Role of Linoleate as an Essential Fatty Acid for the Cat Independent of Arachidonate Synthesis[1]

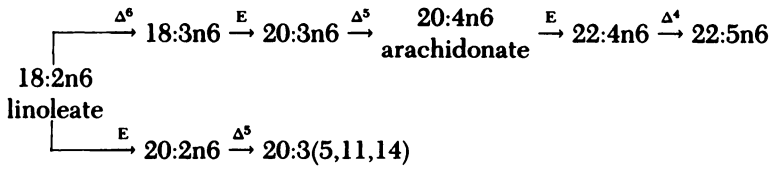
MARNIE L. MacDONALD, QUINTON R. ROGERS AND JAMES G. MORRIS

Departments of Physiological Sciences and Animal Science, University of California, Davis, CA 95616

ABSTRACT To determine the essential fatty acid (EFA) requirements of the cat, specific pathogen-free kittens were fed either a linoleate-deficient diet or one of two diets containing 5% safflower seed oil (SSO) with or without 0.2% tuna oil. The diets were fed for 82–101 weeks beginning at 3 months of age. The results showed that linoleate is an essential fatty acid for the cat. Linoleate deficiency resulted in reduced feed efficiency (in males), high rates of transepidermal water loss, poor skin and coat condition, and fatty liver. These manifestations of EFA deficiency were prevented by SSO. Tuna oil had no additional effect. Analyses of the fatty acid composition of plasma, erythrocytes and liver lipids revealed that linoleate deficiency caused changes that were qualitatively, but not quantitatively similar to EFA deficiency in the rat. When SSO was provided, linoleate was elongated and desaturated at the Δ^5 position to form 20:2n6 and 20:3(5,11,14). However, there was negligible conversion of linoleate to arachidonate. These results indicate that linoleate has specific functions as an EFA, independent of arachidonate synthesis and prostaglandin formation. J. Nutr. *113:* 1422–1433, 1983.

INDEXING KEY WORDS linoleate · arachidonate · essential fatty acids · cat · prostaglandins

All mammals require some fat in the diet. Linoleate meets the essential fatty acid (EFA) requirements of most mammals, including the rat, dog and human (1). In the livers of these species, linoleate is converted to arachidonate by alternating desaturation and elongation. The known pathways for the n6 fatty acids are as follows (2):



where Δ^6 stands for desaturation at the Δ^6 position, and E stands for elongation. Although linoleate at 1–2% of dietary energy cures EFA deficiency in these species, it is not known whether linoleate alone is responsible for the cure or whether arachidonate, synthesized from linoleate, is the physiologically essential fatty acid (3, 4).

In 1975, Rivers and co-workers suggested that the cat could not convert linoleate to arachidonate (5). Cats fed purified diets containing linoleate but lacking arachidonate had low levels of arachidonate in plasma compared to cats fed commercial diets high in arachidonate. When [1-^{14}C]linoleate was injected into cats, there was no evidence for

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incorporation of radioactivity into arachidonate (6). Furthermore, these authors reported signs of EFA deficiency in cats fed a diet high in linoleate (25% safflower seed oil (5, 7).

The essentiality of linoleate for the cat has not been determined previously. The purpose of the present study was to determine whether linoleate, supplied by safflower seed oil, is essential for the cat. Our results confirm that linoleate is not significantly converted to arachidonate in the cat. Nevertheless, linoleate prevents several of the classical signs of EFA deficiency: scaly skin, increased transepidermal water loss and fatty liver. These results provide evidence that linoleate has a specific role as an essential fatty acid independent of requirements for arachidonate and prostaglandins.

MATERIALS AND METHODS

Animals

Specific pathogen-free, domestic short hair kittens, vaccinated against panleukopenia, were weaned onto a diet of commercial canned and dry cat foods, then adapted to a purified diet containing 25% chicken fat. Kittens were housed individually in stainless-steel cages in temperature-controlled rooms with food and water available ad libitum. At 3 months of age, twelve female and twelve male kittens with mean body weights (±SEM) of 1053 ± 105 g and 1083 ± 59 g, respectively, were randomly allocated to three groups of 8 (4 females and 4 males). For 2 weeks, kittens were fed the experimental diets (table 1) with hydrogenated coconut oil instead of hydrogenated beef tallow. Due to low acceptability of diets containing hydrogenated coconut oil, hydrogenated beef tallow was substituted after 2 weeks, and the experiment was begun. For the determination of growth rates, food intake and feed efficiency, cats were weighed every 2 days, and food intake was measured daily for 7 weeks. Thereafter, cats were weighed twice weekly.

One male cat fed diet A was killed after 4 weeks of the study due to a congenital deformity of the spine, and one male cat fed diet C was removed from the study after 12 weeks due to weight loss of unknown cause.

TABLE 1

Composition of experimental diets

Ingredient	Diet A	Diet B	Diet C
		% by weight	
Fat source[1]			
Hydrogenated beef tallow[2]	35	30	29.8
Safflower seed oil[3]	0	5	5
Tuna oil[4]	0	0	0.2
Other ingredients in A, B & C			
Casein[5]		34.0	
Cornstarch[6]		5.46	
Cerelose[7]		7.0	
Sucrose[8]		9.0	
Mineral mix[9]		6.3	
Vitamin mix[10]		2.3	
L-Methionine[11]		0.25	
L-Arginine · HCl		0.5	
Sodium acetate[12]		0.19	

[1] See table 2 for composition. [2] Hydrogenated flaked edible tallow, Bunge Edible Oils, Fort Worth, TX. Gift from Kal Kan Foods Inc., Vernon, CA. [3] Pacific Vegetable Oils International, Inc., Richmond, CA. [4] Gift from Starkist Foods Inc., Terminal Island, CA. [5] Vitamin-free casein, U.S. Biochemical Corp., Cleveland, OH. [6] Melojel, food grade cornstarch, National Starch and Chemical Co., Bridgewater, NJ. [7] Staleydex dextrose, A.E. Staley Mfg. Co., Decatur, IL. [8] Amstar Corp., San Francisco, CA. [9] Supplied in grams per 100 g mineral mix: $CaHPO_4$, 39.0; KCl, 20.0; NaCl, 14.0; $CaCO_3$, 11.0; K_2HPO_4, 9.0; $MgSO_4$, 4.5; $MnSO_4 \cdot H_2O$, 0.384; $ZnSO_4 \cdot 7H_2O$, 0.445; $CuSO_4 \cdot 5H_2O$, 0.080; $FeC_6H_5O_7 \cdot 3H_2O$, 1.00; $Ca_5(IO_6)_2$, 0.015; $SnCl_2 \cdot 2H_2O$, 0.010; Na_2SeO_3, 0.003; $(NH_4)_6Mo_7O_4 \cdot 4H_2O$, 0.004; $CrCl_3 \cdot 6H_2O$, 0.026; $NiCl_2 \cdot 6H_2O$, 0.030; NaF, 0.014; $NH_4VO_3 \cdot 4H_2O$, 0.002; NaCl, 0.487 (carrier for trace minerals). After 7 months of the study, 14 g of NaCl was replaced with $NaHCO_3$ and 10 g of KCl was replaced with $KHCO_3$ (to ensure blood neutrality). [10] Gift from the Dept. of Agriculture and Animal Health, Roche Chemical Division, Hoffman-LaRoche Inc., Nutley, NJ. Supplied per kilogram diet: retinyl acetate, 26,054 IU; cholecalciferol, 2605 IU; 2-ambo-α-tocopherol, 416 IU; cyanocobalamin, 65 μg; and in milligrams: riboflavin, 13; nicotinic acid, 130; calcium pantothenate, 26; menadione, 5.79; folic acid, 13; pyridoxine · HCl, 13; thiamin mononitrate, 31.7; *myo*-inositol, 261; *d*-biotin, 1.3; ascorbic acid (as preservative for thiamin), 521; choline bitartrate, 8190; taurine, 975. [11] For the first 6 months of the study, L-methionine was supplied at 0.75% to meet the requirements for growth. [12] Added to balance arginine · HCl.

All other cats were fed the experimental diets for 82 weeks. After 62 weeks, rooms were dehumidified to 55% relative humidity to accentuate dermal problems. Blood samples were taken at 0, 2, 4, 6, 10, 15, 42 and 80

weeks. Data is reported only for the initial, 10-week and 80-week samples. Males were killed at 82 weeks for the determination of organ weights and liver fatty acid composition.

In a second study, 2 males and one female cat were fed diet B and one male cat was fed diet A for 101 weeks. In this experiment, hydrogenated coconut oil replaced hydrogenated beef tallow in the diets from 0 to 24 weeks. The room was dehumidified after 78 weeks. Because of similar body weights, clinical condition and blood fatty acid composition, these cats were included with those of experiment 1 for the measurements reported in tables 4, 5 and 8.

Experimental diets

The composition of the experimental diets is shown in table 1. Diets were prepared by mixing the dry ingredients, then adding melted hydrogenated beef tallow and mixing again. Tuna oil (diet C) was mixed with safflower seed oil and added last. While warm, the diets were extruded through a meat grinder (Hobart Mfg. Co., Troy, OH) to form pellets, approximately 1 cm in diameter. These were then stored under refrigeration.

The fatty acid composition of dietary fats is shown in table 2. Hydrogenated beef tallow provided only traces of 18:2 (0.32% of kilocalories), and was used to induce a linoleate deficiency. Safflower seed oil (diets B and C) provided linoleate (6.4% of kilocalories). Tuna oil, which was preserved with 0.1% ethoxyquin, provided arachidonate (0.006% of kilocalories) and other n6 and n3 polyunsaturated fatty acids. The manufacturer's analysis of tuna oil was as follows: total fatty matter, 96%; unsaponifiables, 2%; moisture, 1%; iodine value, 130. Vitamin E was provided at approximately four times the NRC requirement (8) to ensure adequacy and to prevent possible changes in blood fatty acids due to vitamin E deficiency (9).

TABLE 2

Fatty acid composition of dietary fats[1]

Fatty acid	Hydrogenated beef tallow	Safflower seed oil	Tuna oil
	% of total fatty acids by weight		
14:0	3.27	0.11	3.46
15:0	0.48	—	0.81
16:0	25.6	6.72	19.7
t16:1	0.34	—	0.77
c16:1	0.66	0.10	6.22
17:0	2.34	—	1.88
18:0	61.3	2.46	5.49
t + c18:1	4.23	11.7	22.9
18:2	0.56	77.7	1.26
20:0	0.54	0.36	0.27
18:3n3	—	0.11	0.35
20:1n9	0.04	0.27	2.47
21:0	0.04	—	—
20:2n6	—	—	0.57
20:4n6	—	—	1.84
20:3n3	—	—	0.42
22:0	0.14	0.19	—
20:5n3	—	—	5.14
24:0	—	0.10	—
22:4n6	—	—	0.70
22:5n6	—	—	1.52
22:5n3	—	—	1.29
22:6n3	—	—	22.6

[1] See text for method of analysis.

Methods

Blood samples were taken from the jugular vein of unanesthetized cats at approximately the same time (1000–1200 hours) on each sampling day. None of the samples were visibly lipemic. Heparinized blood was centrifuged to obtain plasma, and erythrocytes were washed three times with cold 0.9% NaCl. Plasma and washed erythrocytes were stored at −80°. Within 2 weeks of sampling, lipids were extracted with chloroform: methanol:water in the proportions described by Bligh and Dyer (10) with an increased solvent-to-sample ratio of 30:1. Butylated hydroxytoluene (BHT, 0.05%) was added to the solvents. Extracts were stored under nitrogen at −80°. Methyl esters were prepared by saponification followed by esterification with BF_3:methanol (11). Esters were extracted with isooctane and analyzed by gas chromatography on 3.0 m by 2.67 mm stainless-steel columns packed with 10% SP-2330 on 100/120 Chromosorb W AW (Supelco Inc., Bellefonte, PA). Analyses were performed at 180° with a Hewlett-Packard 5700A gas chromatograph equipped with a 7671A auto sampler and 3352A laboratory data system (Hewlett-Packard, Sacramento, CA). A known mixture of simple triglycerides was used as a primary standard. Mixtures

of fatty acid methyl esters (Applied Science, State College, PA and Nu-Chek Prep, Elysian, MN) were chromatographed to determine retention times of the fatty acids of interest. In this chromatographic system, 22:0 preceded 20:4n6; 22:1n9 emerged after 20:4n6; and 24:0 preceded 22:4n6. Values for 22:0, 22:1n9 and 24:0 are not reported. Because 20:4n6 and 20:3n3 cochromatographed, representative samples were also analyzed on 15% OV-275 at 200° in a 6.1 m by 2.67 mm column to determine the contribution of 20:3n3 to the 20:4n6 peak. The amount of 20:3n3 was less than 0.3% by weight in all samples tested.

Livers taken from male cats after death were wrapped in aluminum foil, frozen in liquid nitrogen and stored at −80°. Lipids were extracted with chloroform:methanol (2:1, vol/vol) containing BHT (0.05%). Lipid extracts were analyzed for phosphorous by the method of Bartlett (12) as modified by Kates (13). Phospholipids were separated by thin-layer chromatography on Silica Gel-G, 250 μm (Supelco Inc.) with hexane: ether:acetic acid (90:10:1, vol/vol/vol) as the developing solvent. Phospholipids were extracted by two washes with chloroform: methanol:water (5:5:1, vol/vol/vol) containing BHT. Total lipid extracts and phospholipids were methylated and analyzed by gas chromatography as described above.

Plasma levels of prostaglandins, PGE and PGF, were measured at weeks 59–75 of the study. Blood (12 ml) was collected into heparinized tubes containing 1 mg indomethacin. Plasma was acidified with HCl (final concentration, 0.6 *N*) and protein was sedimented by centrifugation. Lipids were extracted from the supernates with chloroform. Prostaglandins were separated by column chromatography on silicic acid as described by Giri and Krishna (14) and were measured by radioimmunoassay. (Clinical Assays, Cambridge, MA). PGE was measured after conversion to PGB with sodium hydroxide. The antibody cross-reactivities were 100% for PGB_1 and 23% for PGB_2 in the PGB assay; and 100% for $PGF_{2\alpha}$ and 28% for $PGF_{1\alpha}$ in the PGF assay. Values were corrected for recoveries, which were calculated by adding traces of [^{3}H]PGE_2 or [^{3}H]$PGF_{2\alpha}$ to acidified plasmas before extraction.

Basal metabolic rates of cats fasted for 24 hours were measured at weeks 61–77 of the study by indirect calorimetry as described by Kane, Morris and Rogers (in preparation).

Transepidermal water loss was measured at weeks 72–88 of the study as described by Elias and Brown (15). A stream of dry nitrogen, which was passed over a hairless area (1 cm^2) of the pinna of the ear, was analyzed with a Model W electrolytic moisture analyzer (MEECO, Warrington, PA). The nitrogen flow rate was kept constant at 100 ml/minute. Values were corrected for traces of water in the gas, and values for left and right ears were averaged.

At the end of the study, a dermal score was assigned to each cat as follows: 0: no scaling of the skin; normal, glossy hair coat with no thinning; some normal shedding. 1: mild scaling, mainly in the sacral region; normal hair coat and normal shedding. 2: mild scaling as for 1; dry, dull hair coat; normal shedding. 3: excessive scaling; hair coat dull and thin; normal or excessive shedding. 4: severe scaling, not confined to the sacral region but most severe there, with flakes 2–3 mm in diameter; severe shedding, at least twice the normal rate; dry hair coat.

Statistical analyses were performed on a Burroughs B7800 computer (Detroit, MI) (16). Analysis of variance was used to detect significant effects of treatments, and Duncan's multiple-range test was used to detect which means were significantly different at $P < 0.05$. For fatty acid analyses, quantities less than approximately 0.03% by weight were not detectable (ND in tables) and were treated as missing data. When a fatty acid was missing for more than one-half of the cats in any group, the entire group was designated ND in tables.

RESULTS

Growth, food intake and feed efficiency

In female cats there was no significant effect of diet on mean weight gain, food intake, feed efficiency or final body weight (table 3). In males, weight gain and food intake were highest in group B. When feed efficiency was calculated, however, the values for groups B and C were equal and almost twofold higher than the value for group A. Thus dietary li-

TABLE 3

Effect of source of dietary fat on weight gain, food intake and feed efficiency of kittens[1,2]

Diet	Mean wt gain 0–7 weeks	Mean food intake 0–7 weeks	Feed efficiency 0–7 weeks	Final body wt 82 weeks
	g/day	*g/day*	*g weight gain / g food intake*	*g*
Females				
A	16.1[a]	82.2[a]	0.19[a]	2728[a]
B	11.7[a]	56.6[a]	0.21[a]	2915[a]
C	12.5[a]	66.8[a]	0.21[a]	2691[a]
Pooled SEM	1.9	7.8	0.02	226
Males				
A	11.7[a]	78.6[a]	0.15[a]	3747[a]
B	24.8[b]	99.9[a]	0.26[b]	4302[a]
C	17.4[ab]	73.8[a]	0.26[b]	4128[a]
Pooled SEM	2.5	8.8	0.02	334

[1] Values are means; $n = 4$ except males fed diet A ($n = 3$). [2] Means within a response criterion not sharing a common superscript are significantly different, $P < 0.05$.

noleate deficiency reduced feed efficiency in male, but not in female cats. A reduction in feed efficiency also occurs in EFA deficiency in the rat (17). Despite the low feed efficiency of males fed diet A, final body weights at week 80 were not significantly altered by diet. The characteristic early plateau in body weight observed in EFA-deficient rats (18) was not seen in cats.

Signs of EFA deficiency

The effects of diet on transepidermal water loss, dermal score, basal metabolic rate and levels of plasma prostaglandins are shown in table 4. Values for male and female cats were not significantly different and were pooled.

Transepidermal water loss was increased fourfold in cats fed the linoleate-deficient diet. Rates of water loss were low in cats fed diets containing safflower seed oil with or without tuna oil (diets B and C). Large increases in water loss also occur in rats fed linoleate-deficient diets (15).

The skin and coat condition were poor in cats fed diet A, with a subjective dermal score three times that of cats fed the diets containing safflower seed oil. The condition of the linoleate-deficient cats deteriorated after rooms were dehumidified. The skin of these cats was extremely thin and friable, and one female cat fed diet A for 85 weeks developed spontaneous lesions over dorsal and ventral surfaces. Histological examination after 80

TABLE 4

Transepidermal water loss, dermal score, basal metabolic rate and plasma prostaglandin concentrations in cats fed linoleate-deficient or adequate diets[1,2]

Diet	Transepidermal water loss	Dermal score	Basal metabolic rate	Plasma PGE	Plasma PGF
	$mg/(cm^2 \cdot hr)$		$kcal/wt^{3/4}$	*pg/ml*	*pg/ml*
A	0.27[a] (8)	2.2[a] (8)	86.5[a] (4)	104.3[a] (4)	62.2[a] (8)
B	0.07[b] (11)	0.7[b] (11)	86.8[a] (5)	96.5[a] (7)	63.9[a] (9)
C	0.05[b] (7)	0.2[b] (7)	94.0, 98.8	96.5[a] (5)	74.8[a] (5)
Pooled SEM	0.05	0.3	4.7	6.1	6.5

[1] All measurements were made after 60 weeks of the study; number of animals in parentheses. [2] Means not showing a common superscript are significantly different, $P < 0.05$.

weeks revealed moderate to excessive hyperkeratosis in all cats fed diet A, but there was no evidence of the epidermal hyperproliferation commonly observed in EFA-deficient rats (15). Staining with oil Red O, a neutral lipid marker, was not less intense in linoleate-deficient cats, in contrast to deficient rats (15).

The good condition of the cats fed diets adequate in linoleate contrasts with the results of Rivers et al. (5) who reported dry hair coats and severe dandruff in cats fed purified diets containing safflower seed oil; however, other differences in the composition of the purified diets make direct comparisons difficult. Rivers and Frankel (19) also reported abrasions on the hocks of cats fed a diet adequate in linoleate. We observed similar hock lesions in some of the cats from each group. Although there was a higher incidence in group A, the lesions appeared to be due in part to confinement in cages, because they disappeared in cats allowed to run free in 3 m by 5 m rooms.

The basal metabolic rate, which is elevated in EFA-deficient rats (20), was not significantly altered by linoleate status in cats.

Levels of plasma PGE and PGF were measured to determine whether changes in fatty acid composition of the diet altered endogenous prostaglandin levels. Hwang and Carroll reported that higher levels of prostaglandins were produced in the serum of rats fed linoleate than in EFA-deficient rats (21). In the plasma of cats, the levels of PGE and PGF were less than 100 pg/ml, which is a comparable concentration to that in human plasma (22). Circulating prostaglandin levels were not significantly affected by diet.

Other physiological changes in EFA-deficient rats include a notching in electrocardiograms (23), increased systolic blood pressure (24), decreased intraocular pressure (25) and abnormal electroretinograms (26). All of these parameters were assessed in cats fed the experimental diets for longer than 60 weeks, and there was no evidence of abnormal responses in any of the three groups (data not shown).

The organ weights and liver composition of male cats are shown in table 5. Linoleate-deficient cats had enlarged livers that showed fat infiltration on gross pathology. Testis weights were lower in cats fed diet A, but this difference was not statistically significant. The relative weights of other major organs were not affected by diet. Histological changes were observed in several organs of linoleate-deficient cats, including the liver, adrenals, adipose tissue and testis (MacDonald, M. L., Anderson, B., Rogers, Q. R. and Morris, J. G., unpublished data).

TABLE 5

Organ weights and liver composition of male cats fed experimental diets for 82–101 weeks[1]

Measures	Diet A	Diet B	Diet C	Pooled SEM
Body wt, *g*	4070[a]	4266[a]	4000[a]	365
Liver, *% of body wt*	3.95[a]	2.32[b]	2.18[b]	0.18
Kidneys, *% of body wt*	1.02[a]	0.93[a]	0.95[a]	0.06
Heart, *% of body wt*	0.35[a]	0.28[a]	0.33[a]	0.03
Spleen, *% of body wt*	0.31[a]	0.26[a]	0.26[a]	0.03
Testes, *% of body wt*	0.067[a]	0.083[a]	0.092[a]	0.008
Liver composition:				
Water, %	59.5[a]	65.8[b]	66.6[b]	1.8
Lipid, *% of wet wt*	8.34[a]	5.52[ab]	3.76[b]	1.27
Total lipid, *g/100 g body wt*	0.32[a]	0.13[b]	0.08[b]	0.04
Phospholipid, *% of lipid*	10.7[a]	17.7[a]	27.9[a]	5.2

[1] Means not showing a common superscript are significantly different, $P < 0.05$.

The livers of linoleate-deficient cats had a lower water content and a 250% increase in total lipid compared to the other groups. The phospholipid content of liver lipid was reduced in cats fed diet A, but this large difference in means was not statistically significant due to high variability among cats.

Effects of linoleate deficiency on plasma and erythrocyte fatty acids

Changes in the fatty acid composition of plasma and erythrocyte lipids had occurred by 10 weeks of the study (tables 6, 7). Linoleate deficiency resulted in a decrease in

TABLE 6

Effects of experimental diets on plasma fatty acid composition in cats[1,2]

Fatty acid	Initial[3]	Week 10 of study				Week 80 of study			
		Diet A	Diet B	Diet C	Pooled SEM	Diet A	Diet B	Diet C	Pooled SEM
	% of total fatty acids by wt					*% of total fatty acids by wt*			
16:0	11.7 ± 0.2	14.6[a]	11.9[ab]	10.2[b]	1.0	14.4[a]	11.5[b]	12.6[b]	0.47
16:1	1.04 ± 0.06	5.96[a]	2.27[b]	1.36[b]	0.35	7.11[a]	1.68[b]	1.59[b]	0.31
17:0	0.18 ± 0.01	1.09[a]	0.82[b]	0.73[b]	0.09	0.77[a]	0.60[b]	0.70[ab]	0.03
18:0	21.1 ± 0.2	16.4[a]	17.7[ab]	21.3[b]	1.5	17.1[a]	21.2[b]	21.4[b]	0.6
18:1	7.53 ± 0.30	35.1[a]	14.5[b]	14.7[b]	2.0	47.1[a]	13.8[b]	13.4[b]	0.8
18:2n6	46.0 ± 0.4	12.7[a]	42.4[b]	39.7[b]	2.6	7.47[a]	45.3[b]	41.6[c]	0.9
18:3n6	0.14 ± 0.03	0.13[a]	0.21[a]	0.11[a]	0.04	0.17[a]	0.23[a]	0.16[a]	0.05
20:0	0.25 ± 0.01	0.29[a]	0.27[a]	0.31[a]	0.04	0.13[a]	0.19[b]	0.20[b]	0.02
18:3n3	0.04 ± 0.00	0.16[a]	0.05[b]	0.05[b]	0.01	0.25[a]	0.13[a]	0.20[a]	0.04
20:1n9	0.25 ± 0.02	0.60[a]	0.30[b]	0.33[b]	0.07	0.75[a]	0.25[b]	0.28[b]	0.04
21:0	0.18 ± 0.03	0.19[a]	0.06[b]	0.08[b]	0.02	0.30[a]	0.08[b]	0.10[b]	0.04
20:2n6	0.84 ± 0.14	0.66[a]	1.22[ab]	1.42[b]	0.21	0.09[a]	0.73[b]	0.67[b]	0.01
20:3n9	ND[4]	0.46	ND	ND	0.17	1.63	ND	ND	0.23
20:3(5,11,14)	0.80 ± 0.07	0.24[a]	0.68[b]	0.73[b]	0.08	ND	0.59	0.71	0.01
20:3n6	1.79 ± 0.35	0.89[a]	0.77[a]	0.52[a]	0.18	1.10[a]	1.76[a]	1.27[a]	0.31
20:4n6	1.46 ± 0.07	0.71[a]	1.08[ab]	1.49[b]	0.11	0.58[a]	1.34[b]	1.53[b]	0.21
20:5n3	0.32 ± 0.06	0.10[a]	0.06[a]	0.30[b]	0.03	0.17[a]	0.07[a]	1.53[b]	0.30
22:4n6	0.24 ± 0.01	0.13[a]	0.11[a]	0.08[a]	0.01	0.11[a]	0.09[a]	0.16[a]	0.02
22:5n6	0.11 ± 0.01	0.15[a]	0.06[b]	0.13[ab]	0.02	0.08[a]	0.05[a]	0.12[a]	0.03
22:5n3	0.11 ± 0.01	0.08[a]	0.08[a]	0.23[b]	0.02	0.10[ab]	0.04[a]	0.16[b]	0.02
22:6n3	0.71 ± 0.05	0.04[a]	0.03[a]	1.14[b]	0.13	0.07[a]	0.13[a]	1.59[b]	0.12
20:3n9/20:4n6	—	0.87	—	—	0.29	2.89	—	—	0.41
20:3n9/18:1n9	—	0.01	—	—	0.00	0.03	—	—	0.00
20:4n6/18:2n6	0.03 ± 0.00	0.04[a]	0.02[b]	0.02[b]	0.00	0.08[a]	0.03[b]	0.04[b]	0.01

[1] Values are means except where they are ±SEM or pooled SEM; n = 24 for initial group; n = 7 or 8 for each diet. [2] For each fatty acid within a period, means not sharing a common superscript are significantly different ($P < 0.05$). [3] Cats were fed for 80 weeks from 3 months of age. [4] Not detectable.

linoleate (18:2n6) in erythrocytes to 50% of the level in erythrocytes of cats fed diets containing safflower seed oil. By 80 weeks, the level of linoleate had further decreased to 25% of the values for cats fed adequate linoleate. Similar changes occurred in plasma fatty acids. Levels of 11,14-eicosadienoate (20:2n6) and 5,11,14-eicosatrienoate [20:3(5,11,14)], the products of linoleate by elongation and Δ^5 desaturation, were also greatly reduced in linoleate deficiency. The n7 and n9 fatty acids, palmitoleate (16:1), oleate (18:1) and 11-eicosenoate (20:1n9) were increased two- to fivefold in both the plasma and erythrocytes of linoleate-deficient cats. The "triene" of EFA deficiency, 20:3n9, appeared at low levels as early as 6 weeks of the study and was present in all cats fed diet A by 10 weeks. Sinclair et al. (27) and Rivers and Frankel (28) also reported this fatty acid in the tissues of cats fed diets deficient in linoleate. Levels of 20:3n9 were low despite extremely high levels of 18:1, so that the ratio of product to precursor, 20:3n9/18:1, was only 0.03 in the plasma and 0.10 in erythrocytes after 80 weeks. In contrast, Rao and co-workers (29) reported a ratio of 0.38–0.52 in erythrocytes from rats fed linoleate-deficient diets for 8 weeks.

In cats fed a source of linoleate, high levels of linoleate in erythrocytes were accompanied by 9-fold and 15-fold increases in 20:2n6 and 20:3(5,11,14), respectively, when compared to the deficient group. Similar, but less marked increases occurred in the plasma. The conversion of 20:2n6 to 20:3(5,11,14)

TABLE 7

Effects of experimental diets on erythrocyte fatty acid composition in cats[1,2]

Fatty acid	Initial[3]	Week 10 of study				Week 80 of study			
		Diet A	Diet B	Diet C	Pooled SEM	Diet A	Diet B	Diet C	Pooled SEM
	% of total fatty acids by wt					*% of total fatty acids by wt*			
16:0	18.3 ± 0.2	18.2[a]	18.5[a]	17.6[a]	0.5	17.3[a]	17.1[a]	17.9[a]	0.5
16:1	0.45 ± 0.02	3.16[a]	1.14[b]	1.05[b]	0.31	3.42[a]	1.12[b]	1.05[b]	0.14
17:0	0.22 ± 0.00	1.21[a]	1.15[a]	1.13[a]	0.16	0.80[a]	0.84[ab]	0.96[b]	0.05
18:0	18.2 ± 0.2	12.6[a]	15.8[b]	15.6[b]	0.6	14.4[a]	20.1[b]	20.3[b]	0.5
18:1	8.59 ± 0.13	27.5[a]	10.0[b]	8.2[b]	0.9	34.7[a]	8.56[b]	7.99[b]	0.79
18:2n6	31.2 ± 0.3	17.2[a]	34.9[b]	33.4[b]	0.9	9.76[a]	36.8[b]	36.0[b]	0.96
18:3n6	ND[4]	ND	ND	ND	—	0.22	ND	ND	0.02
20:0	0.31 ± 0.01	0.29[a]	0.29[a]	0.25[a]	0.04	ND	0.14	0.12	0.01
18:3n3	0.03 ± 0.00	0.09	ND	ND	0.01	0.69[a]	0.25[a]	0.26[a]	0.21
20:1n9	0.90 ± 0.03	1.34[a]	0.60[b]	0.47[b]	0.06	1.90[a]	0.45[b]	0.46[b]	0.06
21:0	ND	0.45[a]	0.24[a]	0.40[a]	0.10	0.34[a]	0.08[b]	0.09[b]	0.03
20:2n6	1.57 ± 0.05	0.74[a]	2.48[b]	2.24[b]	0.17	0.11[a]	1.69[b]	1.68[b]	0.09
20:3n9	ND	0.77	ND	ND	0.21	3.40	ND	ND	0.44
20:3(5,11,14)	2.69 ± 0.13	1.87[a]	4.78[b]	4.50[b]	0.40	0.45[a]	3.36[b]	2.88[b]	0.48
20:3n6	1.40 ± 0.14	4.05[a]	3.08[a]	6.72[a]	1.22	3.30[a]	1.94[ab]	1.50[b]	0.49
20:4n6	9.97 ± 0.42	6.72[a]	4.59[b]	4.44[b]	0.45	7.76[a]	6.19[a]	6.59[a]	0.68
20:5n3	0.39 ± 0.01	0.54[a]	0.08[b]	0.72[c]	0.05	0.47[a]	0.19[b]	0.15[b]	0.07
22:4n6	1.08 ± 0.04	0.78[a]	0.63[ab]	0.55[b]	0.06	0.30[a]	0.36[a]	0.31[a]	0.04
22:5n6	1.63 ± 0.08	1.31[a]	0.77[b]	1.01[c]	0.08	1.12[a]	0.45[b]	0.55[b]	0.07
22:5n3	0.19 ± 0.01	0.14	ND	0.14	0.03	0.26[a]	0.46[a]	0.31[a]	0.19
22:6n3	0.29 ± 0.03	ND	ND	0.70	0.12	0.11[a]	0.14[a]	1.20[b]	0.09
20:3n9/20:4n6	—	0.12	—	—	0.04	0.46	—	—	0.08
20:3n9/18:1n9	—	0.03	—	—	0.01	0.10	—	—	0.01
20:4n6/18:2n6	0.32 ± 0.02	0.39[a]	0.13[b]	0.13[b]	0.02	0.80[a]	0.17[b]	0.19[b]	0.04

[1] Values are means except where they are ±SEM or pooled SEM; n = 24 for initial group; n = 7 or 8 for each diet. [2] For each fatty acid within a period, means not sharing a common superscript are significantly different ($P < 0.05$). [3] Cats were fed for 80 weeks from 3 months of age. [4] Not detectable.

also occurs in rat liver microsomes (30); however, 20:3(5,11,14) does not accumulate in rat tissues.

As suggested by Sinclair et al. (31) the synthesis of 20:3(5,11,14) indicates that the cat is capable of elongation and Δ^5 desaturation. However, this pathway is a metabolic dead-end in the cat, since there was negligible synthesis of other polyunsaturated fatty acids from linoleate. In the plasma, levels of γ-linolenate (18:3n6), dihomo-γ-linolenate (20:3n6), 22:4n6 and 22:5n6 were not significantly altered by diet. The level of arachidonate was significantly higher in cats fed diets containing safflower seed oil with or without tuna oil, but it is not known whether this represents an increase in total arachidonate or simply a change in the percentage of arachidonate due to changes in the proportions of different lipid classes (32). In erythrocytes, 18:3n6 was not detectable in most groups. The level of 20:3n6 in erythrocytes was highest in group A at week 80, and the level of 22:4n6 was not significantly different among groups at week 80 of the study. Furthermore, feeding a source of linoleate resulted in lower levels of arachidonate and 22:5n6 in erythrocyte lipids. A decrease in arachidonate may be the result of competition with linoleate for incorporation into structural lipids as well as negligible conversion of linoleate to arachidonate. Therefore, the ratio of 20:4n6/18:2n6 was only 0.17 in cats fed the diet with safflower seed oil. These results are in contrast to those obtained in rats, in which linoleate supplementation

results in the accumulation of arachidonate in at least 1:1 ratio in serum (21) and greater than a 5:1 ratio in erythrocytes (33).

In cats fed the diet containing tuna oil (diet C), n3 fatty acids accumulated in plasma and erythrocyte lipids. The small amount of arachidonate in tuna oil (table 2) did not significantly increase the level of arachidonate in blood lipids, probably because of disproportionately large amounts of n3 fatty acids. The levels of other fatty acids were similar in cats fed diets B or C.

Effects of linoleate deficiency on liver fatty acids

Changes in fatty acid composition as a result of linoleate deficiency were more pronounced in liver lipids than in plasma or erythrocyte lipids (table 8). In liver phospholipids from deficient cats, linoleate decreased to 7%, and 20:2n6 and 20:3(5,11,14) were not detectable. Levels of 16:1, 18:1 and 20:1n9 were increased two- to fourfold. The level of 20:3n9 was similar to that of the erythrocytes at week 80 of the study.

High levels of linoleate (36%) in the liver phospholipids of cats fed diets with safflower seed oil resulted in the synthesis of 20:2n6 and 20:3(5,11,14). However, linoleate was not converted to arachidonate in significant quantities, since the levels of arachidonate and other n6 fatty acids were not altered by diet.

The total lipid of the livers of deficient cats was highly monounsaturated, with 18:1 comprising 61% of the fatty acids by weight. The deposition of neutral lipids, with a lesser degree of unsaturation, is a well-known manifestation of EFA deficiency in rats (34).

TABLE 8

Fatty acid composition of liver total lipid and phospholipid from male cats fed the experimental diets for 82–101 weeks[1,2]

	Phospholipid				Total lipid			
Fatty acid	Diet A	Diet B	Diet C	Pooled SEM	Diet A	Diet B	Diet C	Pooled SEM
	% of total fatty acids by wt				*% of total fatty acids by wt*			
16:0	11.4[a]	8.95[a]	11.0[a]	0.9	13.1[a]	12.4[a]	14.0[a]	1.0
16:1	8.37[a]	2.20[b]	1.78[b]	0.45	13.3[a]	4.67[b]	3.27[b]	0.7
17:0	0.48[a]	0.54[a]	0.53[a]	0.07	0.64[a]	0.45[a]	0.58[a]	0.06
18:0	16.3[a]	21.9[b]	24.8[b]	1.1	4.40[a]	9.50[ab]	14.5[b]	1.9
18:1	44.8[a]	17.4[b]	13.0[b]	2.5	61.0[a]	30.8[b]	24.5[b]	3.0
18:2n6	6.99[a]	36.0[b]	32.8[b]	2.3	3.45[a]	36.9[b]	35.4[b]	2.0
18:3n6	0.20[a]	0.36[a]	0.18[a]	0.08	0.11[a]	0.43[a]	0.33[a]	0.12
20:0	0.21[a]	0.37[a]	0.19[a]	0.08	ND[3]	ND	ND	—
18:3n3	0.09[a]	0.08[a]	0.07[a]	0.05	0.13[a]	0.12[ab]	0.08[b]	0.02
20:1n9	1.17[a]	0.60[b]	0.31[b]	0.13	1.22[a]	0.59[b]	0.45[b]	0.07
21:0	0.78[a]	0.21[b]	0.08[b]	0.11	0.72[a]	0.09[b]	0.11[b]	0.04
20:2n6	ND	1.77	1.14	0.33	ND	1.11	0.81	0.16
20:3n9	3.05	ND	ND	0.32	0.62	ND	ND	0.05
20:3(5,11,14)	ND	0.99	0.64	0.23	ND	0.32	0.34	0.11
20:3n6	1.96[a]	3.52[a]	3.18[a]	0.98	0.28[a]	1.29[ab]	1.64[b]	0.37
20:4n6	2.79[a]	3.28[a]	4.40[a]	0.74	0.35[a]	0.92[b]	1.69[b]	0.12
20:5n3	0.05	ND	ND	0.01	0.10	0.04	ND	0.01
22:4n6	ND	0.22	0.19	0.11	0.04[a]	0.06[a]	0.09[a]	0.02
22:5n6	0.12[a]	0.06[a]	0.24[a]	0.06	ND	ND	ND	—
22:5n3	0.20[a]	0.10[a]	0.72[b]	0.12	0.09[a]	0.03[a]	0.30[b]	0.03
22:6n3	0.13[a]	0.10[a]	4.59[b]	0.37	ND	ND	1.79	0.60
20:3n9/20:4n6	1.17	—	—	0.25	1.87	—	—	0.35
20:3n9/18:1	0.07	—	—	0.01	0.01	—	—	0.00
20:4n6/18:2	0.41[a]	0.09[b]	0.14[b]	0.03	0.10[a]	0.02[b]	0.05[c]	0.00

[1] Values are means except where they are pooled SEM. [2] For each fatty acid in phospholipid or in total lipid, means not sharing a common superscript are significantly different ($P < 0.05$). [3] Not detectable.

In total lipid the percentage of arachidonate was lower in group A than in group B. This difference in percentages was due solely to dilution of arachidonate by monounsaturated fatty acids, and was not a result of differences in the total amount of arachidonate in the liver. The total amount of arachidonate (mean ± SEM) was calculated from the amount of liver fat (grams/100 g body weight) and the percentage by weight of arachidonate in total lipid. These values, in milligrams of arachidonate per 100 g of body weight, were 1.12 ± 0.19 for group A; 1.18 ± 0.32 for group B; and 1.33 ± 0.13 for group C. These results demonstrate that the linoleate that accumulated in the livers of cats fed safflower seed oil was not significantly converted to arachidonate.

DISCUSSION

The results of this study demonstrate that linoleate is an EFA for the cat. Cats fed the linoleate-deficient diet had fatty livers, poor skin and coat condition, and marked increases in transepidermal water loss. Basal metabolic rates and final body weights were not affected by linoleate status. This may be due to the presence of some linoleate in diet A. In addition, the cat retains linoleate at higher levels in tissues than does the rat fed a similar concentration of linoleate (33, 35).

The fatty acid composition of blood and liver lipids changes in a manner that was qualitatively, but not quantitatively, similar to that observed in other linoleate-deficient animals. In deficient cats, the appearance of 20:3n9, although in low concentrations, was also a sensitive indicator of linoleate deficiency. The absence of this fatty acid in cats fed diets containing safflower seed oil prompted Rivers et al. (5) to suggest that the Δ^6 desaturase was absent in the cat. It now appears that some 18:1n9 can be converted to 20:3n9, although the activity of this pathway is indeed very low. The complete absence of this fatty acid in cats fed a source of linoleate is probably due to extremely high levels of linoleate in tissues (35–45%). In rats, 20:3n9 is present even when linoleate is supplied at adequate levels, but tissue levels of linoleate rarely exceed 15–20% (21, 33, 35, 36).

The synthesis of 20:3n9 from 18:1n9 suggests that the cat may also be able to synthesize 20:4n6 from 18:2n6, since the same enzymes are responsible for both conversions (2). However, we observed no significant differences in levels of arachidonate despite marked increases in linoleate that occurred in linoleate-supplemented cats. Apparently the activity of this pathway is so low as to be nearly saturated with substrate, even in linoleate deficiency. Furthermore, the small amount of arachidonate that could be synthesized must compete with extraordinarily high levels of linoleate for incorporation into glycerolipids (37, 38).

Sinclair et al. (27) suggested that the cat may have the ability to desaturate polyunsaturated fatty acids at the Δ^8 position. If so, the increase in 20:2(11,14) (20:2n6) when linoleate was supplied should have resulted in an increase in 20:3(8,11,14) (20:3n6) and/or in arachidonate [20:4(5,8,11,14)]. Instead, 20:2n6 was converted to 20:3(5,11,14) via a Δ^5 desaturase. Thus it appears that both the Δ^8 desaturase and the Δ^6 desaturase are virtually absent in the cat, but the Δ^5 desaturase is present. However, the activity of the Δ^5 desaturase may also be relatively low in the cat, because levels of 20:3n6 in cat tissues were similar to levels of 20:4n6. In the rat, in which the Δ^5 desaturase is active, levels of 20:3n6 are extremely low unless this fatty acid is fed (39).

The ability of the investigator to alter levels of linoleate and arachidonate independently in the cat makes it a useful model for studying the specific physiological roles of these two fatty acids. When linoleate was supplied, there was no evidence of synthesis of arachidonate, and levels of plasma prostaglandins remained unaltered. Nevertheless, skin condition and liver fat deposition were improved. This appears to be the first demonstration of the specific effects of linoleate as an EFA, independent of arachidonate synthesis and prostaglandin formation.

Similar results have been reported by Houtsmuller (40) who observed that columbinic acid (*trans*-5,*cis*-9,*cis*-12-18:3), an analogue of linoleate with an additional *trans*-5 double bond, restores the epidermal permeability barrier in rats but is not converted to prostaglandins. Furthermore, in several

studies, the administration of prostaglandins did not correct the increased water loss of EFA-deficient rats (41–43). Houtsmuller and van der Beek (41) concluded that any straight-chain, even-numbered fatty acid with *cis*-n6,9 double bonds will act to maintain the epidermal permeability barrier, and that prostaglandins are not involved in this function. Although both linoleate and arachidonate were effective in the studies by Houtsmuller and co-workers, Hartop and Prottey reported that linoleate was effective but arachidonate was not (42).

Although linoleate prevented several signs of EFA deficiency in the cat, it did not meet the requirements for normal reproduction or spermatogenesis (MacDonald, M. L., Rogers, Q. R., Morris, J. G. and Cupps, P., unpublished data). This supports the idea that arachidonate has an important role in reproduction, as suggested by Rivers and co-workers (5). Because of impaired arachidonate synthesis, the cat can be deficient in arachidonate despite a surplus of linoleate. As a result, arachidonate is also a dietary essential for the cat.

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