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Effect of Dietary α- and γ-Linolenic Acid on Tissue Fatty Acids in Guinea Pigs (41982)

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Abstract. Guinea pigs were fed regular chow diets supplemented with 5% (by weight) safflower oil, evening primrose oil, or linseed oil for 6 weeks. The unsaturated fatty acid content of these oils was 78.9% of 18:2n6, 74.1% of 18:2n6, and 9.2% of 18:3n6, or 21.5% of 18:2n6 and 46.9% of 18:3n3, respectively. In comparison with 18:2n6, dietary supplementation with 18:3n6 significantly increased the tissue levels of 18:3n6 and 20:3n6, whereas dietary 18:3n3 significantly elevated the levels of 18:3n3 in plasma and liver lipids. Dietary 18:3n3 also significantly increased 22:5n3 and 22:6n3 in total phospholipids. The tissue levels of 20:4n6, on the other hand, were not affected by either treatment. These data suggest that both Δ^6- and Δ^5 desaturation of n-6 fatty acids in guinea pigs are low, and that the metabolism of n-3 and n-6 fatty acids may be regulated by two different enzyme systems.

Linoleic acid (18:2n6), a dietary essential for mammals, cannot be synthesized *de novo* but can be further desaturated by Δ^6-desaturase to form γ-linolenic acid (18:3n6). This in turn is elongated to form dihomo-γ-linolenic acid (20:3n6) and subsequently desaturated by Δ^5-desaturase to form arachidonic acid (20:4n6). The rate-limiting step is that of Δ^6-desaturation (1, 2).

Our previous studies (3, 4) have shown that tissue phospholipids in guinea pigs contained large amounts of linoleic acid but only small amounts of long chain, n-6 polyunsaturated fatty acids (PUFAs). One explanation of this finding might be a low level of Δ^6-desaturation in this animal. In the present study, we fed guinea pigs on a γ-linolenic acid enriched diet, to bypass the Δ^6-desaturation step, and then examined whether tissue long chain n-6 fatty acid levels were raised. Since normal guinea pigs also contain low levels of n-3 fatty acids (3, 4), a separate group of animals was supplemented with an 18:3n3 enriched diet in order to examine the basis for the small amounts of n-3 fatty acids in guinea pigs: it might be related to a low dietary 18:3n3 content or to an inability to utilize n-3 fatty acids as a result of a low rate of Δ^6-desaturation.

Materials and Methods. Female Hartley guinea pigs (weighing approximately 650 g) were randomly allocated into three groups of six each. All animals had free access to drinking water which contained 1 g/liter of ascorbic acid, and were given Purina guinea pig chow to which 5% safflower oil (SFO), 5% evening primrose oil (EPO), or 5% linseed oil (LSO) were added. The fatty acid compositions of the chow and the oil-supplemented diets are shown in Table I. After 6 weeks on the respective diets, the animals were fasted overnight and sacrificed by exsanguination under ether anesthesia. Blood was collected by heart puncture into a test tube containing EDTA (1 mg/ml blood) and plasma separated by centrifugation at 4°C. Livers were rapidly excised, rinsed in ice-cold saline, blotted, and frozen.

Tissue lipids were extracted according to the method of Folch *et al.* (5). Tissue lipid extracts were then separated by thin layer chromatography into cholesteryl esters, triglycerides, and total phospholipids (TPL). Lipid fractions were methylated and the composition of fatty acid methyl esters was analyzed by gas–liquid chromatography (6).

The data were subjected to Student Neuman–Keuls' multiple range test (7). Values of $P < 0.05$ were considered to be statistically significant.

Results and Discussion. The rates of growth were similar for all three dietary groups (final weights: SFO, 792 ± 51 g; EPO, 788 ± 39 g; LSO, 818 ± 42 g).

The fatty acid profiles of plasma lipids after dietary treatment are shown in Table II. Dietary 18:3n6 significantly increased the levels of 18:3n6 and 20:3n6, whereas dietary 18:3n3 increased that of 18:3n3. The levels of 22:5n3 and 22:6n3 in plasma TPL were

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TABLE I. FATTY ACID COMPOSITION OF THE DIET AND THE SUPPLEMENTED OILS

Fatty acid	Chow[a]	Oil supplement		
		Safflower	Evening primrose	Linseed
14:0	1.3	0.1	0.1	—
14:1	0.4	—	—	—
16:0	19.1	6.5	5.8	5.5
16:1	2.3	0.1	0.1	0.1
18:0	9.1	2.2	1.9	3.4
18:1	34.0	10.9	8.1	20.9
18:2n6	26.9	78.9	74.1	21.5
18:3n6	—	—	9.2	—
18:3n3	4.4	0.6	—	46.9
others	2.5	0.7	0.7	1.8

[a] The guinea pig chow (No. 5025, Ralston Purina Co., St. Louis, Mo.) contained 18.5% crude protein; 4% fat; 16% fiber; 9% ash; and 3.5% minerals.

also increased by 18:3n3 treatment. The levels of 18:2n6 were not affected by the dietary manipulation. 20:4n6 was higher in the EPO and LSO group TPL than in the SFO group.

Table III shows the fatty acid compositions of liver lipids. In comparison with 18:2n6, 18:3n6 supplementation significantly increased the levels of 18:3n6 and 20:3n6 but not that of 20:4n6 in liver lipids, whereas 18:3n3 feeding significantly increased the levels of 18:3n3 in liver lipids and those of 22:5n3 and 22:6n3 in TPL.

Consistent increases of 18:3n6 and 20:3n6 after 18:3n6 feeding and increases of 18:3n3, 22:5n3, and 22:6n3 after 18:3n3 feeding were also observed in plasma, red blood cell, and liver phospholipid subclasses (data not shown).

In this study, 18:3n6 treatment increased the levels of 20:3n6 but had little effect on 20:4n6 in all the tissues examined. This result suggests that the elongation of 18:3n6 to 20:3n6 takes place in guinea pigs, but the conversion of 20:3n-6 to 20:4n-6 is low. The present observation agrees well with that reported by Stone *et al.* (8), that the guinea pig has low Δ^5-desaturation activity. When animals were treated with SFO alone, despite a high intake of 18:2n6, the levels of neither 18:3n6 nor 20:3n6 were affected. The data are consistent with the proposition that the activity of Δ^6-desaturation in guinea pigs is also low.

Interestingly, the levels of 22:5n-3 and 22:6n3, which can be formed from 18:3n3 by alternate elongation and desaturation (2 elongases and 2 desaturases, Δ^6- and Δ^5- and also Δ^4-desaturase in the case of 22:6n3), were found to be increased in plasma and liver phospholipids following 18:3n3 administration. Although it is widely believed that the same enzyme systems (elongases and desaturases) are involved in both n-6 and n-3 fatty acid metabolism, Maeda *et al.* (9) have found recently that in cell lines the ability to metabolize n-3 and n-6 PUFAs can be selectively lost. Our data agree with *in vitro* studies (8) in suggesting that in guinea pigs, Δ^6- and Δ^5-desaturation of n-6 fatty acids are only weakly active. In contrast there seems to be relatively substantial conversion of 18:3n3 to its long chain desaturated metabolites. There may be two separate mechanisms regulating n-6 and n-3 fatty acid metabolism in this animal or the single system may have much higher activity for n-3 PUFAs. Further experiments are required to settle this issue.

In conclusion, our data suggest that guinea pigs convert only small amounts of linoleic acid to other n-6 PUFAs. One of the reasons for this appears to be low Δ^6-desaturase activity, because a dietary supplement of the product of this enzyme, 18:3n6, was effective in raising tissue levels of both 18:3n6 and 20:3n6. There was little conversion of 20:3n6 to 20:4n6, supporting the previous *in vitro* evidence that Δ^5-desaturation of n-6 fatty

TABLE II. FATTY ACID COMPOSITION OF PLASMA LIPIDS IN GUINEA PIGS FED A REGULAR CHOW SUPPLEMENTED WITH DIFFERENT OILS

	Cholesteryl esters			Triglycerides			Total phospholipids		
Fatty acid	EPO	SFO	LSO	EPO	SFO	LSO	EFO	SFO	LSO
16:0	7.9 ± 0.5	7.7 ± 1.5	2.9 ± 0.8	14.9 ± 2.7	16.2 ± 1.2	16.5 ± 0.9	7.9 ± 0.7	8.8 ± 1.2	8.5 ± 1.6
16:1	1.4 ± 0.5	1.2 ± 0.3	1.5 ± 0.3	2.1 ± 0.9	2.5 ± 0.5	3.6 ± 1.2	0.5 ± 0.09	0.5 ± 0.1	0.6 ± 0.2
18:0	1.0 ± 0.02	0.9 ± 0.4	1.4 ± 0.4	2.3 ± 0.3	2.7 ± 0.4	2.5 ± 0.9	21.6 ± 5.2[a]	24.4 ± 1.7[a]	17.8 ± 1.3[b]
18:1	5.9 ± 1.2[a]	5.9 ± 1.6[a]	9.6 ± 2.3[b]	18.3 ± 3.0	21.4 ± 1.5	22.3 ± 2.2	8.8 ± 0.9[a]	8.6 ± 0.6[a]	10.6 ± 0.4[b]
18:2n6	65.1 ± 3.6	67.8 ± 5.2	63.5 ± 7.6	50.5 ± 4.9[a]	50.3 ± 3.2[a]	35.0 ± 2.4[b]	42.8 ± 4.7[a]	45.5 ± 3.1[a,b]	45.2 ± 1.6[b]
18:3n6	7.3 ± 1.8	—	—	4.0 ± 1.7	—	—	0.9 ± 0.3	—	—
n3	—	1.1 ± 0.4	4.0 ± 0.8[b]	4.5 ± 0.5[a]	4.4 ± 0.4[a]	16.7 ± 4.5	0.6 ± 0.2[a]	1.0 ± 0.5[a]	2.6 ± 0.3[b]
20:2	—	—	—	0.1 ± 0.06	0.2 ± 0.02	—	0.3 ± 0.05	0.2 ± 0.1	—
20:3n6	2.3 ± 0.8	2.2 ± 0.8	—	0.6 ± 0.2	—	—	3.2 ± 0.5[a]	0.6 ± 0.3[b]	—
20:4n6	8.4 ± 2.5	10.1 ± 3.0	6.1 ± 6.0	0.7 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	9.8 ± 1.5[a]	6.4 ± 0.9[b]	9.3 ± 0.9[a]
22:4n6	—	—	—	—	—	—	0.8 ± 0.2	0.7 ± 0.2	—
22:5n6	—	—	—	—	—	—	1.8 ± 0.5	1.2 ± 0.3	1.9 ± 1.5
22:5n3	—	—	—	—	—	—	0.5 ± 0.1[a]	0.4 ± 0.1[a]	1.6 ± 0.4[b]
22:6n3	—	—	—	—	—	—	0.8 ± 0.2[a]	0.6 ± 0.1[a]	1.4 ± 0.3[b]

Note. Values (mg/100 mg total fatty acids determined) (mean ± SD) with different superscripts are significantly different at $P < 0.05$. Abbreviations: EPO = evening primrose oil, SFO = safflower oil, LSO = linseed oil.

TABLE III. FATTY ACID COMPOSITION OF LIVER LIPIDS IN GUINEA PIGS FED A REGULAR CHOW SUPPLEMENTED WITH DIFFERENT OILS

	Cholesteryl esters			Triglycerides			Total phospholipids		
Fatty acids	EPO	SFO	LSO	EPO	SFO	LSO	EPO	SFO	LSO
16:0	22.1 ± 2.1[a]	22.2 ± 2.2[a]	28.1 ± 3.9[b]	19.2 ± 1.1	19.1 ± 1.1	20.1 ± 1.0	10.9 ± 0.5[a]	9.7 ± 0.5[b]	11.1 ± 0.6[a]
16:1	1.5 ± 1.2	1.1 ± 0.3	1.3 ± 0.5	2.8 ± 0.4[a]	2.9 ± 0.7[a]	4.3 ± 0.9[b]	0.5 ± 0.1[a]	0.5 ± 0.07[a]	0.6 ± 0.06[b]
18:0	6.5 ± 1.8[a]	8.7 ± 1.7[a,c]	9.6 ± 1.0[c]	2.3 ± 0.7	2.2 ± 0.3	1.8 ± 0.5	26.1 ± 1.3[a]	28.1 ± 0.9[b]	26.8 ± 0.6[a]
18:1	9.9 ± 1.8[a]	12.9 ± 2.8[a,c]	14.1 ± 1.6[c]	20.0 ± 0.9[a]	21.9 ± 1.1[b]	24.4 ± 1.3[c]	8.4 ± 0.4[a]	8.1 ± 0.2[c]	10.6 ± 0.5[b]
18:2n6	50.6 ± 2.8[a]	48.7 ± 8.3[a]	38.9 ± 2.9[b]	45.2 ± 1.8[a]	46.2 ± 2.8[a]	32.5 ± 1.9[b]	38.1 ± 0.7[a]	41.2 ± 1.5[b]	36.3 ± 0.3[c]
18:3n6	1.3 ± 0.4	—	—	2.7 ± 0.5	—	—	1.7 ± 0.1	—	—
n3	—	—	4.3 ± 0.5	4.1 ± 0.5[a]	3.8 ± 0.3[a]	13.1 ± 1.9[b]	0.7 ± 0.3[a]	0.9 ± 0.3[a]	2.6 ± 0.4[b]
20:2	—	—	—	—	0.3 ± 0.3	0.2 ± 0.02	0.4 ± 0.1[a]	0.8 ± 0.2[b]	0.4 ± 0.1[a]
20:3n6	2.3 ± 0.9	—	—	0.7 ± 0.3[a]	0.1 ± 0.01[b]	—	2.8 ± 0.6[a]	0.9 ± 0.1[b]	0.8 ± 0.2[b]
20:4n6	5.4 ± 2.5	7.5 ± 3.3	4.1 ± 0.5	0.6 ± 0.06	0.5 ± 0.08	0.5 ± 0.09	6.9 ± 0.3[a]	5.9 ± 0.7[b]	6.2 ± 0.4[b]
22:4n6	—	—	—	—	—	—	0.7 ± 0.09[a]	0.7 ± 0.1[a]	0.5 ± 0.06[b]
22:5n6	—	—	—	—	—	—	0.7 ± 0.07[a]	0.7 ± 0.08[a]	0.5 ± 0.07[b]
22:5n3	—	—	—	—	—	—	0.3 ± 0.002[a]	0.3 ± 0.05[a]	0.8 ± 0.09[b]
22:6n3	—	—	—	—	—	—	0.5 ± 0.1[a]	0.5 ± 0.4[a]	0.9 ± 0.08[b]

Note. Values (mg/100 mg total fatty acids determined) (mean ± SD) with different superscripts are significantly different at $P < 0.05$. Abbreviations: EPO = evening primrose oil, SFO = safflower oil, LSO = linseed oil.

acids is very low in guinea pigs (8). The metabolism of n-3 fatty acids may be different in this species.

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