Pure linoleate deficiency in the rat: influence on growth, accumulation of n–6 polyunsaturates, and [1-^{14}C]linoleate oxidation

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Abstract Essential fatty acid deficiency has been widely studied but the extent to which its effects are attributable specifically to deficiency of linoleate as opposed to deficiency of all unsaturated fatty acids is unknown. Our objective was to evaluate the effect of pure linoleate deficiency on growth as well as changes in the metabolism and oxidation of n–6 polyunsaturates. The diets contained 20 energy % fat blended from 3 energy % pure oleate, 2 energy % linoleate (0.01 energy % in the linoleate-deficient group), 0.3 energy % pure α-linolenate, and the balance as palmitate and stearate from fully hydrogenated soybean oil. Thirty-five-day-old rats consumed the two diets for 84 days, after which the linoleate-deficient rats weighed 15% less than the controls ($P < 0.05$), had mild scaling on the paws, and visible hair loss (in a few rats). Compared with the controls, the ratio of eicosatrienoate to arachidonate after 84 days was elevated in liver (170-fold) and serum (520-fold) phospholipids of the linoleate-deficient group. In total, linoleate-deficient rats consumed 122 mg of linoleate and had a net whole body loss of 470 mg n–6 polyunsaturates compared with an intake of 24,130 mg and a net whole body gain of 7206 mg n–6 polyunsaturates in the control group. Linoleate-deficient rats oxidized 11% of an oral bolus of [1-^{14}C]linoleate over 8 h compared with 34% in the control rats ($P < 0.05$). We conclude that pure linoleate deficiency has marked effects on accumulation of n–6 polyunsaturates but induces milder gross symptoms, particularly growth retardation, than classical essential fatty acid deficiency. α-Linolenate and possibly oleate may have a sparing effect on linoleate oxidation from body stores during linoleate deficiency.—**Cunnane, S. C., and M. J. Anderson.** Pure linoleate deficiency in the rat: influence on growth, accumulation of n–6 polyunsaturates, and [1-^{14}C]linoleate oxidation. *J. Lipid Res.* 1997. **38:** 805–812.

Supplementary key words arachidonate • essential fatty acid • eicosatrienoate

Mammals consuming a diet that is either fat-free or that contains dietary fat derived exclusively from saturated fatty acids become deficient in n–6 and n–3 polyunsaturated fatty acids (PUFA). Depending on the experimental conditions, they exhibit to varying degrees the now classical symptoms of essential fatty acid (EFA) deficiency, including growth retardation, scaliness of the skin, impaired water retention, impaired reproduction, and raised ratio of eicosatrienoate-arachidonate (20:3n–9/20:4n–6) in tissue lipids. These symptoms are mostly attributable to the dietary absence of linoleate (18:2n–6) and to the subsequent loss of linoleate and arachidonate (20:4n–6) from membrane lipids (1–5). Despite numerous studies of EFA deficiency, there do not appear to be any reports in which the experimental diets have been made exclusively deficient in linoleate while providing an adequate amount of α-linolenate (18:3n–3) as well as a source of monounsaturates, especially oleate (18:1n–9). Oleate can be synthesized by mammals but it is also a regular component of the mammalian diet. The only diets from which it is absent are those that are EFA-deficient. Hence, it is unclear which symptoms of EFA deficiency are dependent exclusively on the dietary absence of linoleate alone compared with those symptoms elicited or exacerbated by other dietary changes, including the absence of dietary α-linolenate or oleate, effects of added cholesterol, very high saturated fat intake, fasting, etc. (1).

In the case of dietary deficiency of n-3 PUFA, it is possible to provide a diet containing a natural oil such as sunflower or safflower oil that has a range of saturated and unsaturated fatty acids but minimal levels of n–3 PUFA. In fact, the presence of high amounts of linoleate in dietary fat sources used to induce deficiency of n–3 PUFA probably helps exacerbate this condition (4, 6, 7). However, in the case of linoleate deficiency, no natural plant oils or animal fats contain α-linolenate and oleate without also containing linoleate. To reduce

Abbreviations: EFA, essential fatty acid; LC, long chain; PUFA, polyunsaturated fatty acids.

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dietary linoleate to a clearly inadequate level (< 0.1 energy %) while providing adequate α-linolenate as well as a source of oleate and saturates therefore requires reconstituting the dietary fat source from a combination of virtually pure saturated fat, oleate, and α-linolenate. An extensive search of the literature has revealed no published research describing studies with such a diet or the effects of pure linoleate deficiency. Hence, surprisingly, the extent to which symptoms of EFA deficiency are attributable specifically to the deficiency of linoleate is apparently unknown.

Our main objective was to determine the extent to which linoleate is conserved from oxidation during linoleate deficiency, i.e., to determine how effectively linoleate is conserved during linoleate deficiency. This objective arose from our previous study showing that when linoleate is consumed at the minimum recommended intake level of 2 energy %, 76% is oxidized (8). This led us to design this study to determine whether linoleate is better conserved at a lower, deficient intake. Measurement of [1-^{14}C]linoleate oxidation during a 2-day linoleate refeeding period at the end of the 84-day depletion period was used to determine whether supplemental linoleate may be better conserved by linoleate-deficient rats compared with controls. Linoleate oxidation was assessed by the following two methods: recovery of $^{14}CO_2$ after oral dosing with [1-^{14}C]linoleate (9), and whole body fatty acid balance analysis in which the disappearance or apparent oxidation of linoleate is measured as the difference between linoleate intake and accumulation, conversion to n-6 long-chain (LC) PUFA, or excretion (10).

In the apparent absence of previous studies of pure linoleate deficiency, our secondary objective was to describe the effects of pure linoleate deficiency on weight gain and on whole body accumulation of LC fatty acids.

METHODS

Animals and diets

Weanling (21-day-old) male Sprague-Dawley rats were housed individually in stainless steel wire-bottomed cages suspended above a urine and fecal collection tray. They had free access to a powdered diet in glass jars and tap water from a pressure-sensitive nozzle. The diet composition for the control and linoleate-deficient rats was as follows (in g/kg): casein supplemented with methionine at 400 mg/kg diet, 200; sucrose, 555; cellulose, 100; fat blend, 100 (see **Table 1** for the difference between the control and linoleate-deficient blend); American Institute of Nutrition 76 mineral mix, 35; and American Institute of Nutrition 76 vitamin mix, 10. During the first 2 weeks of the study, all the rats consumed the control diet containing an adequate amount of linoleate (2 energy %). After the initial 2-week adaptation period, one group of rats was killed to obtain baseline data on body fatty acid content and the remaining rats were divided into two groups, one continuing to consume the control diet and one switching to the linoleate-deficient diet (0.01 energy % from linoleate). Body weights and food intake were determined three times each week over the ensuing 84-day (12 week) study period. Fecal collections were done near the beginning and at the end of the study period. The purpose of collecting data on food intake, fecal output, and baseline fatty acid values was to determine linoleate balance by use of the whole body fatty acid balance method (10). Linoleate can only be synthesized by rats if they are given a dietary source of 14:2n-6 or 16:2n-6 (11), neither of which were present in the diets used in this study. Therefore, in the absence of endogenous linoleate synthesis, the fate of dietary linoleate can be determined by measuring linoleate intake, accumulation of linoleate itself, accumulation of n-6 LC-PUFA derived from linoleate, and fecal excretion of n-6 PUFA; linoleate disappearance (apparent oxidation) is calculated by difference.

TABLE 1. Fatty acid components and composition of the diets

	Control	Linoleate-Deficient
Components (g/kg diet)		
Hydrogenated soybean oil	71	84
Pure oleate	15	15
Pure α-linolenate	1.5	1.5
Safflower oil	13	0
Fatty acid composition (%)		
16:0	10.3	10.8
18:0	61.6	71.4
20:0	0.4	0.4
18:1n-9	16.1	15.1
18:2n-6	10.0	0.05
18:3n-3	1.5	1.6
< or > 18 carbon PUFA	0	0

Values are the mean of 3 samples.

Experimental and analytical methods

After 84 days on the respective diets, the rats in both the control and linoleate-deficient groups were divided into two subgroups; one group was killed immediately. The rats were anesthetized under ketamine:acepromazine and blood was collected by cardiac puncture. Fatty acid analysis was done on lipid extracts prepared from the intact whole body. At day 84, rats in the second subgroup of control and linoleate-deficient rats were gavaged with 7 μCi [1-^{14}C]linoleate (New England Nuclear) plus a single bolus dose of 0, 56, 112, or 256 mg

of pure linoleate. The 256-mg dose of linoleate was equivalent to the daily intake of linoleate in the controls. The unlabeled and [^{14}C]linoleate were mixed with 75 mg of pure oleate. The control group did not receive the linoleate supplement. Excretion of ^{14}C in breath CO_2 was determined by placing the rats one at a time in a home-built Plexiglas breath collection chamber for 8 h. Each breath chamber was connected through a series of three glass cylindrical CO_2 traps to an air pump that drew room air through the breath chamber at constant flow (400 mL/min). The CO_2 traps each contained a mixture of ethyleneglycol monoethylether-ethanolamine (1:1; Fisher Scientific, Toronto) and trapped the $^{14}CO_2$ such that > 90% was recovered in the first trap, the remaining ^{14}C was recovered in the second trap, and no ^{14}C was recovered from the third trap. Thus, all expired CO_2 was efficiently trapped. After the 8-h CO_2 collection period, the solution in each of the three traps was pooled and four 1-mL aliquots were collected for ^{14}C analysis by scintillation counting. Forty-eight hours after dosing with the [^{14}C]linoleate, liver, brain, and perirenal fat were excised, washed in saline, blotted, weighed, and stored in chloroform at −20°C for lipid analysis. Plasma was stored frozen.

Total lipids from weighed aliquots of the tissue samples were quantitatively extracted into chloroform-methanol (2:1) with a 20% (v/v) saline wash. Liver and plasma total phospholipids and triglycerides were separated by neutral lipid thin-layer chromatography. Fatty acids in carcass and perirenal total lipids and in the separated lipid classes of liver and plasma were transmethylated using 14% boron trifluoride in methanol (Sigma Chemical Co., St. Louis, MO) as previously described (10). Fatty acid methyl ester profiles were acquired by gas chromatography using a 30 m capillary column (Durabond 23, J & W Scientific, Folsom, CA) and a three-stage temperature program from 150 to 220°C.

Data and statistics

Data are expressed as the mean ± SD for 4–12 samples/point, depending on the measurements (see legends to Figs. 1–3 and Tables 1–5). Statistical comparisons between baseline and control or linoleate-deficient groups or between the control and linoleate-deficient groups were done using analysis of variance and Student's *t*-test where appropriate.

RESULTS

Food intake, growth, and skin condition

Total food intake over the 84-day balance period was not significantly different in the controls (2413 g) compared with the unsupplemented linoleate-deficient groups (2445 g). Initial body weight was the same in both groups and was not statistically lower in the linoleate-deficient group until the rats were on the two diets for 80 days. Final body weight after 84 days was 15% lower in the linoleate-deficient group (405 ± 45 vs. 475 ± 30 g in the controls; $P < 0.05$; **Fig. 1**). Overall, the heaviest rat in the study was from the linoleate-deficient group. Mild scaling of the skin on the forepaws occurred in most of the linoleate-deficient rats and some hair loss was observed in 5 of the 24 rats in the linoleate-deficient group. No other visible differences in the two groups were observed. Liver weight was nonsignificantly lower in the linoleate-deficient group (15.4 ± 2.3 vs. 18.4 ± 2.9 g in the control group).

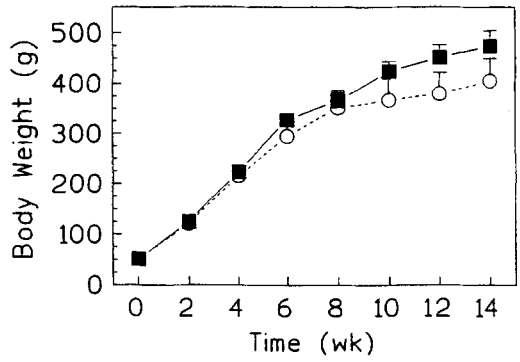

Fig. 1. Weight gain in control (filled squares) and linoleate-deficient (open circles) male rats over 14 weeks from weaning. Each value is the mean ± SD for n = 12 rats/group. Both groups adapted to the control diet during weeks 0–2, after which the control and linoleate-deficient groups were separated. The body weights of the two groups differed significantly ($P < 0.05$) at week 14 (12 weeks after starting the linoleate-deficient diet).

Liver fatty acid profiles

Fatty acid profiles in liver phospholipids reflected linoleate and n-6 LC-PUFA depletion in the linoleate-deficient group as indicated by linoleate and arachidonate levels that were 85% lower than in the control group, and an eicosatrienoate/arachidonate ratio that increased to 3.40 compared with 0.02 in the control group ($P < 0.01$; **Table 2**). The reduced proportion of n-6 PUFA in liver phospholipids of the linoleate-deficient group was replaced mainly by a 1.9-fold increase in percent docosahexaenoate and a 2.7-fold increase in percent oleate. The concentration of total fatty acids in liver phospholipids was not significantly affected by linoleate deficiency (Table 2). In liver triglycerides, the proportion of n-6 PUFA and the eicosatrienoate/arachidonate ratio also reflected linoleate depletion in the linoleate-deficient group. However, in this case, both oleate and total saturates were raised both proportionally and quantitatively, whereas n-3 PUFA declined pro-

TABLE 2. Fatty acid composition of liver phospholipids and triglycerides in control and linoleate-deficient rats

	Phospholipids		Triglycerides	
	Control	Deficient	Control	Deficient
Total (mg/g)	26.5 ± 2.5	27.0 ± 5.3	12.5 ± 3.2[a]	27.3 ± 13.4[b]
	% Composition			
18:2n-6	8.2 ± 0.9[a]	1.2 ± 0.3[b]	7.9 ± 1.8[a]	0.2 ± 0.1[b]
20:4n-6	28.8 ± 2.3[a]	4.0 ± 0.7[b]	1.5 ± 0.4[a]	0.1 ± 0.1[b]
Sum n-6 PUFA	39.4 ± 1.5[a]	5.4 ± 0.8[b]	9.9 ± 2.0[a]	0.3 ± 0.1[b]
18:3n-3	<0.1	<0.1	0.3 ± 0.1	<0.1
22:6n-3	8.1 ± 0.5[a]	15.7 ± 0.9[b]	0.3 ± 0.1[a]	0.1 ± 0.1[b]
Sum n-3 PUFA	8.7 ± 0.5[a]	19.2 ± 0.9[b]	0.6 ± 0.1[a]	0.2 ± 0.1[b]
18:1n-9	6.0 ± 0.5[a]	15.9 ± 3.3[b]	44.8 ± 1.7	47.7 ± 2.2
20:3n-9	0.6 ± 0.1[a]	13.6 ± 6.1[b]	0.2 ± 0.1	0.3 ± 0.1
Sum monounsaturates[1]	11.3	38.1	55.8	61.6
Sum saturates[2]	40.6 ± 1.1	37.3 ± 7.0	33.7 ± 2.4[a]	37.9 ± 2.1[b]

Values are means ± SD; n = 6/group. Different superscripts denote significant differences ($P < 0.05$).
[1]14:1 to 24:1 including 18:1n-9.
[2]14:0 to 24:0.

portionally but remained quantitatively unchanged (Table 2).

Perirenal adipose and whole body fatty acid profiles

In the linoleate-deficient group, linoleate levels in perirenal adipose tissue were reduced by 98% and α-linolenate was 62% lower (**Table 3**). These proportional decreases in adipose tissue PUFA were compensated for by increased oleate. n-6 and n-3 LC-PUFA were virtually undetectable in perirenal adipose tissue and are not reported. In the control group, proportions of all PUFA and the sum of saturates in whole body lipids decreased from baseline values while the proportion of oleate increased by 29% (**Table 4**). Severe decreases in the proportion of n-6 PUFA in whole body lipids occurred with linoleate deficiency resulting in a 20-fold increase in the eicosatrienoate/arachidonate ratio in whole body lipids. In the linoleate-deficient group, α-linolenate was 58% lower but docosahexaenoate was 50% higher than in the control group (Table 4).

TABLE 3. Fatty acid composition of perirenal adipose tissue in control and linoleate-deficient rats

	Control	Linoleate-Deficient
18:2n-6	11.4 ± 0.8[a]	0.2 ± 0.1[b]
18:3n-3	0.8 ± 0.1[a]	0.3 ± 0.1[b]
18:1n-9	46.0 ± 1.5[a]	55.2 ± 0.5[b]
Other monounsaturates[1]	15.7 ± 1.9	16.4 ± 0.7
Sum of saturates[2]	26.1 ± 0.9	27.9 ± 1.5

Values are means ± SD, n = 6/group. Different superscripts denote significant differences ($P < 0.05$).
[1]Includes 14:1 to 24:1 and 20:3n-9; excludes 18:1n-9.
[2]Includes 14:0 to 24:0.

Serum fatty acid profiles

The proportion of total n-6 PUFA in serum phospholipids was 93% lower in the linoleate-deficient compared with control group but this low value more than doubled with a 256-mg linoleate supplement over 48 h (**Fig. 2**). Linoleate supplementation reduced the eicosatrienoate/arachidonate ratio in serum phospholipids in proportion to the amount of linoleate given but, even with the highest linoleate dose, this ratio remained more than 200-fold higher than in the control group. The concentration of total fatty acids in serum phospholipids was not affected by linoleate deficiency or by linoleate supplementation of the deficient rats so fatty acid proportions reflect concentrations for individual fatty acids (Fig. 2). Linoleate rose but arachidonate, n-3 PUFA, and the eicosatrienoate/arachidonate ratio remained low and unchanged in serum triglycerides in the linoleate-deficient groups given linoleate supplementation for 48 h (data not shown).

[1-^{14}C]linoleate oxidation

Oxidation to $^{14}CO_2$ accounted for 33% of the [1-^{14}C]linoleate dose given to the control group and 11% of the dose given to the unsupplemented linoleate-deficient group ($P < 0.01$; **Fig. 3**). Linoleate supplementation of the deficient rats raised the ^{14}C recovered in breath CO_2 to 17, 23, and 25% of the dose given at linoleate supplements of 56, 112, or 256 mg, respectively (all $P < 0.01$ vs. both the unsupplemented linoleate-deficient groups and the control group).

Whole body linoleate balance

Linoleate oxidation was also determined indirectly by calculating its whole body disappearance using fatty

TABLE 4. Fatty acid composition of whole body in control and linoleate-deficient rats

	Baseline	Control	Linoleate-Deficient
18:2n-6	13.9 ± 0.9[a]	10.5 ± 0.8[b]	0.4 ± <0.1[c]
20:4n-6	2.3 ± 0.2[a]	1.3 ± 0.2[b]	0.2 ± <0.1[c]
Sum n-6 PUFA	17.1 ± 1.0[a]	12.0 ± 0.9[b]	0.6 ± <0.1[c]
18:3n-3	0.9 ± 0.2[a]	0.7 ± 0.1[a]	0.3 ± <0.1[b]
22:6n-3	1.0 ± 0.1[a]	0.4 ± 0.1[b]	0.6 ± 0.1[c]
Sum n-3 PUFA	2.3 ± 0.3[a]	1.2 ± 0.1[b]	1.0 ± 0.1[b]
18:1n-9	29.1 ± 1.1[a]	37.5 ± 1.5[b]	51.4 ± 3.6[c]
20:3n-9	0.3 ± 0.1[a]	0.2 ± <0.1[a]	0.6 ± 0.1[b]
Sum n-9 monounsaturates[1]	38.3 ± 4.8[a]	53.8 ± 3.2[b]	67.9 ± 5.9[c]
Sum of saturates[2]	42.3 ± 2.2[a]	33.0 ± 2.6[b]	30.5 ± 2.9[b]

Values are means ± SD, n = 6/group. Different superscripts denote significant differences ($P < 0.05$).
[1] 14:1 to 24:1 including 18:1n-9 and 20:3n-9.
[2] 14:0 to 24:0.

acid balance methodology (**Table 5**). These measurements were only done in the controls and the unsupplemented linoleate-deficient group. After the initial 2-week period for adaptation to the control diet, mean linoleate intake in the linoleate-deficient group over the 84-day balance period was about 1 mg/day or 0.51% of that in the control group (268 mg/day). Whole body accumulation of linoleate itself occurred at 70 mg/day in the control group, whereas the linoleate-deficient group lost linoleate from the whole body at about 5 mg/day. Whole body accumulation of the sum of n-6 LC-PUFA (18–22 carbon PUFA derived from linoleate) occurred in the control group at 10 mg/day compared with a loss of 0.1 mg/day in the linoleate-deficient group. Net conversion to n-6 LC-PUFA equivalent to 3.6% of linoleate intake occurred in the control group but was 0% in the linoleate-deficient group. Summing the accumulation of linoleate and n-6 LC-PUFA with linoleate excretion and subtracting this value from linoleate intake shows that whole body disappearance of linoleate (apparent oxidation) was equivalent to 70% of linoleate intake in the controls (188 mg/day) and 485% of intake in the linoleate-deficient group (7 mg/day; $P < 0.01$). Disappearance of dietary linoleate only accounted for 21% of total linoleate disappearance in the linoleate-deficient group; the remaining 79% was due to depletion of whole body linoleate stores, which were reduced in the linoleate-deficient group at the end of the study to 36% of that present at the beginning.

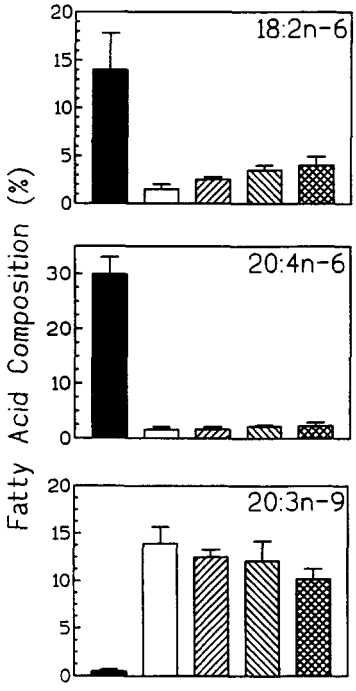

Fig. 2. Changes in serum linoleate (18:2n-6), arachidonate (20:4n-6), and eicosatrienoate (20:3n-9) in linoleate-deficient rats after a 48-h period of graded oral supplement of linoleate at 0 mg (open bars), 56 mg (left hatched bars), 112 mg (right hatched bars), or 256 mg (cross-hatched bars) compared with controls (filled bars). Each value is the mean ± SD for n = 4 rats. All post-supplementation values differed significantly from controls and from the unsupplemented linoleate-deficient group ($P < 0.05$).

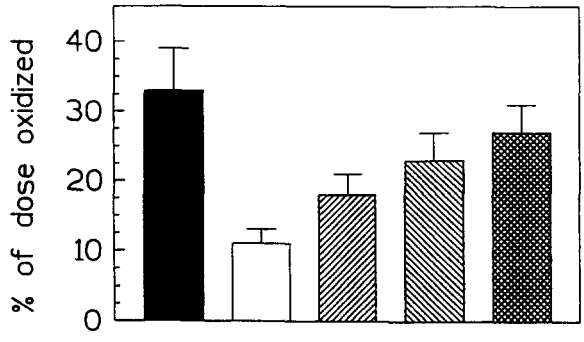

Fig. 3. Recovery of ^{14}C in expired breath CO_2 over an 8-h period in control rats (filled bars), unsupplemented linoleate-deficient rats (open bars), or in linoleate-deficient rats given a single oral supplement of 56 mg linoleate (left hatched bar), 112 mg linoleate (right hatched bar), or 256 mg linoleate (cross-hatched bar). Each value is the mean ± SD for n = 4 rats. Values for each group differed significantly ($P < 0.05$) from each other except for the groups receiving the 112- and 256-mg linoleate supplements.

TABLE 5. Whole body linoleate balance in control and linoleate-deficient rats

Variable	Control	Linoleate-Deficient
Linoleate		
Intake (mg)	24130 ± 84[a]	122 ± 5[b]
Body content (mg)		
Day 0	718 ± 58	718 ± 58
Day 90	7033 ± 1622[a]	261 ± 23[b]
Accumulation (mg)	6315 ± 1623[a]	−457 ± 62[b]
Body weight gain (mg/g)	15.8 ± 4.1[a]	−1.4 ± 0.2[b]
Linoleate intake (%)	26.0 ± 6.7[a]	−375 ± 51[b]
n-6 LC PUFA		
Intake (mg)	0	0
Body content (mg)		
Day 0	166 ± 4	166 ± 4
Day 90	1057 ± 99[a]	153 ± 16[b]
Accumulation (mg)	891 ± 100[a]	−13 ± 16[b]
Body weight gain (mg/g)	2.2 ± 0.3[a]	−0.04 ± 0.05[b]
Linoleate intake (%)	3.6 ± 0.4	0
Linoleate disappearance[1] (mg)	16924 ± 1628[a]	592 ± 64[b]
Body weight gain (mg/g)	42.3 ± 4.1[a]	1.8 ± 0.2[b]
Linoleate intake (%)	70 ± 7[a]	485 ± 53[b]

Values are means ± SD, n = 6/group; 84-day balance period. Different superscripts denote significant differences ($P < 0.05$).

[1]From diet and whole body n-6 PUFA pool; includes fecal excretion of total n-6 PUFA at 1.2% of intake (8).

DISCUSSION

Our objective was to induce pure linoleate deficiency and evaluate its effects on linoleate oxidation to determine whether body stores of linoleate were conserved when dietary linoleate was inadequate. Excretion of ^{14}C in breath CO_2 after an oral dose of [1-^{14}C]linoleate was reduced by 30% in the linoleate-deficient group, resulting in 89% retention of the tracer over 8 h compared with 66% in the control group ($P < 0.01$; Fig. 3). Furthermore, the body content of linoleate in the linoleate-deficient group was only 3.7% of that in the controls so [^{14}C]linoleate retention relative to the whole body content of linoleate was much greater in the linoleate-deficient group. Both points suggest that linoleate is conserved better in linoleate deficiency. Nevertheless, n-6 LC-PUFA were more effectively conserved than linoleate itself because, unlike linoleate, the n-6 LC-PUFA were not significantly reduced from baseline in the whole body of the linoleate-deficient rats (Table 5). The whole body retention of arachidonate contrasted with its marked depletion from plasma and liver which, in the linoleate-deficient group, were 5.4% (serum phospholipids) and 13.9% (liver phospholipids) of that in the control group. Hence, the ability of the rats to gain weight at 85% of control values despite this severe level of arachidonate depletion in liver and plasma seems remarkable and suggests that, under these experimental conditions, plasma and liver fatty acid data may not directly reflect whole body stores.

The $^{14}CO_2$ data for our controls match closely the results reported previously for oxidation of [^{14}C]linoleate in vivo (9, 12–16). Clearly, it is useful to have oxidation data derived both from a ^{14}C tracer and from organs and whole body measurements because the long-term organ or whole body loss of linoleate cannot be directly assessed by an exogenous dose of [^{14}C]linoleate, whereas the whole body measurements cannot resolve linoleate partitioning over a period of several hours but require at least several days. Short-term supplementation with a single bolus dose of linoleate raised linoleate and arachidonate levels but also increased ^{14}C-dietary linoleate oxidation by more than 100% in the linoleate-deficient group (Fig. 3), suggesting that, even in long-term severe linoleate deficiency, supplemental dietary linoleate is only partially conserved and is still readily oxidized.

Diets maximally deficient in linoleate (providing <0.1 energy % as linoleate) but containing α-linolenate and oleate as well as saturates appear not to have been used before. Hansen and Jensen (5) provided a fat-free diet containing 2 energy % as α-linolenate but no oleate and reported improved growth compared with rats consuming a fat-free diet. Bourre et al. (7) evaluated the impact of α-linolenate in rats consuming at least 0.3 energy % as linoleate and observed that α-linolenate provided at 0.4 energy % resulted in minimal symptoms of linoleate depletion, i.e., normal weight gain, and no reproductive impairment or mortality. We have not determined the relative effect of α-linolenate alone compared with the presence of α-linolenate and oleate in the linoleate-deficient rats but we agree that α-linole-

nate probably influences the metabolism of linoleate such that the gross symptoms of EFA deficiency are moderated when low levels of α-linolenate and possibly oleate are present in the diet (5, 7). Whether the requirement for linoleate is dependent in some way on α-linolenate is not clear from this study but is suggested by the study of Bourre et al. (7).

Despite having 0.3 energy % α-linolenate in the diet, α-linolenate in perirenal adipose tissue was reduced by 62% compared with control values. n-3 LC-PUFA were raised in liver phospholipids (Table 2) and in whole body lipids (Table 4) so linoleate deficiency probably facilitated conversion of some α-linolenate to n-3 LC-PUFA (2). In addition, given that whole body linoleate levels were reduced through oxidation in the linoleate-deficient group, it is possible that part of the reduction in fat stores of α-linolenate was also due to increased oxidation, i.e. by being readily β-oxidized itself, α-linolenate may have spared linoleate oxidation and thereby reduced the impact of dietary linoleate deficiency (7). Oleate is also relatively easily oxidized in the rat (9, 12, 16) and it too may have spared linoleate oxidation. Both α-linolenate and oleate are absent from classical EFA deficiency diets, which may contribute to the faster onset of growth retardation and skin lesions in classical EFA deficiency compared with pure linoleate deficiency in which a dietary source of α-linolenate and oleate are provided.

Generally, in EFA deficiency studies, the assumption seems to have been that unsaturated fatty acids such as oleate can be synthesized in adequate amounts and their absence from the diet would not be limiting to the growing rat. Nevertheless, this assumption has not been verified and the desaturation and chain elongation of oleate to eicosatrienoate (n-9) definitely alters the utilization of oleate in EFA or linoleate-deficient animals (4). Oleate is also present in all diets unless they are EFA deficient, i.e., fat-free or based exclusively on saturated fatty acids, so for a well-controlled study of linoleate deficiency, oleate and α-linolenate should also be in the diet. In this study we also attempted to minimize the differences in intake of α-linolenate, oleate, and saturated fatty acids between the controls and linoleate-deficient rats; the lower linoleate in the linoleate-deficient diet was compensated for by changing the level of saturates while α-linolenate and oleate remained the same in both groups (Table 1).

Susceptibility to EFA depletion is a function of many parameters including age and gender, species and strain, duration of depletion, and dietary fat type and level so our current results are not directly comparable to previous studies involving EFA deficiency. Nevertheless, it seems reasonable that the presence of α-linolenate and possibly oleate in our linoleate-deficient group contributed to better weight gain, less hair loss, and fewer skin lesions despite equal or greater n-6 PUFA depletion from body stores than would normally be observed in classical EFA deficiency. Several species of mammals remain resistant to EFA depletion once they achieve mature weight (1, 3, 4). The rats in this study initially weighed about 120 g and gained nearly 300 g after the linoleate-deficient diet was introduced so the relatively normal weight gain for nearly 12 weeks and the mild gross symptoms were not due to the rats being near mature weight at the start of the study.

We conclude that a diet made severely deficient only in linoleate markedly changes whole body and organ fatty acid composition in a manner consistent with that of classical EFA deficiency but does not induce the same level of growth retardation or other gross symptoms. Linoleate was better conserved in the linoleate-deficient rats than in the controls but not as well conserved as n-6 LC-PUFA and this probably contributes to its whole body depletion when diets deficient in linoleate are consumed for long periods. α-Linolenate and possibly oleate may spare linoleate oxidation when linoleate intake is severely deficient.

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