Effect of α-linolenic acid in the human diet on linoleic acid metabolism and prostaglandin biosynthesis

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Abstract The effect of dietary α-linolenic acid intake on linoleic acid metabolism and prostaglandin (PG) biosynthesis was investigated in two groups of six healthy females (25–32 yr). They were given isocaloric formula diets (FD) containing linoleic acid at a constant intake (4% of calories), with different amounts of α-linolenic acid: 0% (FD4/0), 4% (FD4/4), 8% (FD4/8) (group I) and 12% (FD4/12) or 16% (FD4/16) (group II); the diets were given for 2 weeks each. Comparing diet FD4/0 to FD4/16, enrichment of α-linolenic acid was greatest in cholesteryl esters (+6.8% in plasma, +7.1% in low density lipoproteins (LDL), +5.9% in high density lipoproteins (HDL)), less in phosphatidylcholine (+2.5% in plasma, +2.9% in LDL, +2.7% in HDL), and least in platelet lipids (+0.7%). The accumulation of α-linolenic acid was compensated by a decrease of oleic acid. Eicosapentaenoic acid (EPA), which was excluded from the diet, increased in all plasma lipids with augmented α-linolenic acid intake, indicating a chain elongation and desaturation of α-linolenic acid to EPA. However, even at the end of FD4/16, EPA was less than 2% of total fatty acids in all plasma lipids. Plasma linoleic acid levels were constant during all dietary regimes, according to the constant dietary intake of this fatty acid. No replacement of linoleic acid by α-linolenic acid could be observed. The percentage of arachidonic acid in all lipids was unaffected by α-linolenic acid intake. As arachidonic acid was not provided by the diet, it can be concluded that α-linolenic acid does not inhibit chain elongation and desaturation of linoleic acid to arachidonic acid in man. Fatty acids in platelet lipids, except for a small increase of α-linolenic acid and EPA, were barely affected by dietary manipulations. PG metabolites and PGE_2 decreased, −52% and −85%, respectively, in 24-hr urine specimens with increasing α-linolenic acid intake. $PGF_{2\alpha}$ was unaffected up to an α-linolenic acid intake of 8% of calories; thereafter a decrease (−74%), compared to the values during free diet, could be observed. The results of our experiment demonstrate that for α-linolenic acid there is a reduced incorporation into plasma and platelet lipids, and a slower transformation to higher unsaturated fatty acids, compared to the values found with linoleic acid. PG biosynthesis, but not the conversion of linoleic acid to arachidonic acid, is suppressed by α-linolenic acid ingestion. —**Adam, O., G. Wolfram, and N. Zöllner.** Effect of α-linolenic acid in the human diet on linoleic acid metabolism and prostaglandin biosynthesis. *J. Lipid Res.* 1986. **27:** 421–426.

Supplementary key words linoleic acid • linolenic acid • polyunsaturated fatty acid metabolism • liquid formula diets

Modification of dietary fat alters the phospholipid fatty acid composition of plasma and cell membranes and thereby the availability of precursor substances for prostaglandin (PG) biosynthesis (1, 2). Moreover, there is a competition between dietary polyunsaturated fatty acids for desaturating and oxygenating enzymes, which until now has been demonstrated in vitro (3–5) and in animal experiments (6), but only for thromboxane in man (7). Linoleic (18:2, n-6) and α-linolenic (18:3, n-3) acids are the most abundant polyunsaturated fatty acids in Western diets. The same enzymatic systems that transform linoleic to arachidonic acid also transform α-linolenic acid to eicosapentaenoic acid (EPA) by identical metabolic steps of desaturation and chain elongation (8). α-Linolenic acid is a poor substrate for these enzymes and, in vitro, depresses the formation of arachidonic acid (9), by inhibition of the desaturating enzyme, and its conversion to PG (4), by inhibition of cyclooxygenase. Both effects may contribute to a depressed PG biosynthesis during α-linolenic acid feeding. To evaluate the effect of dietary α-linolenic acid on linoleic acid metabolism and PG biosynthesis in human subjects, a precise and constant intake of both fatty acids over a sufficient time period is necessary and the diet has to be devoid of arachidonic acid and EPA. To meet these requirements our experiments were done with liquid formula diets (FD).

Abbreviations: FD, liquid formula diets; arachidonic acid, all-*cis* 5,8,11,14-eicosatetraenoic acid (20:4, n-6); CE cholesteryl esters; EPA, all-*cis* 5,8,11,14,17-eicosapentaenoic acid (20:5, n-3); GLC, gas–liquid chromatography; HDL, high density lipoproteins (density > 1.063 g/ml); LDL, low density lipoproteins (density 1.006–1.063 g/ml); linoleic acid, all-*cis* 9,12-octadecadienoic acid (18:2, n-6); linolenic acid, all-*cis* 9,12,15-octadecatrienoic acid (18:3, n-3); MS, mass spectrometry; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, prostaglandin; TNPDA, tetranorprostanedioic acid.

MATERIALS AND METHODS

Study design

Two groups of six healthy females (normal body weight, age 25–32 yr), were given formula diets (FD) containing a constant amount of linoleic acid (4% of calories) for 6 weeks. α-Linolenic acid, providing 0%, 4%, 8% (group I) and 12% and 16% (group II) of total energy intake, was given in diets of different fat compositions for 2 weeks, to each two volunteers in a different order. The volunteers continued their normal life and physical activities throughout the experiment. They came to the metabolic ward every morning and daily reports of body weight, determination of water and energy intake, frequency and consistency of stools, etc. were recorded. Twenty four-hour urine samples were collected during 6 days under conventional ad libitum diets and throughout the experimental period. Aliquots were taken from every 24-hr urine sample and immediately deep frozen at −20°C for the analyses of eicosanoids. Fasting blood samples were taken twice before the experiment and on the 12th (control) and 14th (experimental) day of each FD-period. The experiments were done with the informed consent of the volunteers and were approved by the ethical committee of the institution. Statistical analyses were performed using the nonparametric two-way analysis of variance (Friedmann test); statistical differences were determined with the Wilcoxon signed-rank test (10). The resulting two-by-two frequency table was evaluated descriptively by the chi-square test (exact-Fisher test) (10).

FD were prepared in isocaloric amounts for each volunteer in our metabolic kitchen. Protein (Hyperprotidine, Guigoz, France) supplied 15%, carbohydrates (oligopolymers of glucose, Maltodextrine, Maizena, FRG) 55%, and fat (mixtures of safflower, olive, and linseed oil) 30% of total energy intake. With the fat, a constant amount of linoleic acid (4% of calories) and the indicated α-linolenic acid intake was provided (**Table 1**). Water intake was allowed ad libitum. Three grams of KCl, 5 g of NaCl, and 0.6 g of cholesterol were included per 2200 kcal of FD. One capsule of vitamins (Protovita, Roche, Grenzach-Whylen, FRG) was given daily and 100 mg of $FeCl_2$ was given every second day.

TABLE 1. Daily nutrient intake with the different liquid formula diets (FD4/0, FD4/4, FD4/8, FD4/12, FD4/16)

	FD4/0	FD4/4	FD4/8	FD4/12	FD4/16
	% of energy intake				
Fat	30	30	30	30	30
Carbohydrates	55	55	55	55	55
Protein	15	15	15	15	15
Linoleic acid	4	4	4	4	4
Linolenic acid	0	4	8	12	16

Methods

Lipoprotein fractions LDL and HDL were separated by ultracentrifugation (11). Cholesteryl esters (CE) and phospholipids in plasma and lipoproteins were extracted (12) and separated by chromatography on silica gel in glass columns (13). The isolation of phospholipid fractions was achieved by thin-layer chromatography (13) on precoated silica gel plates (Schleicher and Schüll, Dassel, FRG). The bands of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were identified on each plate by standards (Sigma, Freiburg, FRG); they were scraped off and eluted from the silica (13). Platelets were isolated from EDTA-plasma by fractional centrifugation, deep frozen at −20°C, and lipids were extracted as described previously (2). Methyl esters were prepared by transmethylation with 5% HCl in CH_3OH (13) and capillary gas–liquid chromatography (GLC) was performed on a Carlo Erba Fractovap Series 4160, equipped with a flame ionization detector, and a Spectra-Physics SP 4100 computing integrator. The capillary glass column (25 m, 0.2 I.D.) was coated with M20 (Chrompack, Berlin, FRG). Most samples were run in duplicate. GLC analysis of all samples was done at least twice, and questionable samples were evaluated on two columns with liquid phases of different polarity (M20 and SP2340, Chrompack, Berlin, FRG). Some samples were additionally reexamined on a Hewlett-Packard Model 5986 GLC–MS system. Thus the accuracy of values under 1% is better than 5% with GLC and better than 1% with GLC–MS. PG metabolites in 24-hr urine samples were determined by GLC measurement of tetranorprostanedioic acid (TNPDA) (14). PGE_2 and $PGF_{2\alpha}$ were determined by specific radioimmunoassays (1). Anti-PGE_2 showed a cross-reactivity to PGE_1 (3.5%) and to PGE_3 (0.2%); anti-$PGF_{2\alpha}$ cross-reacted with PGF_1 (3%) and PGF_3 (3%); the other cross-reactions were below 0.2%. TNPDA, PGE_2, and $PGF_{2\alpha}$ were determined in 24-hr urine samples every second day during each FD.

RESULTS

The most striking finding in our experiment was the difference in the amount of α-linolenic versus linoleic acid found at the end of the α-linolenic acid-rich FD in all lipids we investigated. The concentration of α-linolenic acid increased in all lipids, with the exception of HDL-PE, and the increase was related to augmented intake. However, even with a fourfold higher dietary intake, the levels of α-linolenic acid were far below the percentage of linoleic acid in all lipids. In PC, the relative amount of α-linolenic acid at the end of FD4/16 was below 3% of total fatty acids, while more than 25% linoleic acid was found (**Table 2**). In CE, there was 7% α-linolenic

TABLE 2. Fatty acids in phosphatidylcholine of LDL and HDL

	18:2 (n-6)	18:3 (n-3)	20:4 (n-6)	20:5 (n-3)	18:1 (n-9)	16:0
	% of total fatty acids					
LDL-phosphatidylcholine						
FD4/0	25.0 ± 2.0	0.0 ± 0.0	13.2 ± 1.3	0.2 ± 0.2	20.0 ± 2.0	22.1 ± 3.5
FD4/4	25.2 ± 2.5	0.7 ± 0.3[a]	15.2 ± 2.3	0.2 ± 0.1	19.2 ± 2.0	22.8 ± 3.1
FD4/8	26.2 ± 2.7	1.6 ± 0.2	15.1 ± 1.9	0.2 ± 0.1	16.6 ± 2.8[a]	21.5 ± 1.4
FD4/12	24.4 ± 3.5	1.9 ± 1.0	15.1 ± 2.2	1.4 ± 0.3[a]	11.9 ± 1.6	20.3 ± 1.4
FD4/16	25.0 ± 3.8	2.9 ± 0.9	16.1 ± 2.4	1.5 ± 0.3	9.0 ± 2.0	20.0 ± 1.5
HDL-phosphatidylcholine						
FD4/0	24.0 ± 1.5	0.0 ± 0.0	16.3 ± 2.3	0.0 ± 0.0	16.2 ± 2.2	20.7 ± 1.4
FD4/4	22.8 ± 2.7	0.7 ± 0.2[a]	18.4 ± 3.1	0.6 ± 0.2[a]	16.9 ± 1.6	21.1 ± 1.3
FD4/8	23.4 ± 2.9	1.3 ± 0.3	17.5 ± 3.7	0.6 ± 0.3	16.4 ± 2.0	22.1 ± 1.8
FD4/12	23.8 ± 3.6	2.3 ± 1.2	16.5 ± 6.0	1.6 ± 0.8	11.6 ± 1.7[a]	19.3 ± 2.0
FD4/16	25.5 ± 2.9	2.7 ± 1.3	16.4 ± 4.0	1.8 ± 0.8	11.0 ± 1.0	19.4 ± 1.5

[a]Statistically significant difference ($P < 0.05$) compared to FD4/0.

acid, pointing to a different accumulation of α-linolenic acid, which was related to the linoleic acid content in these two plasma lipids (**Table 3**). The percentage of α-linolenic acid was highest in LDL-CE (7.2%) and somewhat less in HDL-CE (6%) (Table 3). In platelet lipids, α-linolenic acid accumulation was 1.5% at the end of FD4/16 (**Table 4**) and was less than in plasma PC or CE. No incorporation of α-linolenic acid could be observed in HDL-PE at the end of FD4/4 and 4/8 (**Table 5**).

Linoleic acid concentration was not altered after α-linolenic acid ingestion. In fact, dietary α-linolenate did not depress 18:2 (n-6) levels in any lipid we investigated. Apparently α-linolenic acid was incorporated at the expense of oleic acid (18:1, n-9), while saturated fatty acids showed no decrease.

EPA, which was not provided with the diet, increased in LDL-PC from 0.0% (FD4/0) to 1.5% (FD4/16) of total fatty acids and in HDL-PC from 0.0% to 1.8%. Neither of the lipids we investigated showed an increase of EPA, which exceeded 2% at the end of the FD-periods in which α-linolenic acid was provided (Tables 2 and 3).

Arachidonic acid concentration was not depressed by α-linolenic acid ingestion. On the contrary, we observed a small increase of arachidonic acid in LDL-PC, HDL-CE, and platelet lipids. In HDL-PE, α-linolenic acid did not increase during augmented intake and a decrease of 18:1 (n-9) was accompanied by an increase of arachidonic acid in this special lipid fraction.

TNPDA in 24-hr urine, reflecting total body PG biosynthesis (15), decreased from 289 (FD4/0) to 133

TABLE 3. Fatty acids in cholesteryl esters (CE) of the plasma, LDL, and HDL determined in six healthy females at the end of formula diet (FD) periods

	18:2 (n-6)	18:3 (n-3)	20:4 (n-6)	20:5 (n-3)	18:1 (n-9)	16:0
	% of total fatty acids					
Plasma-CE						
FD4/0	47.5 ± 2.4	0.2 ± 0.2	9.0 ± 1.3	0.2 ± 0.1	27.8 ± 1.4	8.9 ± 0.9
FD4/4	46.7 ± 1.8	1.5 ± 0.5[a]	10.6 ± 2.8	0.5 ± 0.2	25.2 ± 1.3	9.0 ± 0.8
FD4/8	48.2 ± 2.4	3.4 ± 0.5	10.3 ± 2.4	0.6 ± 0.2	22.7 ± 1.8[a]	8.7 ± 0.3
FD4/12	51.2 ± 2.4	6.6 ± 1.9	8.3 ± 2.4	1.0 ± 0.3[a]	19.9 ± 1.9	7.7 ± 0.9
FD4/16	53.9 ± 3.4	7.0 ± 2.8	8.4 ± 1.5	1.0 ± 0.5	16.0 ± 2.6	7.0 ± 1.2
LDL-CE						
FD4/0	49.8 ± 2.1	0.1 ± 0.1	9.7 ± 1.8	0.2 ± 0.1	26.9 ± 1.5	7.6 ± 0.7
FD4/4	48.8 ± 2.0	1.5 ± 0.4[a]	10.6 ± 3.0	0.1 ± 0.1	26.7 ± 1.2	8.3 ± 0.6
FD4/8	49.7 ± 3.2	3.2 ± 0.3	10.2 ± 2.5	0.2 ± 0.1	24.1 ± 1.1[a]	8.2 ± 1.0
FD4/12	53.0 ± 2.5	6.3 ± 2.5	9.5 ± 2.4	0.9 ± 0.5[a]	20.0 ± 2.2	7.6 ± 1.2
FD4/16	55.1 ± 3.1	7.2 ± 2.4	9.0 ± 2.0	1.0 ± 0.2	17.9 ± 2.1	7.3 ± 0.4
HDL-CE						
FD4/0	47.6 ± 1.6	0.1 ± 0.1	6.9 ± 1.9	0.1 ± 0.1	29.1 ± 2.4	8.9 ± 0.7
FD4/4	47.2 ± 3.0	1.0 ± 0.5[a]	6.9 ± 1.4	0.2 ± 0.2	27.8 ± 2.0	10.7 ± 1.3
FD4/8	46.5 ± 4.1	2.6 ± 0.5	8.5 ± 2.0	0.1 ± 0.1	24.8 ± 2.1[a]	11.2 ± 2.8
FD4/12	50.0 ± 2.6	5.9 ± 1.8	9.9 ± 2.8	0.8 ± 0.4[a]	17.8 ± 3.2	8.3 ± 1.1
FD4/16	52.1 ± 3.3	6.0 ± 2.2	9.2 ± 2.7	0.8 ± 0.2	16.4 ± 1.3	6.8 ± 3.0

[a]Statistically significant difference ($P < 0.05$) compared to FD4/0.

TABLE 4. Fatty acids in platelet total lipids

	18:2 (n-6)	18:3 (n-3)	20:4 (n-6)	20:5 (n-3)	18:1 (n-9)	16:0
			% of total fatty acids			
Platelet lipids						
FD4/0	4.5 ± 0.7	0.8 ± 0.2	33.6 ± 2.2	0.5 ± 0.1	16.8 ± 2.2	9.9 ± 1.2
FD4/4	4.7 ± 0.7	0.9 ± 0.2	34.9 ± 3.0	0.5 ± 0.1	16.5 ± 2.1	9.3 ± 0.9
FD4/8	4.5 ± 0.4	1.2 ± 0.6	33.1 ± 2.0	0.5 ± 0.1	16.7 ± 2.1	9.3 ± 0.9
FD4/12	6.1 ± 0.8	1.4 ± 0.8	35.5 ± 1.8	0.7 ± 0.3	19.1 ± 0.4	11.4 ± 0.4
FD4/16	6.3 ± 0.7	1.5 ± 0.9[a]	35.4 ± 2.4	0.8 ± 0.5	18.5 ± 1.4	11.4 ± 0.4

[a]Statistically significant difference ($P < 0.05$) compared to FD4/0.

(FD4/16) μg/day with α-linolenic acid intake. Under the same experimental conditions, PGE_2 in 24-hr urine was depressed from 254 to 39 ng/day, while no change was found for $PGF_{2\alpha}$ in 24-hr urine up to 8% α-linolenic acid intake. During FD4/12 and FD4/16, $PGF_{2\alpha}$ in 24-hr urine decreased to 147 and 139 ng/day (**Table 6**).

DISCUSSION

Our results demonstrate differences for the incorporation of linoleic and α-linolenic acids into different plasma and platelet lipids in man. With FD4/16,α-linolenic acid intake exceeded that of linoleic acid four times, but in none of the lipids that were investigated did α-linolenic acid reach the percentage of linoleic acid.

In rats fed diets in which 8% ethyl linolenate was the sole source of fat, there was an accumulation of α-linolenic acid of 1.4% in platelet PC and of 0.8% in liver PC (15). This accumulation is about the same order of magnitude as we observed in man under comparable experimental conditions at the end of FD4/8. When rats were maintained on a diet with equal amounts of 4% linoleic and α-linolenic acid, only 0.2% linolenate could be detected in platelet PC (15). In pigs (5), the incorporation of 18:2 (n-6) into liver lipids was 17-fold greater compared to that of 18:3 (n-3) during all feeding periods with different amounts of these two fatty acids. In man, supplementation of a conventional diet with 30 ml/day of linseed oil for 6 weeks (about 8% of α-linolenic acid) increased the concentration of α-linolenic acid from 0.6% (pre-experimental value) to 1.4% (after 2 weeks) and 1.8% (after 6 weeks) in plasma phospholipids (7). This again was in the same range as we found in our volunteers.

The metabolic pathways for long-chain fatty acids normally include oxidation to CO_2 and H_2O, secretion by the liver via VLDL or bile (especially in phosphatidylcholine), and incorporation in membrane phospholipids. Until now there had not been established a preference for 18:3 (n-3) compared to 18:2 (n-6) in humans or animals. Lands et al. (16) reported on a preferential handling of polyunsaturated fatty acids by 1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase, but found no differences among the polyunsaturated fatty acids of the n-6 and n-3 series. Absorption studies showed a 100% resorption of dietary n-3 fatty acids when the feces were monitored for fatty acid loss (17). However, when uptake was measured in rat chylomicrons, a lower concentration of α-linolenic acid was observed, compared to linoleic acid (18). Oette (17) found an incorporation of α-linolenic acid into liver phospholipids 5 hr postprandially, proven by liver biopsy after an acute 100-ml linseed oil load. At no time in this experiment (1–26 hr) did α-linolenic acid incorporation into liver lipids of man exceed 12% of the 18:3 (n-3) dose, while in plasma free fatty acids a higher percentage of α-linolenic acid was observed. Monitoring $^{14}CO_2$ production in these experiments revealed a higher oxidation rate for α-linolenic acid than for linoleic acid. Taken together, these observations indicate that low α-linolenic acid concentrations in plasma are due to a deposition of α-linolenic acid in certain tissues (e.g., intestinal mucosa triglycerides) and/or by preferential oxidation of α-linolenic acid.

Apparently there were also differences in the amount of α-linolenic acid incorporated in CE of plasma, LDL, and HDL compared to phospholipids, especially HDL-PE. A delayed incorporation in PE compared to PC was reported for α-linolenic (9, 15) and arachidonic acid (19) in

TABLE 5. Fatty acids in phosphatidylethanolamine of HDL

	18:2 (n-6)	18:3 (n-3)	20:4 (n-6)	20:5 (n-3)	18:1 (n-9)	16:0
			% of total fatty acids			
FD4/0	8.4 ± 2.3	0.7 ± 0.2	27.1 ± 6.1	0.2 ± 0.1	25.0 ± 5.0	12.0 ± 2.4
FD4/4	7.9 ± 3.0	0.9 ± 0.7	29.7 ± 4.1	0.3 ± 0.2	21.0 ± 5.2	13.8 ± 3.4
FD4/8	6.9 ± 2.0	0.7 ± 0.7	29.6 ± 3.2	0.6 ± 0.2	20.9 ± 2.5	11.7 ± 0.9

TABLE 6. PGE_2, $PGF_{2\alpha}$, and PG metabolites (PGM) in 24-hr urine of six females[a]

	FD4/0	FD4/4	FD4/8	FD4/12	FD4/16
PGE_2 (ng/day)	254 ± 48	202 ± 35	190 ± 27	69 ± 21	39 ± 11
$PGF_{2\alpha}$ (ng/day)	450 ± 83	397 ± 77	471 ± 92	174 ± 59	121 ± 31
PGM (μg/day)	289 ± 98	183 ± 48	133 ± 30	147 ± 32	139 ± 19

[a]The given values (mean ± SD) are determinations (n = 42) on every second day during the indicated FD periods.

rats, and for arachidonic acid in human platelets (15). The biochemical basis for these differences has not been understood until now. It may be speculated that the observed differences are a reflection of different turnover rates of fatty acids in these lipids. The smaller accumulation of α-linolenic acid in HDL-CE compared to LDL-CE may indicate that α-linolenic acid, transported to peripheral body tissues via LDL, is lost by oxidation or incorporation into lipids (e.g., triglycerides) and not available for the reverse transport from peripheral tissues via HDL (20).

In our experiments, no replacement of linoleic acid by α-linolenic acid could be observed. Increasing the intake of α-linolenic acid had no effect on the percentage of linoleic acid found in plasma or platelet lipids. A decrease of linoleic acid in plasma lipids, which had been reported in animals during α-linolenic acid feeding (3), could not be confirmed in man under our experimental conditions. The body weight of our volunteers was stable within ± 1 kg during the experimental periods. Thus, lipolysis was blocked during most of the day and liberation of linoleic acid from body fat stores could not contribute significantly to the linoleic acid supply. In agreement with our results, no effect of α-linolenate feeding on linoleic acid concentration in plasma and body lipids was found in pigs (5) and in human subjects (7) during experimental periods of 3 weeks. Only when linseed oil was given for a longer period (4 weeks) was a small decrease of 18:2 (n-6) and 20:4 (n-6) observed in one report (7).

Arachidonic acid levels were not reduced in our volunteers after ingestion of α-linolenic acid-rich FD. This contrasts to findings in vitro where the formation of 20:4 (n-6) was reduced by 18:3 (n-3) (21, 22). In vivo experiments in the rat revealed the preferential conversion of 18:2 (n-6) to 20:4 (n-6) and its acylation into phospholipids from platelets and liver versus the analogous transformation of 18:3 (n-3) to 20:5 (n-3) and its incorporation into phospholipids (15). This was true when sufficient 18:2 (n-6) was given with the diet. When ethyl linolenate was given to rats as the sole source of fat, 20:5 (n-3) could readily be detected in platelet and liver phospholipids, and 20:4 (n-6) levels were depressed (15). Also, in pigs, no appreciable 20:5 (n-3) formation could be observed when 18:2 (n-6) and 18:3 (n-3) were given with the diet (5). Recently an in vitro study in rat kidney cells gave ample evidence that suppression of 20:4 (n-6) synthesis by 18:3 (n-3) was readily overcome by increasing the concentration of available 18:2 (n-6) (21).

Polyunsaturated fatty acid ingestion did not depress the amounts of saturated fatty acids either in CE or in phospholipids or platelet lipids. Increasing levels of α-linolenic acid were accompanied by depressed 18:1 (n-9) concentrations, so the sum of 18:3 (n-3) and 18:1 (n-9) was fairly constant in all lipids we investigated, again with the exception of HDL-PE.

Several in vitro and in vivo studies showed that uptake into plasma lipids (15), metabolism (5), and transformation to other fatty acids (15) was slower for 18:3 (n-3) compared to 18:2 (n-6). Despite negligible EPA formation and low α-linolenic acid concentration in the plasma lipids, PG biosynthesis was depressed in our volunteers during α-linolenic acid ingestion. Healthy females who were given a linolenic acid supply of 0%, 4%, or 20% of total energy intake with FD showed an excretion of TNPDA of 123 ± 32, 175 ± 34, and 352 ± 76 μg/day, respectively (23). In agreement with the results from animal experiments (3), PG biosynthesis in humans is stimulated by linoleic acid intake. In the present experiment we found a dose-related reduction of TNPDA in 24-hr urine with FD4/4 and FD4/8, while with a higher intake of α-linolenic acid no greater decrease of TNPDA was observed. This decrease of PG biosynthesis occurred after the fourth day of augmented α-linolenic acid ingestion, indicating a diminution of total body PG biosynthesis (14). A diminution of PG formation during α-linolenic acid feeding may be the result of either reduced formation, and thereby reduced availability of arachidonic acid for PG biosynthesis, or competitive inhibition of PG-synthesizing enzymes by n-3 fatty acids. The percentage of arachidonic acid was not reduced in any lipid we investigated during α-linolenic acid ingestion. In contrast to the findings in vitro (4), the biosynthesis of arachidonic acid was not inhibited and the availability of the precursor for PG biosynthesis was not reduced. Therefore, it can be concluded that the reduction of PG biosynthesis was the result of the inhibition of cyclooxygenase by n-3 fatty acids. In vitro EPA is about ten times more effective than α-linolenic acid with regard to inhibition of cyclooxygenase (24). Our experiments revealed a surprisingly low conversion of α-linolenic to EPA. A substantial increase of

EPA was only found with an α-linolenic intake of 12% and 16%; concomitantly a substantial decrease of PGE_2 and $PGF_{2\alpha}$ could be observed. Therefore it is difficult to determine whether α-linolenic acid or its metabolic product EPA was responsible for the inhibition of PG biosynthesis.

Under our experimental conditions, only a decrease of PGE_2 was found with increasing α-linolenic acid intake to 8%, while the amount of $PGF_{2\alpha}$ remained unaltered. But with an α-linolenic acid supply of more than 8%, the amount of $PGF_{2\alpha}$ in 24-hr urine was also reduced. Obviously the formation of PGE_2 and $PGF_{2\alpha}$ was inhibited to a different degree by α-linolenic acid. PGE_2 promotes sodium excretion in the kidney and increases renal blood flow (22) by vasodilation. Therefore an effect of n-3 fatty acids in the diet on renal sodium excretion and on PG-dependent body functions may be assumed.

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