Substantial carbon recycling from linoleate into products of de novo lipogenesis occurs in rat liver even under conditions of extreme dietary linoleate deficiency

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Abstract **A significant portion of the β-oxidized carbon skeleton of some polyunsaturated fatty acids can be recycled into de novo lipogenesis, i.e., cholesterol, saturates and monounsaturates. The recycling of carbon from linoleate was quantified in liver lipids of severely linoleate-deficient rats to determine whether it is more likely to be a function of redundancy or could be obligatory. After 13 wk on a control (2 energy % linoleate) or severely linoleate-deficient (<0.05 energy % linoleate) diet, 7 μCi [1-^{14}C]linoleate was given by gavage and the rats were killed 48 h later. A second linoleate-deficient group received an oral bolus of 256 mg linoleate as a supplement with the radiotracer. In comparison to the controls, ^{14}C recovery in liver total lipids of the linoleate deficient group was increased about 5-fold with increased dpm/g in linoleate (13.7-fold higher), arachidonate (2.7-fold higher) and products of de novo lipogenesis (3.5-fold higher). In livers of control rats, ^{14}C distribution was: 41% arachidonate, 29% linoleate, 22% sterols, 3% oleate, 3% palmitate, and 2% stearate. In livers of linoleate-deficient rats, ^{14}C distribution was: 63% linoleate, 19% arachidonate, 11% sterols, 4% oleate, 3% palmitate, and <1% stearate. Thus, in controls, equivalent amounts of ^{14}C were in products of de novo lipogenesis as in linoleate (29–30%), and in livers of linoleate-deficient rats, a similar proportion of ^{14}C was in products of de novo lipogenesis as was converted to arachidonate (18–19%). We conclude that carbon recycling into de novo lipogenesis accounts for a significant, obligatory component of linoleate metabolism even during extreme linoleate deficiency.—**Cunnane, S. C., K. Belza, M. J. Anderson, and M. A. Ryan. **Substantial carbon recycling from linoleate into products of de novo lipogenesis occurs in rat liver even under conditions of extreme dietary linoleate deficiency. *J. Lipid Res.* 1998.** 39: **2271–2276.**

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The influence of essential fatty acid deficiency on the health and metabolism of experimental animals and humans has been extensively studied. However, at least two fundamental issues remain to be adequately addressed: *i*) the differences between the effects of essential fatty acid deficiency compared to linoleate (18:2n–6) deficiency per se, and *ii*) the quantitative impact of carbon recycling on the bioavailability of polyunsaturates.

Concerning the first issue, until recently (1), there appear to have been few, if any, reports describing the effects of linoleate deficiency specifically, i.e., the use of diets excluding linoleate but providing adequate energy from fat including oleate (18:1n–9) and α-linolenate (18:3n–3). Nevertheless, essential fatty acid deficiency has been the primary means of extrapolating the requirement for linoleate. Against a background of combined linoleate and α-linolenate deficiency and using plateau values in tissue fatty acid profiles as the measure of linoleate adequacy, the apparent requirement for linoleate in male rats seems to be at least 1 energy % but could be as high as 2.4 energy % (2–5). However, in the presence of an adequate intake of α-linolenate and using normal growth and reproduction as the measure of linoleate adequacy, linoleate intake at 0.4% of energy seems sufficient (5). Despite severe depletion of n–6 polyunsaturates from body stores (1), pure linoleate deficiency from shortly after weaning causes less severe growth retardation and other gross symptoms than provision of an essential fatty acid deficient or fat-free diet (2, 3, 5). These studies suggest that essential fatty acid deficiency is not synonymous with linoleate deficiency and may overestimate the true requirement for linoleate per se (1, 5).

In contrast to the near absence of studies on linoleate deficiency per se, the requirement for α-linolenate itself has been investigated, an anomaly that seems to have arisen from the availability of edible oils for experimental diets that are naturally deficient in α-linolenate whereas there are none in use that are naturally deficient only in linoleate. Hence, aside from work on n–3 polyunsaturates, virtually all research into the significance of essential fatty acids using models of nutrient deficiency seems to have

Abbreviations: GC, gas chromatography; HPLC, high performance liquid chromatography.

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involved a multiple, non-specific deficiency of unsaturated 18 carbon fatty acids from three distinct fatty acid families (linoleate, α-linolenate, oleate).

Concerning the second issue, we and others have observed that carbon from several polyunsaturates can be recycled into lipids synthesized de novo (6–10). In the case of α-linolenate given to neonatal rats, recycling captures a much larger proportion of the carbon skeleton of α-linolenate than is incorporated into longer chain n–3 polyunsaturates derived from α-linolenate (11), suggesting that this pathway could be biologically significant. Tracer methods were used to make these observations, which should minimally perturb the pathways involved, but the possibility remains that extensive β-oxidation and recycling of carbon from some polyunsaturates occurs because of redundancy, i.e., because they are generally present in the diet (and body stores) in excess of actual requirements.

Our primary objective in the present study was, therefore, to quantify carbon recycling from [^{14}C]linoleate during specific linoleate deficiency in the rat. The rationale was that if negligible recycling was observed during linoleate deficiency, then it was more likely that recycling normally occurs as a result of redundancy of linoleate due to it being consumed in excess of requirement; if recycling still occurred during severe and prolonged linoleate deficiency, it would be more likely to be obligatory and would need to be accounted for in determining the bioavailability and metabolic fate of linoleate, as well as its true dietary requirement. Fecal excretion of linoleate was measured to provide a more complete assessment of the bioavailability of linoleate during adequate and deficient linoleate intake.

METHODS

Animals and diets

Weanling male Sprague-Dawley rats (21 days old) were housed individually in stainless steel wire-bottomed cages as described previously (1). A semi-purified diet containing 2 energy % linoleate was provided for the first 2 weeks. Two energy % linoleate was chosen as the control level based on published nutrient requirements for the rat (4). At 35 d old, the rats were divided so that one group remained on the control diet while the other was switched to a diet maximally deficient in linoleate. Apart from higher palmitate (16:0) and stearate (18:0) to compensate for the elimination of dietary linoleate in the linoleate deficient group, both diets contained the same amount of α-linolenate (0.3 energy %), oleate (3 energy %), total fat (20 energy %), and other macro- and micronutrients (1).

Experimental and analytical methods

After 84 days on the respective diets, the rats were gavaged with 7 μCi [1-^{14}C]linoleate (New England Nuclear) and anesthetized under ketamine:acepromazine 48 h later. A subgroup of linoleate-deficient rats [designated linoleate deficient (+)] was given an oral bolus of unlabeled linoleate with the radiotracer. The 256-mg linoleate dose was estimated to be equivalent to the daily linoleate intake of the control rats. The control group did not receive the supplemental linoleate bolus. The purpose was to determine the influence of providing a short-term linoleate supplement on [^{14}C]linoleate metabolism. Total fecal output was collected during the final 48 h of the study.

Total liver lipids were quantitatively extracted into chloroform–methanol 2:1 and then saponified to separate sterols from fatty acids. An aliquot of the nonsaponifiable fraction was dried under N_2, weighed, and the ^{14}C content was determined by liquid scintillation counting (Beckmann LS6000SC with quench correction). An aliquot of the fatty acid fraction was transmethylated using 14% boron-trifluoride in methanol (Sigma Chemical Co., St. Louis, MO) and analyzed by capillary gas chromatrography (GC) (1). A further aliquot containing 2 mg of total fatty acids was separated into its component fatty acids by reversed phase high performance liquid chromatography (HPLC) using two C18 columns in series (Zorbax ODS 5 μ, 4.6 mm i.d. × 25 cm, Jones Chromatography Ltd., New Road, Hengoed, Mid Glamorgan, UK). The flow rate was 0.8 ml/min. Optimal fatty acid separation was obtained using a solvent gradient of acetonitrile–chloroform shifting from 75:25 to 60:40 over a 25-min run time. A stream splitter sent 25% of the eluant to an evaporative light scattering detector (Varex Corp., Burtonville, MD) and 75% for collection and subsequent GC analysis and liquid scintillation counting. The HPLC detector operated at 71°C. Elution times and purity of the main fractions of interest (linoleate, arachidonate, oleate, stearate, palmitate) were determined using standards and GC analysis.

Measurements on fecal total lipids were obtained in the last 2 days of the study and were only done in the control and the linoleate-deficient groups. Fecal total lipids were extracted from a 48-h collection of dried, ground fecal homogenates into chloroform–methanol 2:1 for 1 h in a water bath heated to 60°C. Heating was used to ensure better solubility and recovery of palmitate and stearate during extraction. The samples were centrifuged at 1000 rpm (10 min) and reheated for 15 min at 60°C. The organic phase was decanted and lipids remaining in the fecal pellet were re-extracted under the same conditions and pooled with the first extract. The pooled organic phases were partitioned against 0.8% KCl, separated, weighed, and an aliquot was transmethylated using boron-trifluoride. A second aliquot was used to determine ^{14}C content. A third aliquot was saponified and the nonsaponifiable fraction (sterol-rich) was dried, weighed, and the ^{14}C content was determined.

Statistical analysis

All data are expressed as the mean ± SD of 5–6 samples/group. Statistical comparisons were done using analysis of variance and Student's *t* test where appropriate.

RESULTS

Data on the growth, tissue fatty acid profiles, and ^{14}C excretion in breath of these animals have previously been reported (1); the present report specifically addresses ^{14}C in fecal and liver lipids as they relate to evidence for carbon recycling from [1-^{14}C]linoleate.

Based on food intake measurements, linoleate intake was 287 mg/d while its excretion was 5 mg/d giving a bioavailability of unlabeled dietary linoleate of 98.3% in the controls. In the linoleate-deficient group, there was no detectable excretion of linoleate, resulting in 100% bioavailability of the trace intake (1.4 mg/d) in this group. The loss of ^{14}C in fecal lipids over 48 h was 2.5% in the controls and 2.6% in the linoleate-deficient group giving a bioavailability of the tracer of 97.5% and 97.6%, respectively (**Table 1**).

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TABLE 1. Bioavailability of dietary fatty acids during linoleate deficiency

	Control	Linoleate Deficient
Intake (mg/d)		
Linoleate	287 ± 10[a]	1.4 ± 0.1[b]
Palmitate	295 ± 10	314 ± 13
Stearate	1767 ± 62[a]	2090 ± 84[b]
Oleate	462 ± 16	439 ± 18
Excretion (mg/d)		
Linoleate	5 ± 1	ND
Palmitate	56 ± 11	75 ± 26
Stearate	434 ± 78	614 ± 213
Oleate	13 ± 3[a]	5 ± 1[b]
Bioavailability (%)		
Linoleate	98.3	100
Palmitate	81.0	76.1
Stearate	75.4	70.6
Oleate	97.2	98.9
^{14}C in fecal lipids		
dpm/g	43360 ± 24080	35620 ± 11450
% of dose	2.5 ± 1.6	2.6 ± 0.4

^{14}C in fecal total lipids was determined 48 h after an oral bolus of [1-^{14}C]linoleate. Values are given as means ± SD, n = 5–6/group; ND, not detected (<0.01% of fatty acids). Different superscript letters for paired data denote significantly different values ($P < 0.05$).

Compared to the controls, liver total lipids contained 5-times more ^{14}C in the linoleate-deficient group and 2.3-times more ^{14}C in the linoleate-deficient (+) group; these values represented 2, 10, and 4.6% of the total tracer given, respectively (**Fig. 1**).

The concentration of linoleate in liver total lipids was reduced to 7% of control values in the linoleate-deficient group but rebounded rapidly to 30% of control values in the linoleate-deficient (+) group. Compared to controls, liver linoleate contained 13.7-times more ^{14}C in the linoleate-deficient group and 4.9-times more ^{14}C in the linoleate-deficient (+) group (**Table 2**). Given the higher tracer recovery and lower concentration of linoleate in the linoleate-deficient group, the specific activity in liver linoleate was raised 172-fold in the linoleate-deficient group and 15-fold in the linoleate-deficient (+) group. Similarly for liver arachidonate, concentrations were lower, ^{14}C was higher and, consequently, specific activity was raised 20-fold in the linoleate-deficient group. Intermediate values were obtained in the linoleate-deficient (+) group (Table 2).

The amount of ^{14}C and its specific activity in three liver long chain fatty acids (palmitate, stearate, oleate) and in liver total sterols was determined as the measure of carbon recycling from [1-^{14}C]linoleate into de novo lipogenesis. Amongst these components of liver total lipids, only the concentrations of palmitate and oleate were affected by linoleate deficiency (89% increase for both). ^{14}C recovery was 2- to 7-times higher in these lipids in the linoleate-deficient group than in the controls with intermediate values in the linoleate-deficient (+) group. Specific activities were 2- to 5-times higher in the linoleate-deficient group than in the controls but did not differ between controls and the linoleate-deficient (+) group except for the sterols (**Table 3**).

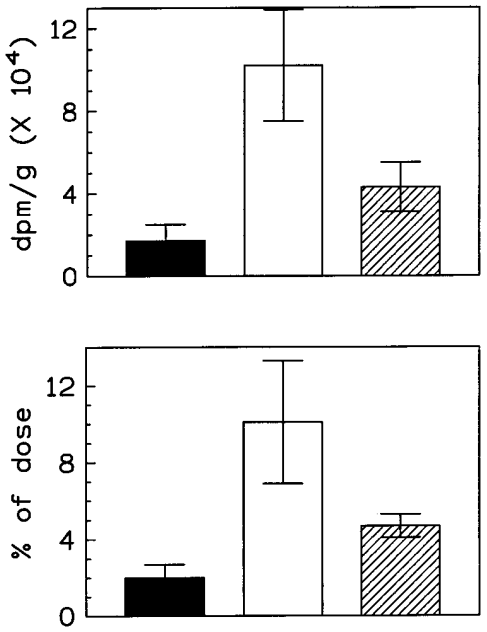

Fig. 1. ^{14}C recovered from liver total lipids 48 h after an oral dose of [1-^{14}C]linoleate given to control rats (□), rats deficient in linoleate (■) or rats deficient in linoleate given 256 mg linoleate with the radiotracer (▨). Each bar in both panels represents the mean ± SD (n = 5–6) and is statistically different from the other two bars ($P < 0.05$).

Of the total ^{14}C recovered in liver lipids of the controls, 41% was in arachidonate, 30% was distributed in lipids synthesized de novo, and 29% was in linoleate (Table 2, Table 3, and **Table 4**). In the linoleate-deficient groups, significantly higher amounts of ^{14}C were present in n–6 polyunsaturates (80–82%) and correspondingly lower amounts in lipids synthesized de novo (18–20%). Despite the lower % dose of ^{14}C in lipids synthesized de novo in the linoleate-deficient groups, similar amounts of ^{14}C were

TABLE 2. ^{14}C in liver n–6 polyunsaturates 48 h after an oral dose of [1-^{14}C]linoleate

	Control	Linoleate Deficient	Linoleate Deficient (+)*
Linoleate			
mg/g	3.0 ± 0.5	0.2 ± 0.1	1.0 ± 0.5
dpm/g	3961 ± 490[a]	54340 ± 6722[b]	19417 ± 2432[c]
Specific activity (dpm/mg × 10^3)	1.3 ± 0.3[a]	223 ± 119[b]	20 ± 1[c]
% ^{14}C**	29 ± 6[a]	63 ± 3[b]	49 ± 6[c]
Arachidonate			
mg/g	6.2 ± 1.3[a]	0.9 ± 0.2[b]	1.3 ± 0.4[a]
dpm/g	5831 ± 1224[a]	15849 ± 3326[b]	11771 ± 2470[b]
Specific activity (dpm/mg × 10^3)	0.9 ± 0.4[a]	18 ± 9[b]	9 ± 1[c]
% ^{14}C**	41 ± 5[a]	19 ± 4[b]	31 ± 6[c]

Values are given as means ± SD, n = 5/group. Different superscript letters for paired data denote significantly different values ($P < 0.05$).

*Linoleate-deficient plus a 256-mg bolus of linoleate with the tracer.

**Recovered in liver total lipids, based on dpm/g.

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TABLE 3. ^{14}C in liver lipids synthesized de novo 48 h after an oral dose of [1-^{14}C]linoleate

Lipid	Control	Linoleate-Deficient	Linoleate-Deficient (+)*
Palmitate			
mg/g	7.5 ± 2.0^a	14.2 ± 3.8^b	14.1 ± 7.1^{a,b}
dpm/g	440 ± 116^a	1962 ± 526^b	1181 ± 591^b
Sp act dpm/mg	59 ± 11^a	138 ± 37^b	84 ± 60^a
% ^{14}C**	3 ± 1	3 ± 1	3 ± 2
Stearate			
mg/g	5.7 ± 3.0	2.5 ± 1.1	5.4 ± 2.3
dpm/g	220 ± 114^a	528 ± 226^b	205 ± 86^a
Sp act dpm/mg	39 ± 20^a	211 ± 90^b	38 ± 16^a
% ^{14}C**	2 ± 1	<1	<1
Oleate			
mg/g	9.0 ± 1.7^a	17.0 ± 2.6^b	20.2 ± 3.1^b
dpm/g	440 ± 83^a	3018 ± 453^b	1181 ± 179^c
Sp act dpm/mg	49 ± 9^a	178 ± 27^b	58 ± 9^a
% ^{14}C**	3 ± 1	4 ± 1	3 ± 1
Sterols			
mg/g	12.5 ± 4.9	7.4 ± 3.0	10.0 ± 6.8
dpm/g	3080 ± 3030^a	9050 ± 2920^b	5830 ± 2820^{a,b}
Sp act, dpm/mg	247 ± 97^a	1227 ± 498^b	578 ± 395^c
% ^{14}C**	22 ± 4^a	11 ± 2^b	14 ± 3^b

Values are given as means ± SD, n = 5/group. Different superscript letters for paired data denote significantly different values ($P < 0.05$).

*Linoleate-deficient plus a 256-mg bolus of linoleate with the tracer.

**Recovered in liver total lipids, based on dpm/g.

present in liver lipids synthesized de novo as were recovered in liver arachidonate (18–19%; Tables 2, 3).

DISCUSSION

Using rats made specifically and severely linoleate deficient, our primary aim was to quantify the recycling of carbon from [^{14}C]linoleate in liver lipids. In controls, we observed that carbon recycling was equivalent to liver incorporation of the [^{14}C]linoleate itself. Linoleate-deficient rats accumulated 3-times more ^{14}C in products of de novo lipogenesis (dpm/liver or % of dose given) than the controls, an amount equivalent to that metabolized to [^{14}C]arachidonate. Given the severe depletion of n–6 polyunsaturates in the linoleate-deficient group yet equivalent recovery of the tracer in products of de novo lipogenesis as in arachidonate, it seems that carbon recycling from linoleate is not only obligatory, e.g., is not spillover because of excess intake, but is quantitatively significant. Thus, carbon recycling seems to be a biologically important pathway in linoleate metabolism and needs to be accounted for in determining the bioavailability of linoleate and the proportion of linoleate that is obligatorily metabolized outside the n–6 family of polyunsaturates. A study equivalent to the present one but assessing recycling of a tracer dose of α-linolenate during the deficiency of n–3 polyunsaturates has not yet been done but current evidence also strongly implicates recycling as an important pathway in the metabolism of n–3 polyunsaturates (8–11).

Essential fatty acid deficiency induces an increase in activity of the delta-6 and delta-5 desaturases resulting in increased conversion of linoleate to arachidonate (3). We have confirmed this (Table 3) with a 2.7-fold increase in [^{14}C]arachidonate (dpm/g) and a 20-fold increase in the specific activity of liver arachidonate in the linoleate-deficient group. Nevertheless, the 13.7-fold increase in liver [^{14}C]linoleate and the 172-fold increase in the specific activity of liver linoleate clearly shows that the predominant lipid response to severe and prolonged linoleate deficiency was a doubling of the proportion of [^{14}C]linoleate that was esterified in liver lipids. During linoleate-deficiency, linoleate esterification appears to be more sensitive to the effects of a single oral linoleate bolus than desaturation/chain elongation because its esterification decreased 65% whereas its conversion to arachidonate only decreased 26% in the linoleate-deficient (+) group (Table 2). Therefore, despite the marked esterification of supplemental linoleate in linoleate deficiency, it seems that maintaining arachidonate tissue levels is more important than restoring linoleate levels.

The aim of quantifying carbon recycling from [^{14}C]linoleate was to estimate its importance relative to com-

TABLE 4. Distribution of ^{14}C between n–6 polyunsaturates and lipids synthesized de novo in the liver 48 h after an oral dose of [1-^{14}C]linoleate

	Control	Linoleate Deficient	Linoleate Deficient (+)*
^{14}C in n–6 polyunsaturates**			
dpm/liver (×10^3)	216 ± 119^a	1305 ± 401^b	577 ± 213^c
% of ^{14}C in liver total lipids***	70 ± 10^a	82 ± 9^b	80 ± 8^b
% of dose	1.4 ± 0.5^a	8.4 ± 2.6^b	3.7 ± 0.5^c
^{14}C in lipids synthesized de novo****			
dpm/liver (×10^3)	88 ± 73^a	267 ± 71^b	144 ± 63^a
% of ^{14}C in liver total lipids***	30 ± 9^a	18 ± 3^b	20 ± 3^{a,b}
% of dose	0.6 ± 0.2^a	1.7 ± 0.5^b	0.9 ± 0.1^c

Values are given as means ± SD, n = 5–6/group. Different superscript letters for paired data denote significantly different values ($P < 0.05$).

*Linoleate-deficient plus a 256-mg bolus of linoleate with the tracer.

**Sum of linoleate and long-chain n–6 polyunsaturates.

***Based on dpm/g.

****Sum of sterols, 14–18 carbon saturates, and 14–18 carbon monounsaturates.

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plete β-oxidation in the obligatory catabolism of linoleate. On a mass balance basis, 65–70% of linoleate intake consumed at 2 energy % disappears, i.e., cannot be accounted for in the pool of n–6 polyunsaturates in the body (1, 12). Using [^{14}C]linoleate, β-oxidation and excretion in breath over 8 h accounted for 34% of the [^{14}C]linoleate given (1). Fecal excretion accounted for another 2.5% (Table 1). Within the liver alone, <1% of the [^{14}C]linoleate dose was recycled to de novo lipogenesis (Table 4). Assuming a similar degree of carbon recycling in carcass lipids as in the liver, the partitioning of linoleate at a normal linoleate intake of 2% of energy would be about 30% to n–6 polyunsaturates, 35–40% to CO_2, and 30–35% to de novo lipogenesis. In severe and prolonged linoleate deficiency, about half as much linoleate was partitioned towards breath CO_2 (1), but about 3-times as much was recycled into liver lipids synthesized de novo (Table 4).

In both the control and linoleate-deficient groups, sterols contained 60–70% of the recycled linoleate carbon in liver lipids (Table 3). This agrees with other reports in which carbon from tracer amounts of α-linolenate appears in brain cholesterol of suckling rats in amounts that markedly exceed its incorporation into brain docosahexaenoate (9–11). Linoleate and α-linolenate are more easily β-oxidized than other long chain fatty acids (13, 14) and may be more ketogenic as well (15). The linoleate-deficient rats in the present study consumed the same amount of food as the controls and excreted less carbon from [^{14}C]linoleate in breath CO_2 (1) so they did not recycle linoleate carbon due to increased ketosis and fatty acid β-oxidation caused by energy deficit.

During the suckling/neonatal period, ketones are important substrates not only for energy but also for de novo lipogenesis (16). Hence, by being ketogenic substrates, 18 carbon polyunsaturates appear to be available for cholesterol synthesis. The apparently obligatory recycling of carbon from linoleate into de novo lipogenesis in adult rats may therefore be a residue of a pathway that is functionally more important in neonatal life. It is unclear whether this level of recycling from linoleate is comparable to that from other long chain fatty acids such as palmitate; this is the subject of current research in our laboratory.

Two key points emerge from this study and the previous related study (1): *i*) a value of 2 energy % linoleate appears to exceed the actual requirement for linoleate because it is based on essential fatty acid deficiency (4) which, by being a dual nutrient deficiency, is more severe than linoleate deficiency per se (1, 5), and *ii*) the apparent bioavailability of linoleate when consumed at 2 energy % is about 30%. The obligatory loss of 70% of linoleate intake is split about equally between β-oxidation to breath CO_2 and carbon recycling. Hence examining linoleate β-oxidation on the basis of tracer recovery in breath CO_2 misses about 50% of the β-oxidized linoleate carbon that is recycled into de novo lipogenesis.

Assessing β-oxidation and recycling of linoleate is potentially a useful way to determine the true requirement for linoleate per se, which could be equivalent to the dietary level at which its bioavailability is highest, i.e., when linoleate is recycled/oxidized the least and most efficiently retained in the body pool of n–6 polyunsaturates. This information becomes more important under conditions of energy deficit, weight cycling, or when key co-factors such as zinc are deficient, because the loss of linoleate and α-linolenate to β-oxidation and recycling markedly increases and can prevent normal accumulation of these fatty acids in body tissues despite their apparently adequate intake (7, 17, 18).

The use of tissue saturation with long chain polyunsaturates as a method to determine linoleate (or α-linolenate requirements) yields a higher value in some tissues than in others (3, 5). There is no a priori reason why the plateau level of a long chain polyunsaturate in a tissue should reflect the optimal intake level of a precursor such as linoleate. As long as a margin of safety above a minimal requirement is satisfied, optimal cellular function is unlikely to depend on a specific saturation level of one or two components as other components such as cholesterol can readily be adjusted to maintain adequate function. In fact, the tissue saturation level of long chain polyunsaturates may strain the organism's ability to maintain optimal membrane function because of potentially increased requirements of other components such as antioxidants. Furthermore, Bourre et al. (5) elegantly showed that the tissue saturation level of n–6 polyunsaturates clearly depends on other nutrients, e.g., α-linolenate, which permits functional outcomes such as growth and reproduction to be adequately maintained on much less dietary linoleate (0.4% of energy) than was required to achieve tissue saturation of arachidonate (as high as 2.4% of energy for some tissues).

Others have also reported that the presence of α-linolenate in the diet reduces the impact of essential fatty acid deficiency on growth and overall health of rats (19). Hence, it appears to be harder to induce linoleate deficiency than essential fatty acid deficiency. This is reminiscent of the difficulty in obtaining reproducible gross symptoms of α-linolenate deficiency without multi-generation studies and points not to a diminished importance of these fatty acids but to the efficacy of conservation of the long chain polyunsaturates that helps prevent deficiency symptoms during inadequate intake of the 18 carbon precursors (1).

We would go further than to note that α-linolenate should be present in the diet if one is evaluating linoleate deficiency or metabolism of n–6 polyunsaturates. Oleate is also present in all diets except those that are essential fatty acid-deficient or fat-free. Recent evidence suggests that without a dietary source of oleate, rats cannot sustain 'normal' tissue oleate levels (20). This is not to say that there is necessarily a dietary requirement for oleate, but that the absence of dietary oleate may increase losses of linoleate to β-oxidation and, therefore, increase the apparent requirement for linoleate. Thus, to determine the true effects of linoleate or α-linolenate and the specific symptoms of their respective deficiencies and to relate these results to normal diets, oleate should also be present in the diet.

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In the future, emerging evidence that essential fatty acid deficiency is not equivalent to linoleate deficiency per se will hopefully discourage use of the former as a tool to investigate the metabolism of and requirement for polyunsaturates.

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REFERENCES

1. Cunnane, S. C., and M. J. Anderson. 1997. Pure linoleate deficiency in the rat: influence on growth, accumulation of n–6 polyunsaturates, and [^{14}C]linoleate oxidation. *J. Lipid Res.* **38:** 805–812.
2. Alfin-Slater, R. B., and L. Aftergood. 1968. Essential fatty acids reinvestigated. *Physiol. Rev.* **48:** 758–784.
3. Holman, R. T. 1971. Essential fatty acid deficiency. *Prog. Chem. Fats Other Lipids.* **9:** 275–348.
4. Reeves, P. G., F. H. Nielsen, and G. C. Fahey, Jr. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A rodent diet. *J. Nutr.* **123:** 1939–1951.
5. Bourre, J-M., M. Piciotti, O. Dumont, G. Pascal, and G. Durand. 1990. Dietary linoleate and polyunsaturated fatty acids in rat brain and other organs. Minimal requirements of linoleic acid. *Lipids.* **25:** 465–472.
6. Cunnane, S. C., S. C. R. Williams, J. D. Bell, S. Brookes, K. Craig, R. A. Iles, and M. A. Crawford. 1994. Utilization of [U-^{13}C]polyunsaturated fatty acids in the synthesis of long chain fatty acids and cholesterol accumulating in the neonatal rat brain. *J. Neurochem.* **62:** 2429–2436.
7. Cunnane, S. C., and J. Yang. 1995. Zinc deficiency impairs whole body accumulation of polyunsaturates and increases the utilization of [1-^{14}C]linoleate for de novo lipid synthesis in pregnant rats. *Can. J. Physiol. Pharmacol. Physiol.* **73:** 1246–1252.
8. Sheaff-Greiner, R. C., Q. Zhang, K. J. Goodman, D. A. Gissani, P. W. Nathanielsz and J. T. Brenna. 1996. Linoleate, α-linolenate, and docosahexaenoate recycling into saturated and monounsaturated fatty acids is a major pathway in pregnant or lactating adults and fetal or infant rhesus monkeys. *J. Lipid Res.* **37:** 998–1008.
9. Sinclair, A. J. 1975. Incorporation of radioactive polyunsaturated fatty acids into liver and brain of the developing rat. *Lipids.* **10:** 175–184.
10. Dhopeswarkar, G. A., and C. Subramanian. 1975. Metabolism of α-linolenic acid in developing brain. I. Incorporation of radioactivity from [1-^{14}C]α-linolenic acid into brain fatty acids. *Lipids.* **10:** 238–243.
11. Menard, C. R., K. J. Goodman, T. Corso, J. T. Brenna, and S. C. Cunnane. 1998. Recycling of carbon into lipids synthesized de novo is a quantitatively important pathway of [U-^{13}C]α-linolenate utilization in the developing rat brain. *J. Neurochem.* In press.
12. Cunnane, S. C., and M. J. Anderson. 1996. The majority of linoleate in the growing rat is partitioned towards oxidation or storage in visceral fat. *J. Nutr.* **127:** 146–152.
13. Leyton, J., P. J. Drury, and M. A. Crawford. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Br. J. Nutr.* **57:** 383–393.
14. Gavino, G. R., and V. Gavino. 1991. Rat liver mitochondrial carnitine palmitoyl transferase activity towards long chain polyunsaturated fatty acids and their CoA esters. *Lipids.* **26:** 266–271.
15. Emmison, N., P. A. Gallagher, and R. A. Coleman. 1995. Linoleic and α-linolenic acids are selectively secreted in triacylglycerol by hepatocytes from neonatal rats. *Am. J. Physiol.* **269:** R80–R86.
16. Edmond, J., N. Auestad, R. A. Robbins, and J. D. Bergstrom. 1985. Ketone body metabolism in the neonate: development and the effect of diet. *Fed. Proc.* **44:** 2359–2364.
17. Chen, Z-Y., and S. C. Cunnane. 1993. Refeeding after fasting increases apparent oxidation of n–6 and n–3 fatty acids in pregnant rats. *Metabolism.* **42:** 1206–1211.
18. Chen, Z-Y., C. R. Menard, and S. C. Cunnane. 1995. Weight cycling decreases whole body accumulation or polyunsaturates in young rats. *Br. J. Nutr.* **75:** 583–591.
19. Hansen, H. S., and B. Jensen. 1983. Urinary prostaglandin E_1 and vasopressin excretion in essential fatty acid-deficient rats: effect of linolenic acid supplementation. *Lipids.* **18:** 682–690.
20. Bourre, J-M., O. L. Dumont, M. E. Clement, and G. A. Durand. 1997. Endogenous synthesis cannot compensate for absence of dietary oleic acid in rats. *J. Nutr.* **127:** 488–493.

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