC (n = 9)	CTL (n = 10)	W-CYC (n = 10)
C14:0	0.99 (0.15)	0.97 (0.08)	1.06 (0.19)	1.07 (0.09)	0.72 (0.09)†	1.12 (0.07)	1.24 (0.14)
C16:0	17.46 (2.75)	15.94 (1.90)	18.06 (3.18)	16.73 (1.47)	16.36 (0.67)	17.67 (1.84)	15.97 (1.49)
C16:1 *tr*	1.23 (0.16)	1.18 (0.09)	1.09 (0.14)	1.33 (0.14)	0.62 (0.12)†	1.43 (0.24)	1.17 (0.24)
C16:1n-9	0.70 (0.08)	0.70 (0.05)	0.67 (0.06)	0.76 (0.06)	0.35 (0.07)†	0.84 (0.15)	0.68 (0.13)
C16:1n-7	1.71 (0.64)	1.36 (0.43)	2.10 (0.80)	1.57 (0.41)	0.75 (0.20)†	1.86 (0.43)	2.01 (0.67)
18:0	10.82 (0.79)	10.68 (1.67)	9.32 (0.94)	9.64 (1.03)	11.82 (2.21)	7.87 (1.09)	9.37 (1.02)
C18:1 *tr*	5.51 (1.03)	5.88 (0.88)	5.42 (1.86)	5.90 (0.97)	4.90 (0.34)	5.32 (0.72)	6.41 (0.78)
C18:1n-9	27.70 (1.36)	28.92 (2.78)	31.28 (1.86)	30.71 (3.07)	23.46 (4.17)†	33.77 (2.96)	29.82 (3.02)
C18:1n-7	2.85 (0.36)	2.79 (0.40)	2.97 (0.28)	2.94 (0.27)	2.03 (0.10)†	2.89 (0.71)	2.76 (0.75)
C18:2n-6	7.32 (0.72)	7.14 (0.30)	6.79 (1.19)	7.38 (0.79)	12.37 (0.84)†	7.47 (0.53)	7.78 (0.61)
C20:4n-6	12.53 (1.01)	12.09 (1.50)	9.20 (1.30)†	10.66 (0.24)	14.61 (2.75)†	9.43 (1.51)	10.31 (1.30)
C22:4n-6	0.17 (0.03)	0.15 (0.02)	0.12 (0.02)	0.12 (0.04)	0.18 (0.02)*	0.11 (0.01)	0.11 (0.02)
C22:5n-6	0.08 (0.01)	0.08 (0.03)	0.13 (0.07)	0.06 (0.02)	0.13 (0.12)	0.06 (0.02)	0.07 (0.02)
C18:3n-3	0.41 (0.06)	0.40 (0.06)	0.44 (0.14)	0.44 (0.07)	0.88 (0.22)†	0.49 (0.06)	0.50 (0.11)
C22:5n-3	0.48 (0.03)	0.45 (0.06)	0.35 (0.10)	0.43 (0.06)	0.59 (0.08)	0.41 (0.06)	0.48 (0.09)
C22:6n-3	5.48 (0.36)	5.54 (0.94)	4.85 (0.74)	4.83 (0.89)	5.56 (0.92)	4.17 (0.84)	5.51 (1.04)
Total sat	29.11 (2.52)	27.79 (1.61)	28.64 (2.98)	27.64 (1.51)	29.08 (1.89)	26.87 (1.31)	26.77 (1.71)
Total mono	34.56 (1.71)	35.62 (3.12)	39.04 (1.84)	37.96 (3.18)	28.26 (4.43)†	41.15 (3.02)	37.28 (3.02)
Total *trans*	7.86 (1.21)	8.42 (0.97)	7.80 (1.03)	8.76 (1.14)	6.54 (0.45)†	8.38 (0.68)	9.26 (0.87)
Total n-6	20.66 (1.82)	20.25 (1.51)	16.99 (2.06)†	18.92 (1.99)	27.95 (2.54)†	17.68 (1.99)	19.11 (1.49)†
Total n-3	6.71 (0.35)	6.74 (1.00)	5.92 (0.86)	6.06 (1.00)	7.62 (0.96)†	5.32 (0.92)	6.84 (1.08)†
Total fatty acids (mg/g)	31.15 (2.49)	29.14 (3.61)	33.07 (3.56)	27.27 (3.70)	29.24 (5.80)	31.78 (5.09)	26.73 (2.93)

Data are expressed as mean ± SD.
*$P < 0.05$; $P < 0.01$, between the control (CTL) and the weight cycled (W-CYC) animals.

WC accelerated oxidation of 18:2n-6 and 18:3n-3 but it acted more effectively for the former.

The present study supported our previous observation[13] and showed that WC tended to increase saturates and monounsaturates in carcass lipids. It is possible that 18:2n-6 and 18:3n-3 are preferentially released from triacylglycerols of adipose tissue and carcass during partial food restriction and subsequently oxidized in muscle mitochondria.[22–24] During refeeding, the lipogenesis of saturated and monounsaturated fatty acids is probably stimulated in liver and contributes to the higher concentration of 16:0, 16:1n-7, and 18:1n-9, and consequently this results in lower proportions of 18:2n-6 and 18:3n-3 in serum.[17] In fact, the rates of 16:0 and 16:1n-7 synthesis increased by 2 and by 8 fold, respectively, in rats fed a fat-free diet for 23 hr after a 48 hr fasting.[25] Thus, a smaller proportion of 18:2n-6 or 18:3n-3 may be diverted to triacylglycerol synthesis in adipose tissue and carcass compared with saturates and monounsaturates during refeeding.

In summary, we observed a consistent decline in 18:2n-6 of adipose and carcass in young[17] and adult rats associated with WC induced by 100% food restriction or 76% food restriction followed by ad libitum refeeding. Selective depletion of this fatty acid from carcass and adipose tissue in the weight-cycled rats is probably due to several possibilities including greater mobilization, less re-esterification, or preferential oxidation. Hence, it is of interest to know whether in humans undergoing the inevitable weight regain after prolonged intake of low caloric diets there is evidence of depletion of 18:2n-6 from adipose tissue.

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