Specific linoleate deficiency in the rat does not prevent substantial carbon recycling from [^{14}C]linoleate into sterols

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Abstract **Compared with classic essential fatty acid deficiency or the feeding of a fat-free diet, little is known about specific linoleate deficiency in the rat. Carbon recycling into de novo lipogenesis has been reported to be an obligatory feature of linoleate metabolism in the liver, even in extreme linoleate deficiency (LA-D). The present study had two objectives: *1*) to report a brief summary of the tissue n−6 polyunsaturated fatty acid (PUFA) profiles in specific LA-D, and *2*) to quantify whole body carbon recycling from [^{14}C]linoleate in specific LA-D. Rats consumed a linoleate-deficient diet for 12 weeks and then received a bolus of [1-^{14}C]linoleate by gavage. In linoleate-deficient rats, the triene/tetraene ratio in several organs increased by 18- to 100-fold. The amount of ^{14}C appearing in organ sterols (dpm/g) of linoleate-deficient rats was 2- to 10-fold higher than in the controls and equaled 16.3% of the [^{14}C]linoleate dose given, compared with 7.4% in the controls. We conclude that a similar amount (about 10%) of the carbon skeleton of linoleate is normally recycled into lipids synthesized de novo, as remains in the whole body pool of n−6 polyunsaturates.**—Cunnane, S. C., D. Trotti, and M. A. Ryan. **Specific linoleate deficiency in the rat does not prevent substantial carbon recycling from [^{14}C]linoleate into sterols. *J. Lipid Res.* 2000.** 41: **1808–1811.**

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Linoleate (18:2n−6) is widely recognized as a nutrient needed for normal mammalian growth and development. Much of what is known about the significance of linoleate in mammalian health and development has been learned from studies using the two classic models of dietary depletion of polyunsaturates, either essential fatty acid deficiency (EFA-D) or total dietary fat deficiency induced by a fat-free diet. Unfortunately, neither of these models is specific to linoleate because, in addition to linoleate deficiency (LA-D), they both also induce deficiency of α-linolenate (18:3n−3) and oleate (18:1n−9). α-Linolenate is a nutrient distinct from linoleate. Oleate can be synthesized endogenously but apparently not in sufficient quantities during rapid development (1). Hence, the portion of the effects of EFA-D or total fat deficiency that is attributable to depletion of linoleate alone is really unknown without studying specific linoleate deficiency.

We reported the effects of specific linoleate (18:2n−6) deficiency in the rat on growth and some tissue fatty acid profiles (2). Despite severe depletion of n−6 polyunsaturates from tissue lipids, gross symptoms including skin lesions and growth retardation were relatively mild in LA-D compared with those usually reported for classic EFA-D or total fat deficiency. Subsequently, we described the effects of LA-D on appearance of ^{14}C in breath CO_2 and in individual liver fatty acids and sterols after an oral dose of [1-^{14}C]linoleate (3). The aim of that study was to assess the significance of earlier reports of carbon recycling from polyunsaturated fatty acids (4, 5) by using a model in which dietary and tissue levels of linoleate were reduced to a minimum prior to assessing carbon recycling. Carbon recycling accounted for a significant proportion of the metabolism of the carbon skeleton of linoleate during normal linoleate intake (30%) and during extreme and specific LA-D (18%). Hence, carbon recycling from linoleate into lipids synthesized de novo in the liver occurred as an apparently obligatory process in linoleate metabolism (3).

Our objectives in extending the description of specific LA-D in the rat were, first, to report proportions of linoleate, arachidonate and n−9 eicosatrienoate as a measure of the severity of the dietary LA-D and, second, to report quantitative, whole body data for carbon recycling from [^{14}C]linoleate into sterols in several organs and remaining carcass. These results should provide a perspective on the actual bioavailability of linoleate.

MATERIALS AND METHODS

Animal housing and diets

Twenty-one-day-old male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) were housed individually in stainless

Abbreviations: EFA-D, essential fatty acid deficiency; LA-D, linoleate deficiency.

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TABLE 1. Organ weights (g) of rats consuming a control or linoleate-deficient diet

	Control	Linoleate Deficient
Liver	18.4 ± 2.9[a]	15.4 ± 2.3
Brain	2.0 ± 0.1	1.8 ± 0.1
Testes	1.6 ± 0.1	1.3 ± 0.1[b]
Heart	1.3 ± 0.1	1.3 ± 0.1
Carcass	432 ± 21	331 ± 31[b]

[a] Mean ± SD (n = 5 or 6/group).
[b] $P < 0.05$ (vs. corresponding control; Student's unpaired *t*-test).

steel wire-bottomed cages as described previously (2). Free access to food and water was provided. Lights were off between 7 PM and 7 AM. Humidity was 50–55% and the room temperature was 21–22°C.

A semipurified diet was used that contained (g/kg): 200 casein, 555 sucrose, 100 cellulose, 100 fat blend, 35 AIN 76 mineral mix, and 10 AIN 76 vitamin mix. The fat was a blend of hydrogenated soybean oil, pure oleate, pure α-linolenate, and safflower oil (controls only) that provided 2 energy % from linoleate, 0.3 energy % from α-linolenate, and 3 energy % oleate. Two energy % linoleate is considered to meet the linoleate requirement of the growing rat. After 2 weeks on the control diet, that is, at 35 days old, the rats were divided so that one group remained on the control diet while the other was switched to a diet maximally depleted specifically of linoleate. The fatty acid profile of the control diet was palmitate (10.3%), stearate (61.6%), arachidate (0.4%), oleate (16.1%), linoleate (10.0%), and α-linolenate (1.5%). The fatty acid profile of the linoleate-deficient diet was palmitate (10.8%), stearate (71.4%), arachidate (0.4%), oleate (15.1%), linoleate (0.05%), and α-linolenate (1.6%). To compensate for the lower linoleate in the linoleate-deficient diet, palmitate (16:0) and stearate (18:0) were proportionately increased but, otherwise, the dietary fatty acids were virtually identical. There was no detectable content of long-chain polyunsaturates in either diet (<0.01% of total fatty acids).

Experimental and analytical methods

After 84 days on the respective diets, the rats were gavaged with 7 μCi of [1-^{14}C]linoleate (American Radiolabeled Chemicals, St. Louis, MO; specific activity, 55 mCi/mmol) and killed 48 h later under ketamine-acepromazine anesthesia. Organs were dissected and weighed. Samples of skin and adipose tissue were taken but the total amounts of skin and adipose tissue were not determined.

Tissue total lipids were quantitatively extracted into chloroform–methanol 2:1 and were then saponified to separate the sterol-rich fraction (hereafter described as "sterols") from fatty acids. An aliquot of the nonsaponifiable sterol fraction was dried under nitrogen gas and weighed, and the ^{14}C content was determined by liquid scintillation counting with appropriate reference and quench standards (LS6000SC; Beckman, Palo Alto, CA). An aliquot of the fatty acid fraction of the organ and carcass total lipid extracts was trans-methylated with boron trifluoride in methanol (Sigma, St. Louis, MO) and the fatty acid methyl esters were analyzed by capillary gas chromatography (2). ^{14}C distribution in individual fatty acids was not determined for the organs reported here, but this information for liver has been previously reported from this study (3).

Statistical analysis

All data are expressed as means ± SD of five or six samples per group. Statistical comparisons were done by analysis of variance and Student's *t*-test where appropriate.

RESULTS

Growth of the LA-D rats in the present study has been previously reported as being reduced by 15%, mostly in the final 4 weeks of the overall 14-week study (2). LA-D did not affect organ weights, apart from the testes, which were 19% lighter than in the control group ($P < 0.05$, **Table 1**). LA-D also did not affect the concentration of total fatty acids in any organs except testes (+32%; **Table 2**). As expected, n−6 polyunsaturates were reduced in various organs of the linoleate-deficient group, linoleate by 78–97% and arachidonate (20:4n−6) by 37–100%. The classic marker of dietary LA-D is eicosatrienoate (20:3n−9), which was increased 3- to 28-fold in various organs of the LA-D rats. Hence, the triene/tetraene (eicosatrienoate/arachidonate) ratio increased 18-fold (skin) to 100-fold (testes) in the LA-D group (Table 2).

Accumulation of total ^{14}C in organ total lipids was 5- to 10-fold higher in liver, brain, heart, and testes, and was

TABLE 2. Comparison of organ fatty acid content and profile of linoleate (18:2n–6), arachidonate (20:4n–6), and eicosatrienoate (20:3n–9) in rats given a control or linoleate-deficient (LA-D) diet[a]

	Diet	Testes	Heart	Skin	Brain	Adipose
Total fatty acid content (mg/g)						
	Control	11.5 ± 1.5[b]	23.8 ± 1.4	29.1 ± 1.7	36.0 ± 2.5	856 ± 19
	LA-D	15.2 ± 1.1[c]	23.6 ± 3.8	30.2 ± 2.4	34.7 ± 5.6	848 ± 40
Fatty acid profile (%)						
18:2n–6	Control	4.3 ± 0.9	15.6 ± 1.2	13.7 ± 1.2	0.7 ± 0.2	10.5 ± 0.8
	LA-D	0.8 ± 0.1[c]	3.4 ± 1.1[c]	0.9 ± 0.3[c]	0.1 ± 0.0[c]	0.3 ± 0.0[c]
20:3n–9	Control	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
	LA-D	5.6 ± 0.3[c]	8.6 ± 4.9[c]	0.6 ± 0.2[c]	2.6 ± 0.2[c]	0.1 ± 0.0
20:4n–6	Control	17.1 ± 2.1	21.5 ± 1.0	1.3 ± 0.3	10.1 ± 0.4	0.2 ± 0.0
	LA-D	5.0 ± 1.0[c]	5.8 ± 1.1[c]	0.2 ± 0.1[c]	6.4 ± 0.4[c]	0.0 ± 0.0[c]
20:3n–9/20:4n–6	Control	0.01 ± 0.00	0.02 ± 0.01	0.15 ± 0.04	0.02 ± 0.00	0.0
	LA-D	1.12 ± 0.21[c]	1.48 ± 0.33[c]	3.00 ± 0.59[c]	0.41 ± 0.09[c]	—

[a] Liver and plasma data have been reported previously (2).
[b] Mean ± SD (n = 5 or 6/group).
[c] $P < 0.05$ (vs. corresponding control; Student's unpaired *t*-test).

3.4-fold higher in the whole body lipids of the LA-D rats compared with the controls (**Table 3**). Accumulation of ^{14}C in organ sterols (dpm/g) increased 2- to 10-fold in the LA-D rats (**Table 4**). The proportion of ^{14}C in organ total lipids that was recovered in organ sterols ranged from 5% in the heart to 67% in the skin of controls, and from 5% in the heart to 57% in the skin of LA-D rats. In total lipids of the body as a whole, 35.9% of the [^{14}C]linoleate was recovered as [^{14}C]sterols in the controls whereas the corresponding value in the LA-D group was 29.3% (NS). Of the dose of [1-^{14}C]linoleate that was administered, 7.4% appeared in whole body sterols in the controls compared with 16.3% in the LA-D group ($P < 0.05$; Table 4). In summary, 48 h after dosing, the fate of an oral dose of [^{14}C]linoleate in adult control rats is that approximately 78% is completely β-oxidized to CO_2, 10% remains in the whole body pool of n−6 polyunsaturates, 10% is recycled into products of de novo lipogenesis, and 2% is excreted in the feces. In LA-D rats, the main difference is that less ^{14}C is completely β-oxidized and more is retained in tissue lipids, that is, about 42% of linoleate is β-oxidized to CO_2 and 35% remains in the whole body pool of n−6 polyunsaturates. However, about 21% is still recycled into products of de novo lipogenesis, while 2% still appears in the feces (**Table 5**).

DISCUSSION

Carbon recycling from [^{14}C]linoleate into liver lipids synthesized de novo increases during LA-D (2). In the present study, we quantified carbon recycling from [^{14}C]-linoleate into sterols in several organs and in the body as a whole. Despite less oxidation to $^{14}CO_2$ in LA-D rats (2), twice as much ^{14}C was recovered in organ and whole body sterols as in the controls (Table 4). However, the proportion of ^{14}C recovered in sterols was not increased (36% in controls vs. 29% in LA-D), and so sterol synthesis from linoleate was not stimulated per se in LA-D. Rather, LA-D increased the retention of ^{14}C in all tissue lipids, whether n−6 polyunsaturates or lipids synthesized de novo. Hence, greater conservation of linoleate during LA-D still carries with it an obligatory increase in carbon recycling that doubles relative to that in controls (Tables 4 and 5). Given the relatively extreme linoleate depletion achieved in this study, this indicates that carbon recycling is probably an obligatory process in linoleate metabolism.

Passage of carbon through the tricarboxylic acid cycle can result in exchange of the tracer between different metabolites without a net transfer of carbon. Because linoleate cannot be synthesized de novo, the appearance of ^{14}C in sterols that was originally in linoleate must be, in a net sense, a unidirectional process. Our present data are con-

TABLE 3. Distribution of ^{14}C in total lipids of individual organs, carcass, and whole body of control and linoleate-deficient (LA-D) rats 48 h after an oral dose of [1-^{14}C]linoleate

	Diet	dpm/g	% Total Dose[a]
Liver	Control	14,090 ± 6,710[b]	2.0 ± 0.7
	LA-D	84,530 ± 17,060[c]	10.1 ± 3.1[c]
Brain	Control	1,030 ± 190	0.01 ± 0.00
	LA-D	4,820 ± 2,450[c]	0.05 ± 0.02[c]
Heart	Control	7,919 ± 2,938	0.06 ± 0.02
	LA-D	76,408 ± 25,451[c]	0.64 ± 0.20[c]
Testes	Control	2,339 ± 505	0.02 ± 0.00
	LA-D	21,705 ± 11,409[c]	0.16 ± 0.08[c]
Skin	Control	6,927 ± 4,847	ND
	LA-D	16,878 ± 9,187	ND
Adipose	Control	12,890 ± 8,670	ND
	LA-D	25,300 ± 19,610	ND
Carcass[d]	Control	5,805 ± 1,866	18.2 ± 5.9
	LA-D	18,978 ± 2,341[c]	44.7 ± 7.4[c]
Whole body	Control	6,832 ± 2,076	20.3 ± 6.3
	LA-D	22,999 ± 4,410[c]	55.6 ± 8.3[c]

ND, Not determined.
[a] Percentage of [1-^{14}C]linoleate dose in feces was reported previously (2) as 2% in both groups.
[b] Mean ± SD (n = 5 or 6/group).
[c] $P < 0.05$ versus corresponding control.
[d] Whole body including skin, and adipose tissue but excluding liver, heart, testes, brain.

TABLE 4. Distribution of ^{14}C in organ sterols of control and linoleate-deficient (LA-D) rats 48 h after an oral dose of [1-^{14}C]linoleate

	Diet	dpm/g	% of Organ ^{14}C in Sterols	% of Total Dose Administered
Liver	Control	3,080 ± 3,030[a]	21.9 ± 10.1	0.4 ± 0.2
	LA-D	9,050 ± 2,920[b]	10.7 ± 3.2[b]	0.9 ± 0.4[b]
Brain	Control	210 ± 91	20.4 ± 8.7	<0.1
	LA-D	902 ± 506[b]	18.7 ± 10.2	<0.1
Heart	Control	435 ± 206	5.5 ± 2.2	<0.1
	LA-D	4,170 ± 3,444[b]	5.5 ± 3.6	<0.1
Skin	Control	4,654 ± 4,029	67.2 ± 49.3	ND
	LA-D	9,659 ± 6,421	57.2 ± 39.6	ND
Carcass[c]	Control	2,509 ± 1,028	43.2 ± 18.3	7.0 ± 3.9
	LA-D	7,150 ± 723[b]	37.7 ± 5.0	15.4 ± 2.1[b]
Whole body	Control	2,477 ± 1,004	35.9 ± 13.7	7.4 ± 4.3
	LA-D	6,795 ± 1,084[b]	29.3 ± 8.4	16.3 ± 1.9[b]

[a] Mean ± SD (n = 5 or 6/group).
[b] $P < 0.05$ (versus corresponding control).
[c] Whole body including skin but excluding heart, testes and brain.
ND, not determined.

TABLE 5. Distribution of ^{14}C 48 h after an oral dose of [1-^{14}C]linoleate given to control and linoleate-deficient rats

	Control	Linoleate Deficient
Whole body		
n–6 polyunsaturates	9.8 ± 4.1[a]	35.1 ± 9.6
De novo lipogenesis[b]	10.5 ± 4.5	20.5 ± 6.3
Breath[c]	77.6 ± 10.1	42.4 ± 3.0[d]
Feces[e]	2.1 ± 1.2	2.0 ± 0.1

[a] Mean ± SD; n = 5 or 6/group.
[b] Sterol data from Table 5 plus estimate from liver fatty acid data (2).
[c] Difference between dose administered and recovery 48 h later in fecal or whole body lipids.
[e] From ref. 2.
[d] $P < 0.05$ (vs. corresponding control).

sistent with the increase in cholesterol synthesis from labeled water when linoleate-enriched diets are consumed (6). Hence, there is a net transfer of linoleate carbon to sterols (or fatty acids) when the ^{14}C appears in the newly synthesized lipids. This carbon transfer from linoleate (or α-linolenate) (4, 5, 7) to lipids synthesized de novo does not necessarily increase lipid synthesis or levels. However, it does mean than carbon recycling is as quantitatively important in linoleate metabolism as conversion to long-chain n−6 polyunsaturates.

Because β-oxidation appears to play a significant role in liver (2) and whole body homeostasis of linoleate (8) (Table 5), the absence of all the dietary 18 carbon-unsaturated fatty acids in EFA-D deficiency appears to exacerbate their depletion from body stores and magnifies the resulting deficiency symptoms. Supplemental α-linolenate improves the growth of EFA-deficient rats (9, 10), so it seems reasonable that specific LA-D would be less severe than EFA-D or total fat deficiency.

In contrast to EFA-D or total fat deficiency, the absence of only α-linolenate from the diet is well recognized as producing the relatively mild gross symptoms of deficiency of n−3 polyunsaturates. As we have now shown, the absence of only linoleate from the diet during specific LA-D also produces relatively mild symptoms of deficiency of n−6 polyunsaturates. In order that only linoleate would be absent from the diet of the linoleate-deficient rats, saturates, oleate and α-linolenate were added to the diet. Fully hydrogenated fats (triglycerides) containing no trans-fatty acids are available commercially but purified triglycerides of oleate or α-linolenate are quite expensive on a scale needed for dietary studies, and so the free fatty acid form of oleate and α-linolenate was used for convenience and for economy. We are not aware of any reason why LA-D would be influenced by the free or esterified form of these two fatty acids.

Decreased linoleate and arachidonate and increased eicosatrienoate and eicosatrienoate/arachidonate in different tissues are classic measures of the severity of LA-D or EFA-D. The changes in the percent composition of these fatty acids in the present study (Table 2) were consistent with severe linoleate depletion but nevertheless varied across tissues. Among lean tissues of the linoleate-deficient rats, linoleate and arachidonate were decreased the most in skin while eicosatrienoate was highest in testes and heart. The actual increase in eicosatrienoate and the eicosatrienoate/arachidonate ratio was the least in skin relative to the control values (Table 2). Hence, there appears to be no direct relation between the severity of the gross symptoms of specific LA-D and the change in fatty acid profile of the skin or other tissues.

Further study of the fate of carbon recycled from linoleate may reveal biological functions of linoleate that are not presently known.

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