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Accumulation of polyunsaturates is decreased by weight-cycling: whole-body analysis in young, growing rats

BY ZHEN-YU CHEN*, CHANTALE R. MENARD AND STEPHEN C. CUNNANE†

Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 1A8, Canada

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Whole-body fatty acid analysis in rats has previously shown that 50–70% of dietary linoleate and α-linolenate is β-oxidized to CO_2 and that this value increases with refeeding after a single episode of fasting. Our hypothesis was that repeated fasting–refeeding or weight-cycling would increase the β-oxidation of linoleate and α-linolenate thereby depleting their whole-body levels. In rats consuming 3% energy as linoleate and 0·15% energy as α-linolenate during a 16 d balance period, 19% of the linoleate consumed accumulated in weight-cycled rats compared with 34% in the free-fed controls ($P < 0·01$). Similarly, 11% of the α-linolenate consumed accumulated in the weight-cycled rats compared with 22% in the controls ($P < 0·01$). Arachidonate and docosahexaenoate also accumulated to lower extents in the weight-cycled rats than in the controls. In contrast, whole-body accumulation of palmitate, stearate and oleate was not different between the weight-cycled group and the controls when measured as a proportion of intake or relative to weight gain. Thus, whole-body depletion of linoleate and α-linolenate did not occur *per se* but the partitioning of linoleate and α-linolenate was significantly altered by weight-cycling resulting in lower whole-body accumulation and higher apparent oxidation of all polyunsaturates especially linoleate and α-linolenate.

Fasting: Refeeding: β-Oxidation: Linoleate: α-Linolenate

Despite several decades of research on the metabolism of linoleate (18:2*n*-6) and α-linolenate (18:3*n*-3) and a number of *in vivo* (Lynn & Brown, 1959; Leyton *et al.* 1987) and *in vitro* (Bjorntorp, 1968; Gavino & Gavino, 1991) studies showing that labelled linoleate and α-linolenate are readily β-oxidized to CO_2, knowledge of the influence of nutritional status on their partitioning between accumulation and β-oxidation has only recently begun to emerge. Useful quantitative information on the utilization of linoleate and α-linolenate has come from whole-body fatty acid balance analysis because this approach accounts for the total intake, accumulation and excretion of polyunsaturates. Since linoleate and α-linolenate cannot be synthesized by mammals, this method allows fatty acid disappearance (apparent oxidation) to be determined as the difference between intake and accumulation plus excretion (Yang *et al.* 1994). Using whole-body fatty acid balance analysis in pregnant rats we have shown previously that during a 5 d period of refeeding after a single 48 h episode of fasting, only saturates and monounsaturates show net whole-body accumulation; the total dietary intake of linoleate and α-linolenate consumed during the refeeding period completely disappears, i.e. is apparently oxidized (Chen & Cunnane, 1993).

We hypothesized that if rats fail to accumulate linoleate and α-linolenate during one

* Present address: Department of Biochemistry, Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

† For reprints.

cycle of fasting–refeeding, repeated cycles of fasting and refeeding, e.g. weight-cycling, would exacerbate the refeeding-induced increase in oxidation of linoleate and α-linolenate and could potentially deplete whole-body stores of these two essential nutrients. Whole-body accumulation relative to intake was calculated by difference after quantifying (1) fatty acid composition of the diet, (2) daily feed intake, (3) whole-body fatty acid content at the beginning and the end of the study period, and (4) fatty acid output in the faeces. Since β-oxidation was not determined *per se*, the calculated value of whole-body disappearance of linoleate and α-linolenate more accurately represents their 'apparent' oxidation (Chen & Cunnane, 1993).

Details concerning weight gain, serum hormone and fatty acid profiles, and fatty acid concentration in individual organs have been reported from this study (Chen *et al.* 1995); the present report focuses entirely on the intake, whole-body accumulation and apparent oxidation of polyunsaturates, especially linoleate and α-linolenate.

EXPERIMENTAL METHODS

Animals and diets

All procedures were approved by the University of Toronto Animal Care Committee. Male Sprague-Dawley rats were housed individually in stainless steel wire-bottomed cages. They all consumed tap water and a pelleted, non-purified, rodent chow diet (Ralston-Purina, St Louis, MO, USA). At the beginning of the study the rats selected for weight-cycling (W-CYC) were chosen at random and underwent 24 h complete fasting followed by *ad lib.* refeeding for 3 d. This procedure was repeated on four consecutive occasions (weight cycles) over a total of 16 d. The control (CTL) group was allowed continuous free access to the chow.

The rodent chow contained 50 g fat/kg of the following fatty acid composition (g/kg fat): palmitate (16:0) 204, palmitoleate (16:1*n*-7) 24, stearate (18:0) 79, oleate (18:1*n*-9) 292, linoleate 306, arachidonate (20:4*n*-6) 3, α-linolenate 29, eicosapentaenoate (20:5*n*-3) 12, and docosahexaenoate (22:6*n*-3) 11. Body weight, feed intake and faeces output were determined daily. For slaughter, rats from both groups were chosen at random, anaesthetized with CO_2 and exsanguinated, at three time points: the start of the study, after 24 h fasting, and after refeeding for 3 d, in each of the four weight cycles. Blood was collected and the serum was separated. Liver, perirenal and epididymal adipose tissues were removed, washed with saline, and frozen at −20°. Carcass (whole body − (liver + adipose tissue + blood)) was also retained for analysis.

Lipid extraction and fatty acid analysis

Total lipids were extracted within 2 d of obtaining the tissues. Fatty acid composition was analysed by capillary GLC after conversion of the extracted lipids to their methyl esters using BF_3 in methanol. Triheptadecanoin was added as an internal standard to a portion of each total lipid extract to quantitate total fatty acids as previously described (Chen & Cunnane, 1992). For the carcass samples, three portions of homogenized carcass were extracted. The fatty acid value for each carcass sample was averaged from the three portions. Whole-body content of individual fatty acids was calculated from the values in liver, serum, adipose tissue and carcass. To determine fatty acid accumulation, animals were paired on a weight basis at the beginning of the balance period; one of the pair was killed at the start of the balance period and the other of the same pair was killed at the end of the balance period. Accumulation of each fatty acid over the 16 d balance period was determined by the difference in whole-body fatty acid content between the two paired animals.

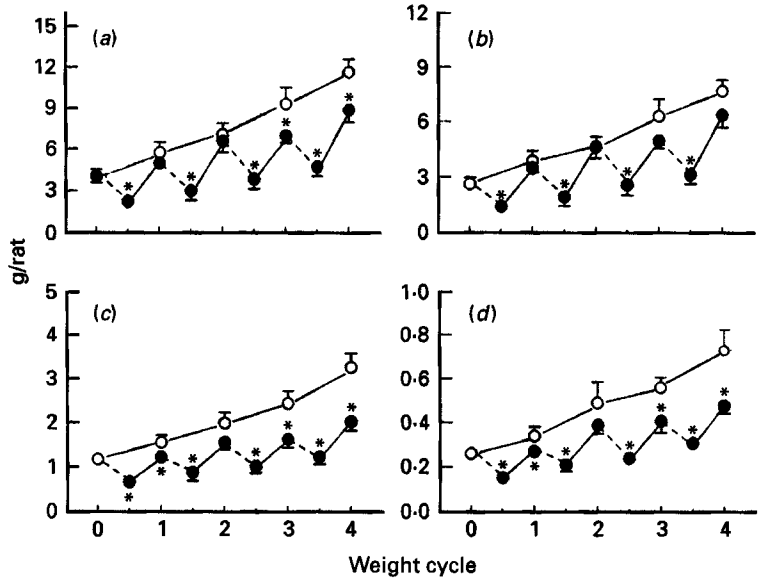

Fig. 1. Changes in total whole-body fatty acid content (g/rat) during weight-cycling in rats. Each weight cycle consisted of 24 h fasting (●——●) followed by 3 d refeeding (●——●) and is compared with free-feeding (○——○). Values are means for four or five rats, with standard deviations represented by vertical bars. (a), Total fatty acids; (b), saturated+monounsaturated fatty acids (14:0+14:1+16:0+16:1*n*-7+18:0+18:1*n*-9+18:1*n*-7); (c), total *n*-6 polyunsaturated fatty acids (18:2*n*-6+20:3*n*-6+20:4*n*-6+22:4*n*-6+22:5*n*-6); (d), total *n*-3 polyunsaturated fatty acids (18:3*n*-3+20:5*n*-3+22:5*n*-3+22:6*n*-3). *Mean values were significantly different from control, $P < 0.01$. For details of procedures, see pp. 584–585.

Statistics

All data are expressed as means and standard deviations. ANOVA followed by Student's *t* test (two tailed) was used for statistical evaluation.

RESULTS

Feed intake and body-weight gain

Cumulative feed intake in the W-CYC group was 24% lower than in the CTL group. Body weight was initially the same in both groups (105 (SD 8) g) and remained similar during the first two weight cycles. Weight-cycling reduced body-weight gain during the third and fourth weight cycles so that over the study period weight gain in the W-CYC group was 24% lower than in the CTL group (+98 (SD 4) g in CTL *v.* +74 (SD 6) g in the W-CYC group; Chen *et al.* 1995). Serum β-hydroxybutyrate and free fatty acid concentrations increased significantly during each fasting cycle but returned to control levels during refeeding. In the W-CYC group, serum insulin and triacylglycerol concentrations decreased during fasting but also returned to control values during refeeding (Chen *et al.* 1995).

Fatty acid accumulation

A full account of the changes in percentage fatty acid composition in serum, liver, adipose tissue and remaining carcass has been reported previously (Chen *et al.* 1995). Whole-body fatty acid content (g/whole body) decreased significantly during each fasting period but returned to control levels during refeeding in the first and second but not the final two weight cycles (Fig. 1). In the CTL group, final whole-body total fatty acid accumulation

Table 1. *Whole-body intake, content and accumulation of major fatty acids in weight-cycled (W-CYC) and control (CTL) rats*†

(Mean values and standard deviations for four or five rats per group)

		Intake (mg)		Whole-body content (mg) Day 1		Whole-body content (mg) Day 16		Accumulation‡ (%)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total fatty acids	CTL	16537	1239	4050	463	11690	961	46	5
	W-CYC	12011	937			8906	910	40	4
Sum SFA+MUFA§	CTL	10528	791	2616	335	7710	610	48	5
	W-CYC	7646	597			6399	676	49	5
16:0	CTL	3350	252	887	110	2601	199	51	4
	W-CYC	2441	191			2170	259	53	11
18:0	CTL	1296	97	313	17	884	98	44	7
	W-CYC	941	74			674	71	38	6
18:1*n*-9	CTL	4823	362	922	121	3028	259	44	4
	W-CYC	3504	274			2581	252	50	4
Sum *n*-6 PUFA‖	CTL	5115	383	1174	128	3258	325	41	5
	W-CYC	3716	290			2025	325	23**	2
18:2*n*-6	CTL	5055	380	968	117	2685	252	34	3
	W-CYC	3672	287			1666	160	19**	3
20:4*n*-6	CTL	50	4	163	14	454	87	582	146
	W-CTC	37	3			290	39	343*	79
Sum *n*-3 PUFA¶	CTL	894	67	260	25	722	171	52	9
	W-CYC	649	51			482	34	34*	3
18:3*n*-3	CTL	483	36	56	9	164	15	22	2
	W-CYC	351	27			96	8	11**	1
22:6*n*-3	CTL	178	13	135	15	367	65	130	29
	W-CYC	129	10			248	14	88*	8

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Mean values were significantly different from those for controls: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

† Each of a total of four weight cycles consisted of 24 h fasting followed by 72 h refeeding to satiety.

‡ (Day 16 − day 1)/intake × 100.

§ (14:0+14:1+16:0+16:1*n*-7+18:0+18:1*n*-9+18:1*n*-7).

‖ (18:2*n*-6+20:3*n*-6+20:4*n*-6+22:4*n*-6+22:5*n*-6).

¶ (18:3*n*-3+20:5*n*-3+22:5*n*-3+22:6*n*-3).

after the 16 d balance period was 7640 mg, of which 67% was saturates plus monounsaturates, 27% was *n*-6 polyunsaturates and 6% was *n*-3 polyunsaturates. In contrast, final whole-body total fatty acid accumulation in the W-CYC rats was 4856 mg, of which 78% was saturates plus monounsaturates, 18% was *n*-6 polyunsaturates and 4% was *n*-3 polyunsaturates (Table 1). Thus, after the four weight cycles, total whole-body fatty acid accumulation was 36% less than during free feeding and shifted to a higher proportion of saturates and monounsaturates while the proportion of polyunsaturates was decreased.

Expressed as mg fatty acid accumulated/g body weight gained during the study period, W-CYC rats gained 1% less total saturated and monounsaturated fatty acids (NS) but 46% less total *n*-6 polyunsaturates and 55% less total *n*-3 polyunsaturates (both $P < 0{\cdot}01$ *v.* CTL). Thus, irrespective of the slower body-weight gain in the W-CYC rats, accumulation of total polyunsaturates was significantly less in this group. During refeeding of the W-CYC rats, only palmitate and oleate returned to the control values; the whole-

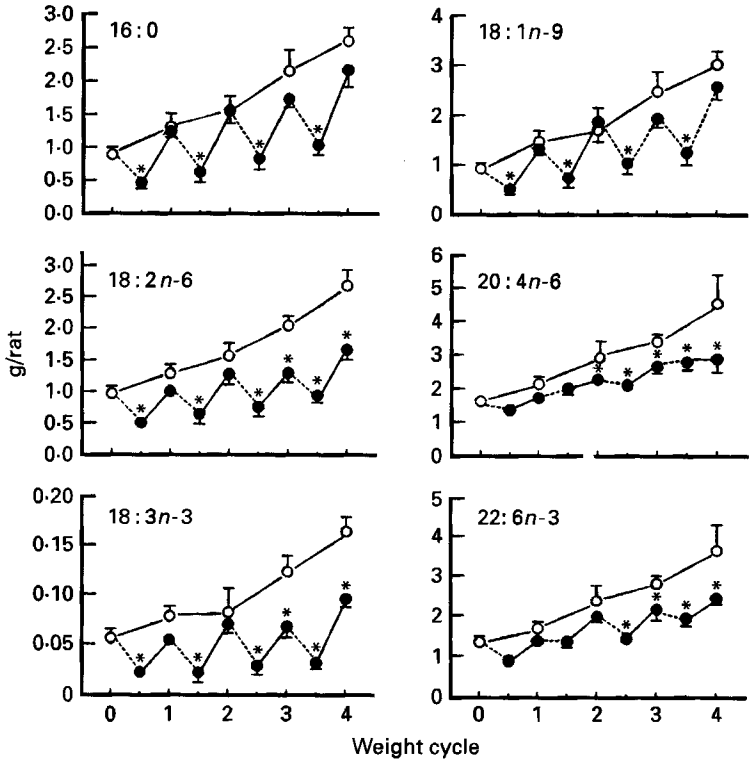

Fig. 2. Changes in whole-body content of individual long-chain fatty acids during weight-cycling in rats caused by 24 h fasting (●---●) followed by 3 d refeeding (●——●) compared with free-feeding (○——○). *Mean values were significantly different from control, $P < 0.01$. For details of procedures, see pp. 584–585.

body content of linoleate, α-linolenate, arachidonate and docosahexaenoate was significantly less in the W-CYC rats than in the CTL rats during the third and fourth weight cycles (Fig. 2).

Total whole-body fatty acid accumulation as a proportion of dietary fat intake was 46% in the CTL and 40% in the W-CYC rats (not significantly different; Table 1). Accumulation of total saturates and monounsaturates as a proportion of their dietary intake was the same in both groups (48–49%). However, accumulation of total *n*-6 and *n*-3 polyunsaturates was 23% and 34% of intake respectively in the W-CYC rats, compared with 41% and 52% respectively in the CTL rats ($P < 0.05$). Relative to intake, linoleate (19%) and α-linolenate (11%) were accumulated the least in the W-CYC rats compared with the CTL rats (34% and 22% respectively, $P < 0.01$). The accumulation of arachidonate was also reduced in the W-CYC rats but was still greater than intake indicating that net synthesis of arachidonate continued but at a lower rate than in the CTL group. However, accumulation of docosahexaenoate in the W-CYC rats was 12% less than intake indicating that there was no net synthesis of docosahexaenoate in the W-CYC rats.

Apparent oxidation

Apparent oxidation or net disappearance was calculated using the equation: disappearance = (intake − (accumulation + excretion)). For both families of polyunsaturates, accumulation values for the sum of all members of each family were also determined (see Table 1). Faecal excretion of the main dietary fatty acids (palmitate, oleate, linoleate) was included

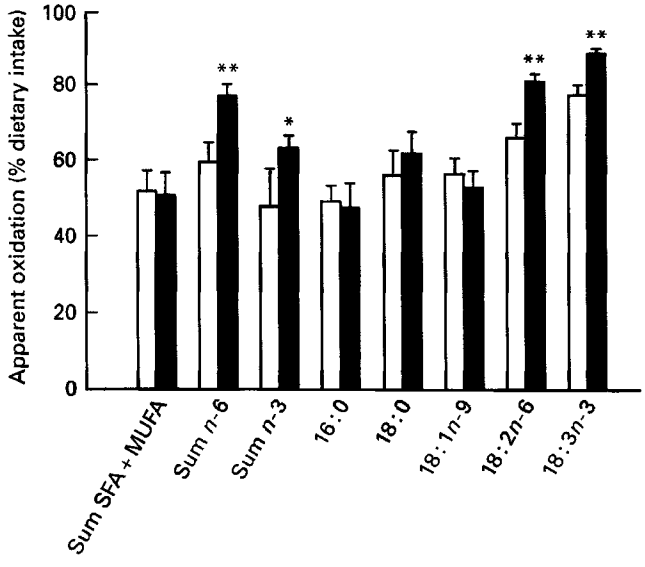

Fig. 3. Changes in apparent oxidation (disappearance) of long-chain fatty acids during weight-cycling in rats caused by 24 h fasting followed by 3 d refeeding (■) compared with free-feeding (□). Values are means for four or five rats, with their standard deviations represented by vertical bars. Mean values were significantly different from controls: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$. Sum of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), 14:0+14:1+16:0+16:1*n*-7+18:0+18:1*n*-9+18:1*n*-7; sum *n*-6, 18:2*n*-6+20:3*n*-6+20:4*n*-6+22:4*n*-6+22:5*n*-6; sum *n*-3, 18:3*n*-3+20:5*n*-3+22:5*n*-3+22:6*n*-3. For details of procedures, see pp. 584–585.

in the disappearance data and accounted for < 2% of fatty acid disappearance over the balance period. Relative to intake, net whole-body disappearance of total saturates plus monounsaturates combined was not significantly affected by weight-cycling during the study period (Fig. 3). In contrast, disappearance of the sum of *n*-6 or *n*-3 polyunsaturates in the W-CYC rats was significantly increased compared with the CTL rats. Among individual dietary fatty acids, greater disappearance occurred for both linoleate and α-linolenate in the W-CYC rats than in the CTL rats. However, no difference was observed for net disappearance of palmitate, stearate or oleate between the two groups (Fig. 3).

Since β-oxidation must be at least a partial function of body-weight gain, the impact of weight-cycling on apparent oxidation relative to weight gain over the balance period was also determined. Even when expressed relative to weight gain, apparent oxidation of linoleate, α-linolenate or the sum of *n*-6 or *n*-3 polyunsaturates was greater in the W-CYC than in the CTL group.

DISCUSSION

The objective of the present study was to establish whether weight-cycling in the rat disrupts whole-body partitioning of linoleate and α-linolenate between accumulation and disappearance (apparent oxidation). Comparison of whole-body energy content with energy intake provides a valid measure of energy balance in laboratory rats (Rothwell & Stock, 1982). Calculation of apparent oxidation from fatty acid balance data yields similar results to tracer data for β-oxidation of fatty acids which cannot be synthesized (linoleate and α-linolenate) and therefore also appears to be a valid measure of their whole-body partitioning between β-oxidation and tissue accumulation (Chen & Cunnane, 1993; Cunnane *et al.* 1993; Yang *et al.* 1994). For fatty acids such as palmitate, which was consumed in the diet but can also be synthesized *de novo*, this balance methodology has

limited applications because it can only assess net changes in the content of a fatty acid and not actual β-oxidation or synthesis *per se*.

In the present study our primary observation was that, in comparison with free feeding, weight-cycling decreased whole-body accumulation of linoleate and α-linolenate, and significantly increased their apparent oxidation relative to dietary intake. The lower quantitative accumulation of linoleate and α-linolenate during weight cycling reflected a proportional decrease in the main pool of these fatty acids (mainly in adipose tissue but also in the carcass) with each repeated weight cycle (Chen *et al.* 1995). We have also previously commented on the fact that although accumulation of both linoleate and α-linolenate was significantly reduced by weight-cycling, the reduction in linoleate occurred during the fasting phase with slight recovery during the refeeding phase of each weight cycle, whereas the opposite occurred for α-linolenate, i.e. net loss during refeeding but partial recovery during fasting (Chen *et al.* 1995). An explanation for this difference in the effects of weight-cycling on the utilization of linoleate and α-linolenate is not apparent at the moment.

Since the rats in the present study were still growing, the reduced concentration of polyunsaturates in the W-CYC rats was partly offset by the slower change in body weight and total fatty acid pool size. Hence, our hypothesis that weight-cycling would actually deplete whole-body levels of linoleate and α-linolenate was not confirmed. However, the loss in whole-body content of linoleate and α-linolenate caused by the 24 h fasting stage of each weight cycle was incompletely reversed during the refeeding stage, resulting in lower net whole-body accumulation of these two fatty acids. The diet provided about 15 g linoleate/kg (3% energy) which was enough to sustain adequate growth and linoleate accumulation in the CTL group but when feed intake was cyclical normal tissue accumulation of linoleate was not maintained. Perhaps the use of pregnant rats in our earlier study (Chen & Cunnane, 1993) was an important factor influencing the severity of the fatty acid changes during fasting–refeeding which prevented the accumulation of any dietary linoleate and induced an actual depletion of whole-body α-linolenate during the 5 d refeeding period.

Weight-cycling also decreased accumulation of arachidonate and docosahexaenoate compared with free feeding, indicating that the whole-body loss of linoleate and α-linolenate was not through significant diversion towards chain elongation and desaturation. In fact, after excluding arachidonate consumed from the diet, calculated net whole-body synthesis of arachidonate was 63% lower in the W-CYC rats than in the CTL rats. Since accumulation of docosahexaenoate in the W-CYC rats was less than its intake in this group (113 *v.* 129 mg), there was clearly no net synthesis of this important *n*-3 polyunsaturate in the W-CYC rats. Hence, weight cycling does not impair whole-body accumulation of linoleate and α-linolenate because of increased conversion to longer-chain polyunsaturates.

In contrast, whole-body loss of the saturates and monounsaturates (mainly palmitate, stearate, and oleate) during fasting was completely reversed during the refeeding period of each weight cycle, presumably as a result of a combination of both dietary supply and lipogenesis. Fasting–refeeding induces *de novo* fatty acid synthesis (Allman *et al.* 1965) which is one mechanism by which the total body content of saturates and monounsaturates could be maintained in the W-CYC rats despite the fluctuating dietary energy availability in this group. However, no such mechanism exists to synthesize and replace linoleate and α-linolenate. Hence, we have proposed that the diet may contain an apparently adequate amount of linoleate and α-linolenate but if feed intake fluctuates markedly, impaired accumulation and a relative deficiency of linoleate and α-linolenate could still be induced (Chen *et al.* 1995).

Previous research has suggested that in humans with unrestricted food intake, linoleate is β-oxidized more easily than saturated fatty acids. Jones & Schoeller (1987) studied the

effect of dietary fat on BMR and on the thermogenic effect of food in seven healthy humans. Consumption of a diet rich in linoleate led to fat oxidation contributing more to the thermogenic effect of food than when dietary linoleate was lower. Higher linoleate intake was associated with lower carbohydrate oxidation, higher fat oxidation and higher respiratory quotient compared with lower linoleate intake (Jones & Schoeller, 1987). Similar results have recently been reported in rats (Su & Jones, 1993; Early & Spielman, 1995; Takeuchi *et al.* 1995) suggesting that increased fat oxidation while consuming a higher intake of linoleate occurs across mammalian species. The implication for our present results is that lower accumulation of whole-body polyunsaturates during weight-cycling may tend to permit more fat accumulation during the refeeding phase than would otherwise occur with free feeding.

Mice consuming diets containing beef tallow have greater energy gain and greater efficiency of weight gain compared with mice consuming maize oil, again suggesting that the linoleate in maize oil is not as readily accumulated as the saturates and monounsaturates in beef tallow (Mercer & Trayhurn, 1987). In the perfused rat liver, linoleate is known to decrease triacylglycerol output and to increase ketone-body production, compared with palmitate (Kohout *et al.* 1971). It appears that the greater β-oxidation of linoleate may contribute to its hypocholesterolaemic and hypotriacylglycerolaemic effects in both animals and humans by diverting more fatty acid C towards CO_2 and less toward lipoprotein and triacylglycerol synthesis (Beynen & Katan, 1985). Liver carnitine palmitoyltransferase (*EC* 2.3.1.21) was observed to have a higher activity towards linoleate and α-linolenate than saturates and monounsaturates (Gavino & Gavino, 1991). Leyton *et al.* (1987) showed that within 8 h of oral dosing, ^{14}C oleate was β-oxidized faster than linoleate or α-linolenate, but after 8 h it was oxidized slower than α-linolenate but still faster than linoleate.

Overall, these studies agree with our present observations and indicate that increased losses of linoleate and α-linolenate during weight-cycling are probably due mainly to increased β-oxidation to CO_2. Our present findings re-emphasize the importance of nutritional status and long-term energy intake as major determinants of the partitioning of linoleate and α-linolenate between accumulation and β-oxidation (Cunnane *et al.* 1993). They also indicate that the slower whole-body accumulation of linoleate and α-linolenate during weight cycling is not due to increased synthesis of arachidonate and docosahexaenoate because whole-body levels of these two longer-chain polyunsaturates were significantly reduced in the W-CYC rats. Less synthesis of arachidonate and docosahexaenoate and slower weight gain would presumably help to reduce the rate of depletion of linoleate and α-linolenate in the W-CYC rats.

From the whole-body fatty acid balance results presented here, we conclude that weight-cycling disproportionately reduces the whole-body accumulation of *n*-6 and *n*-3 polyunsaturates, especially linoleate and α-linolenate, while permitting accumulation of saturates and monounsaturates at a rate not statistically different from that of the free-fed controls. As a result, the fatty acid composition of most organs including liver and adipose tissue shifted significantly towards lower unsaturation (Chen *et al.* 1995) which may have important consequences for the regulation of membrane receptor-mediated functions including fatty acid synthesis and storage. If confirmed in human studies, these changes in the utilization of polyunsaturates combined with stimulation of lipogenesis may contribute to the difficulty of sustaining weight loss during dieting.

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