Retention of Linoleic Acid in Carcass Lipids of Rats Fed Different Levels of Essential Fatty Acids

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Rats of an inbred Sprague-Dawley strain were fed purified diets with low (0.3% of total energy), normal (3%) or high (10%) content of essential fatty acids (EFA) for at least three generations. Two 30-day-old rats with similar weights were chosen from one litter. One was killed; weight increase and food consumption of the other rat was measured for 15 days. Total lipid content and fatty acid composition in total lipid and lipid classes were determined in both rats. Seven pairs of rats from each group were treated in the same way. Calculations based on amount of linoleic acid ingested and retained in the carcass lipids showed that 50% of the ingested linoleic acid was retained in the low EFA rats compared to 10–15% in the normal and high EFA rats.
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The dietary level of EFA influences the utilization and metabolism of linoleic acid (1,2). Thus, a low level of EFA in the diet leads to low levels of linoleic acid in the tissue lipids. However, studies by Alling and coworkers (3) indicated that rats fed diets with low levels of EFA accumulated more of the dietary linoleic acid in the body fat than did rats fed a diet with a normal EFA content. In previous short-term studies, we have shown that rats fed low levels of EFA (0.3% of total energy) retained more ^{14}C-activity in the carcass from a single oral or intravenous dose of ^{14}C-labeled linoleic acid than rats fed normal (3%) or high (10%) levels (4,5). Using diets similar to those described by Alling and coworkers (3,6), we aimed in the present balance study to measure the retention of dietary linoleic acid in the body fat during a longer period in rats fed balanced diets with a low, normal or high content of EFA.

MATERIALS AND METHODS

Diets. The rats were fed pelleted, purified diets supplying 0.3% (low), 3% (normal) or 10% (high) of total energy as EFA (Table 1). The fatty acid composition of the diets is shown in Table 2. The ratio between linoleic and linolenic acid was 4:1 in both the normal and high EFA diets. The contribution of EFA from the fish protein and wheat starch preparations in the low EFA diet also was determined. One g of either fish protein or wheat starch was hydrolyzed with 7 M hydrochloric acid after addition of ethanol. The mixture was heated on a steam bath for one hr and the homogenate was extracted with ethyl ether and ethyl ether/petroleum ether (1:1, v/v). Methyl esters were prepared as described below and quantitatively determined by gas liquid chromatography (GLC), using heptadecanoic acid (17:0) and Δ7,10,13,16-docosatetraenoic acid (22:4ω6) as internal standards. The ratio between linoleic acid and total ω3 acids was ca. 7:1 in the low EFA diet.

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Rats. Rats from an inbred Sprague-Dawley strain were used (3,6). Females were mated at the age of 90 days. On the third or fourth day after birth, each litter was reduced to six animals, usually three males and three females. The dam and her offspring were housed together for 25 days. The pups then were separated from the mother and fed the experimental diets. Male rats from the fourth generation or more on the experimental diets (second for the high EFA group) weighing within ±2 SD of the mean representative of the given diet and age were selected. Two rats aged 30 days and with similar weights were chosen from one litter. One was killed immediately (30-day reference). The other (45-day) was placed alone in a cage and food consumption and weight were measured for 15 days. The rat then was killed. Gastrointestinal contents of the 30- and 45-day-old rats were removed and the carcasses were frozen until lipid analysis was performed. Seven pairs of rats from each dietary group were treated in the same way.

Quantitative determination of lipids. Samples of the diets were extracted with chloroform/methanol (C/M) (2:1, v/v) and the lipid content was determined gravimetrically according to Folch et al. (7). The frozen carcass was thawed for 15–30 min at room temperature and cut into thin (3–5 mm) slices with a knife. The slices were put into a 2-l Erlenmeyer flask containing 1 l C/M (1:1, v/v). After standing overnight with magnetic stirring, the solvent

TABLE 1

Composition of Diets

	Low EFA	Normal EFA	High EFA
Energy percent EFA	0.3	3.0	10.0
Energy percent fat	20	20	20
Energy percent protein	15	15	15
Constituents, g/kg diet			
Fish protein	175	175	175
Wheat starch	608	608	608
Sucrose	51	51	51
Fat mixture	91	91	91
Hydrogenated tallow	91	67.2	16.8
Sunflower seed oil	—	28.3	57.0
Linseed oil	—	5.5	17.2
Salt mixture[a]	39	39	39
Vitamin mixture[b]	2.0	2.0	2.0
Choline chloride	2.0	2.0	2.0
Cellulose	35	35	35

[a]USP 17 + per kg of salt mixture 0.088 g $KAl(SO_4)_2 \cdot 12\ H_2O$ 0.28 g NaF; 0.009 g $NaAsO_2$; 0.022 g $Na_2B_4O_7 \cdot 10\ H_2O$ and 0.0031 g $Na_2MoO_4 \cdot 2\ H_2O$. Selenium content was analyzed and found to be 0.46 mg/kg in all diets.

[b]Vitamins in 1 kg of diet: retinol 300 μg; ergocalciferol 12.5 μg; thiamin 50 mg; riboflavin 20 mg; pyridioxine 20 mg; nicotinamide 200 mg; panthotenic acid 100 mg; p-aminobenzoic acid 100 mg; menaquinone 10 mg; biotin 1 mg; folic acid 5 mg; cyanocobalamin 0.005 mg; myoinositol 1,000 mg, and tocopheryl acetate 500 mg.

TABLE 2

Fatty Acid Composition of Diets

Fatty acid	Wt % fatty acids		
	Low EFA	Normal EFA	High EFA
14:0	1.7	1.4	0.5
16:0	27.2	23.2	11.0
18:0	64.3	53.4	18.9
20:0	2.7	2.3	0.9
22:0	0.8	0.7	0.7
Σ Saturated	97.9	81.7	32.0
18:1ω9	0.7	5.0	16.9
18:2ω6	1.3	10.8	41.5
18:3ω3	tr	2.5	9.6
ω6/ω3	7[a]	4.3	4.3
mg 18:2/100 g diet	117	898	3490

[a]Based on separate analyses of fish protein and wheat starch.

was filtered into a 2-l flask. The slices were re-extracted with 500 ml C/M for 5–6 hr and homogenized in a Waring blender; the homogenate again was extracted with 500 ml C/M. The solvent was filtered into the flask and the volume adjusted to 2 l. The total lipid content in an aliquot of the extract then was determined gravimetrically after the extract had been freed from nonlipid contaminants by phase partition (8). The remainder of the carcass was hydrolyzed with 7 M hydrochloric acid after addition of some ethanol. The mixture was heated on a steam bath overnight or until the carcass was dissolved. Ten ml of this homogenate was extracted with 25 ml ethyl ether and 30 ml ethyl ether/light petroleum (1:1, v/v). The solvent then was evaporated and the lipid-like material weighed. This material represented 2–5% of the total carcass lipid. The quantity of fatty acids in this residue also was determined by GLC using heptadecanoic acid (17:0) and Δ7,10,13,16-docosatetraenoic acid (22:4ω6) as internal standards (see below) and was found to represent 1–2% of the total carcass fatty acids. The fatty acid composition of the homogenate did not show any important differences compared to the fatty acid composition of the C/M extract.

For quantitative determination of lipid classes, aliquots of the C/M extracts of the reference and experimental rats on the respective diets were pooled. Lipid phosphorus was assayed by a Bartlett method (9). Both cholesterol esters (10) and triglycerides (11) were determined colorimetrically.

Separation of lipids. The lipids of the pooled C/M extracts were separated into lipid classes by thin layer chromatography (TLC) (12). Portions of the cleaned lipid extracts containing 5–10 mg lipid were applied as 15-cm broad bands on 20 × 20 cm thin layer plates coated with 0.2 mm silica gel 60 (E. Merck AG, Darmstadt, West Germany). The plates were developed with 100 ml light petroleum/diethyl ether/glacial acetic acid (85:15:1, v/v/v). The plates were sprayed with water and the bands containing total phospholipids (PL) and triglycerides (TG) were transferred to glass tubes with Teflon screw caps. The tubes were dried overnight in a vacuum desiccator over P_2O_5.

Fatty acid analysis. The TG fatty acids were transmethylated with 1 ml of 2 vol% sulphuric acid in dry methanol at 100 C for 1 hr. The fatty acids of the PL were transmethylated with 1 ml of 0.1 M sodium methylate (12), whereas the fatty acids in the diet and carcass lipids were transmethylated using 14% BF_3 (13). The methyl esters were injected into a Hewlett-Packard 5830A gas chromatograph equipped with a flame ionization detector, a 25 m capillary column coated with P 1000 (Supelco, Bellefonte, Pennsylvania) and a Hewlett-Packard 18835B capillary inlet system. Helium was used as the carrier gas (2 ml/min). The column temperature was programmed as follows: 1 min at 100 C, then 30 C/min to 170 C and finally 4 C/min up to 210 C. The peaks were identified by comparing retention times with those of commercial GLC reference standards (Nu-Chek-Prep, Elysian, Minnesota) and quantified with a Hewlett-Packard electronic integrator model 18850A.

Identification of 18:2 isomers. Fatty acid methyl esters from the pooled C/M extracts of the low EFA rats were fractionated according to unsaturation by TLC on Silica Gel G plates containing 12.5% silver nitrate (14). The fractions containing monoenes and dienes were scraped off and gas-chromatographed. The dienes were further fractionated according to chain length by preparative scale gas chromatography (GC) (14). The 18:2 fraction was dissolved in hexane and subjected to ozonolysis. A sample of the reaction mixture, containing aldehydes and aldehyde esters was then analyzed by GC (14).

Calculation of linoleic acid retention in carcass. The amount of linoleic acid ingested by the 45-day-old rat was calculated from food consumption and fatty acid composition of the diets. The amount of fatty acids in the linoleic acid series retained in the carcasses was calculated from the lipid content × wt percent ω6 acids. By subtracting the amount in the 30-day-old reference rat from that in the 45-day-old rat, the retention of ingested linoleic acid by the tissues could be calculated.

Statistical analyses. One-way variance analysis was used to test whether any differences between the groups existed. If significant differences were found ($P < 0.05$), the Student-Newman-Keuls' test was used to compare the different groups. A paired t-test was used to detect any differences between the 30- and 45-day-old rats on each diet (15).

RESULTS

Body weight and fat content. Weight and food consumption data of the experiment are shown in Table 3. For two rats on the high EFA diet, the weight increase was substantially lower than for the other high EFA rats. The reason for this is not known, but could be due to some infection. These rats were excluded from the subsequent analyses. The initial weights of the 30-day-old reference and experimental rats were similar within each diet group. The 30-day-old rats of the low EFA group weighed significantly less than the normal EFA rats, whereas there were no significant differences between the normal and high EFA groups. At the end of the experiment, the low EFA and the normal EFA groups still differed in weight. The 45-day-old rats of the high EFA group weighed significantly less than those of the normal EFA group. The weight increase was lower in both the low and

TABLE 3

Weight and Food Consumption Data[a,b]

EFA level in diet	Reference rats, 30-day weight (g)	Experimental rats				
		30-day weight (g)	45-day weight (g)	Weight increase (g)	Food consumption (g)	Feed efficiency[c]
Low (n = 7)	64.5 ± 2.8**	64.6 ± 4.3**	133 ± 7.4**	68.7 ± 4.7*	213 ± 16	0.32 ± 0.03
Normal (n = 7)	78.4 ± 4.1	78.4 ± 4.3	156 ± 10	77.1 ± 8.2	226 ± 29	0.34 ± 0.02
High (n = 5)	79.8 ± 6.9	79.2 ± 6.4	142 ± 12*	63.1 ± 8.6**	171 ± 21*	0.37 ± 0.02*
	75.5	74.3[d]	104	29.7	135	0.22
	78.5	77.4[d]	98	20.4	110	0.19

[a]Results are mean ± SD; n = number of animals.

[b]Differences from normal EFA group: **, $P < 0.01$; *, $P < 0.05$. Absence of asterisk in the low EFA or high EFA row indicates a non-significant ($P > 0.05$) difference from the normal EFA group.

[c]g Weight increase per g food consumed.

[d]These rats grew poorly and were excluded from further analyses.

TABLE 4

Lipid Concentration and Fatty Acid Composition in Total Carcass Lipids from 30- and 45-day-old Rats[a]

Fatty acid	Low EFA group (n = 7)		Normal EFA group (n = 6)		High EFA group (n = 5)	
	30-day	45-day	30-day	45-day	30-day	45-day
			wt% fatty acid			
10:0	0.7 ± 0.6	trace[b]	1.5 ± 0.2	trace	1.6 ± 0.3	trace
12:0	2.4 ± 0.7	trace	3.0 ± 0.4	trace	2.8 ± 0.6	trace
14:0	4.4 ± 0.6	2.2 ± 0.2	4.2 ± 0.5	2.3 ± 0.1	3.2 ± 0.5	1.9 ± 0.2
16:0	30.6 ± 0.5	29.6 ± 0.9	27.8 ± 0.7	30.6 ± 0.8	19.5 ± 0.6	24.1 ± 1.6
16:1ω7	9.0 ± 0.6	10.5 ± 0.6	5.2 ± 0.6	8.4 ± 0.5	2.2 ± 0.3	3.9 ± 0.5
18:0	7.8 ± 0.7	7.4 ± 0.7	9.4 ± 0.6	7.9 ± 0.3	7.3 ± 0.4	8.0 ± 0.5
18:1ω9	41.1 ± 0.9	45.6 ± 0.9	31.7 ± 0.9	37.9 ± 1.0	23.2 ± 0.4	25.8 ± 0.6
18:2ω6	1.5 ± 0.2	1.8 ± 0.2	12.0 ± 1.4	8.5 ± 0.9	31.2 ± 0.9	28.8 ± 2.6
18:3ω3	trace	trace	1.4 ± 0.2	1.2 ± 0.2	4.9 ± 0.5	4.3 ± 0.4
20:3ω9	1.5 ± 0.2	1.5 ± 0.3	trace	trace	trace	trace
20:4ω6	1.1 ± 0.2	1.2 ± 0.3	2.9 ± 0.3	2.2 ± 0.2	2.6 ± 0.1	2.5 ± 0.1
22:6ω3	trace	trace	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.05	0.7 ± 0.4
Σ ω6	2.6 ± 0.3	3.0 ± 0.5	14.9 ± 1.6	10.7 ± 1.0	33.8 ± 0.8	31.3 ± 2.5
Σ ω3	trace	trace	2.4 ± 0.3	2.1 ± 0.2	6.4 ± 0.4	5.0 ± 0.5
ω6/ω3	—	—	6.2 ± 0.2	5.2 ± 0.4	5.3 ± 0.3	6.3 ± 0.5
			Lipid in carcass (mg/per g)			
	77.5 ± 9.4	66.7 ± 10.9	70.6 ± 6.3	65.4 ± 5.6	83.7 ± 4.1	74.4 ± 4.4

[a]Values are mean and SD; n = number of animals.

[b]Trace = < 0.1%.

high EFA group than in the normal EFA group. The food consumption was lower in the low EFA and high EFA groups than in the normal EFA groups but the differences were significant only between the two latter groups. The feed efficiency was higher in the high EFA group (Table 3).

For each dietary group, the fat concentration of the 30-day-old rats was higher than that of the 45-day-old rats (Table 4). The differences were significant ($P < 0.05$) only for the high EFA group. The 30-day-old rats from the high EFA group had a significantly higher body fat content ($P < 0.05$) than the rats of the normal EFA group, but at the end of the experiment there were no significant differences between the groups. The differences in fat content could be attributed to variations in the TG concentration.

Fatty acids. The fatty acid composition of the carcass fat was related to the differences in the dietary levels of EFA (Table 4). The differences were most pronounced for linoleic acid and monounsaturated fatty acids. The 18:2 fraction in the carcass lipids of the low EFA rats contained about 90% linoleic acid. Small amounts of 18:2ω7 (6.0%) and possibly 18:2ω9 (1.8%) also were detected.

The fatty acid composition in the TG and PL was determined in pooled samples, as the variations in the total fatty acid composition were small within the groups

TABLE 5

Fatty Acid Composition in Phospholipids from Pooled Total Carcass Lipids

	Low EFA		Normal EFA		High EFA	
Fatty acid	30-day	45-day	30-day	45-day	30-day	45-day
			Wt% fatty acid			
16:0	19.8	19.5	22.0	22.7	21.5	21.9
16:1	5.8	4.8	1.7	2.5	1.1	1.3
18:0	17.6	18.2	20.1	18.6	22.3	21.6
18:1	27.1	25.7	13.7	16.0	11.1	10.9
18:2ω6	6.4	6.6	13.2	14.2	17.4	17.6
18:3ω3	0.9	0.7	0.6	0.6	0.8	0.7
20:3ω9	8.8	9.8	0.9	1.0	0.1	n.d.[a]
20:3ω6	1.1	1.2	1.4	1.3	1.1	1.1
20:4ω6	7.0	8.1	15.0	12.7	13.7	14.3
20:5ω3	1.1	tr[b]	0.6	0.9	0.4	0.4
22:4ω6	0.6	0.6	1.4	0.9	1.2	1.2
22:5ω6	1.2	1.2	0.9	0.8	0.3	0.6
22:5ω3	0.2	0.4	2.0	1.6	2.8	2.4
22:6ω3	2.4	3.3	6.6	6.4	6.2	6.1
Σ ω6	16.3	17.7	31.9	29.9	33.7	34.8
Σ ω3	4.6	4.4	9.8	9.5	10.2	9.6
20:3ω9/20:4ω6	1.3	1.2	<0.1	<0.1	<0.1	<0.1
			Phospholipids in carcass (μmol/g)			
	16.1	14.5	16.3	14.9	17.3	13.3

[a]n.d., Not detected.
[b]tr = <0.1%.

(Table 4). In the triglycerides, the levels of saturated, monoenoic, linoleic and linolenic acids were closely related to those found in the total carcass lipids. Apart from traces of arachidonic acid, no other long chain PUFA could be detected in the TG.

The PL showed a different and more stable fatty acid composition (Table 5). The level of saturated fatty acids was similar in all groups. Eicosatrienoic acid, 20:3ω9, was detected only in the PL and the level decreased with increasing amounts of dietary EFA. The triene/tetraene ratio was 1.2–1.3 in the low EFA group and below 0.1 in the other groups. Other markers of EFA deficiency, e.g., monoenes, also decreased with increasing levels of EFA in the diet. The level of linolenic acid was uninfluenced by the dietary supply, but the percentage of the long chained metabolites 22:5ω3 and 22:6ω3 was higher in the normal EFA and high EFA groups than in the low EFA rats. The level of 22:5ω6 was increased in the low EFA group, reflecting the low supply of ω3 acids.

Linoleic acid retention. Data on the amount of linoleic acid retained in the carcass lipids of the rats are given in Table 6. A much higher percentage of the ingested linoleic acid was retained in the carcass lipids of the low EFA rats compared to the normal EFA or high EFA rats.

DISCUSSION

In a study of this type, it is very important to have rats with minimal variations in body fat content and fatty acid composition. For all diets, the initial weight of the experimental rats in most cases did not differ by more than 1 g. The breeding system resulted in small variations in body fat content and fatty acid composition. This strongly justifies the assumption that the body fat content and fatty acid composition of the 30-day-old experimental rats were similar to those of the 30-day-old reference rats.

As expected, the low EFA rats weighed significantly less than the normal EFA rats, both at 30 and 45 days of age. The 30-day-old high EFA rats were selected to weigh within ±2 SD of the standard mean of the normal rats at that age, since no growth curve yet had been established for the high EFA group. However, at 45 days of age, the high EFA rats weighed 9% less ($P < 0.05$) than the normal EFA rats (Table 3). To test if this was due only to chance, weight development was followed up to 90 days of age for a larger group of high EFA rats. The results showed that these rats weighed 10–15% less ($P < 0.05$) than the normal EFA rats at corresponding ages (Becker, W., unpublished observations).

At least two reasons for the retarded growth of the high EFA rats can be given. One obvious contributing factor was the lower food intake in the high EFA compared to the normal EFA group. In a subsequent similar experiment (Becker, W., unpublished observations), the food consumption of the high EFA group also was lower than that of the normal EFA group, but only by 10%, compared to 30% in the present study. This lower food intake could have been due to presence of oxidation products, since elevated peroxide values (40–80 meq/kg) were found in this diet after longer periods of storage. However, Kaunitz and collaborators (16,17) fed rats diets with 20% by weight of different mildly oxidized fats containing 10–324 meq/kg peroxides for periods up to 96 wk

TABLE 6

Deposition of ω6 Acids in Carcass Lipids[a,b]

EFA level in diet	ω6 acids			% of Retained ω6 acids in:[c]	
	Consumed (g)	Deposited (g)	Retention (%)	PL	TG
Low (n 5 7)	0.25 ± 0.02	0.12 ± 0.02	48.9 ± 8.6**	63	37
Normal (n = 6)	2.59 ± 0.33	0.27 ± 0.11	10.3 ± 3.9	65	35
High (n = 5)	5.97 ± 0.73	0.96 ± 0.27	15.9 ± 4.0	22	78

[a]Values are means and SD; n = number of animals.

[b]Differences from the normal EFA group: **, $P < 0.01$. Absence of asterisk in the low EFA or high EFA row indicates a nonsignificant ($P > 0.05$) difference from the normal EFA group.

[c]Calculated from pooled lipid extracts. PL, phospholipid; TG, triglyceride.

and found no negative effect on growth or food consumption in rats fed these rancid fats compared to rats fed the corresponding fresh fats.

Another, and in our opinion more important, explanation is that the high EFA diet had a specific stimulatory effect on the brown adipose tissue. In separate studies, it was found that these rats have higher levels of thermogenin in the brown fat adipose tissue compared to rats fed the other diets (18) and a higher energy expenditure following a subcutaneous injection of norepinephrine (19). These data strongly suggest that an increased capacity for nonshivering thermogenesis could be a major reason for the lower weight of these animals.

The EFA content of the carcass lipids correlated well with the dietary level. This was most clear in the TG. In the PL, the percentage of individual or total ω6 and ω3 acids was no more than twice as high in the normal EFA rats as in the low EFA rats, despite a 10-fold higher dietary supply of EFA to the normal EFA rats. In the high EFA group, the level of linoleic and total ω6 and ω3 acids was similar to that of the normal EFA group. This probably is due to the fact that the ratio between, rather than absolute amounts of, linoleic and linolenic acids in the diet, which was the same in these two latter diets, regulates their subsequent metabolism (1,2,20).

It is known that the lipids of rats fed fat-free diets contain isomeric unsaturated fatty acids that are uncommon in normal rats (21). Sand and coworkers (21) fed weanling EFA-normal rats a fat-free, casein-sucrose diet and analyzed the occurrence of unsaturated isomers in the carcass lipids after different periods on this diet. After two months on this diet, only about half of the 18:2 fraction was linoleic acid, the rest being a mixture of 18:2ω7, 18:2ω9 and 18:2ω10. After six mo, the linoleic acid accounted for only about 20% of the 18:2 fraction. Our own analyses showed that linoleic acid constituted almost 90% of the 18:2 acids, indicating that the other isomers are formed only after long-term fat-free feeding or when the supply of EFA is lower than 0.2–0.3% of total energy.

The results of this study show that rats fed a diet with a marginal EFA content retain more of an ingested amount of linoleic acid than rats fed sufficient or high amounts of EFA. This is in agreement with earlier observations by Alling et al. (3). They fed similar diets, containing 0.07, 0.75 or 3.0% of total energy as EFA, to rats and calculated that rats from the 0.07% group retained ca. 50–70% of the linoleic acid fed compared to 5–20% in the other two groups. In our study, the retention was about 50% in the low EFA group and around 10% in the normal EFA group.

Previous studies with rats fed the same diets also showed that the carcass retention of a single oral dose of 1-^{14}C-linoleic acid was significantly greater in rats fed the low EFA diet than those fed the normal EFA or high EFA diets (4). Further studies also showed that the larger retention seen in the low EFA rats was due to a higher incorporation of ^{14}C into the PL than into the TG, whereas the opposite was found in the normal and high EFA groups (22). Similar observations were made by Catala and Brenner (23), who found in fat-deficient rats a specific incorporation of oral unlabeled linoleic acid into the PL of the liver and other viscera during the first 48 hr of administration, whereas the incorporation into the TG was small in these tissues.

Data from the present study also show that in the low EFA rats more of the retained ω6 acids was deposited in the PL and less in the TG, whereas in the high EFA rats the opposite was found (Table 6). In contrast to the results from the ^{14}C-tracer study (22), however, there were no differences between the low EFA and normal EFA group in this respect. The reason for this discrepancy is not clear, but it could be that the long-term incorporation of ω6 acids at this dietary level is different from that of a single tracer dose. One possible explanation of the present results is that the level of linoleic acid in the normal EFA diet (2.2% of total energy) is below the optimum for the growing rat and that most of the retained dietary linoleic acid is needed for the synthesis of membrane PL.

In conclusion, the results of the present balance study and of the experiments with labeled linoleic acid agree with respect to the prinipal differences between the dietary groups, e.g., the low EFA rats retained more of a given dose of linoleic acid in their tissues than rats fed the other two diets.

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