Effects of supplementation with fish oil–derived n−3 fatty acids and γ-linolenic acid on circulating plasma lipids and fatty acid profiles in women[1–3]

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ABSTRACT

Background: Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and γ-linolenic acid (GLA) have lipid-modifying and antiinflammatory properties. The effects of supplement mixtures of these fatty acids on plasma lipids and the fatty acid compositions of serum phospholipids have received little attention.

Objective: The objective was to determine the effects of different levels of GLA supplementation together with a constant intake of EPA plus DHA on the triacylglycerol-lowering effect of EPA plus DHA alone and on the fatty acid patterns (eicosanoid precursors) of serum phospholipids.

Design: Thirty-one women were assigned to 1 of 4 groups, equalized on the basis of their fasting triacylglycerol concentrations. They received supplements providing 4 g EPA+DHA (4:0, EPA+DHA:GLA; control group), 4 g EPA+DHA plus 1 g GLA (4:1), 2 g GLA (4:2), or 4 g GLA (4:4) daily for 28 d. Plasma lipids and fatty acids of serum phospholipids were measured on days 0 and 28.

Results: Plasma triacylglycerol concentrations were significantly lower on day 28 than on day 0 in the 4:0, 4:1, and 4:2 groups. LDL cholesterol decreased significantly (by 11.3%) in the 4:2 group. Dihomo-γ-linolenic acid increased significantly in serum phospholipids only in the 4:2 and 4:4 groups; however, total n−3 fatty acids increased in all 4 groups.

Conclusions: A mixture of 4 g EPA+DHA and 2 g GLA favorably altered blood lipid and fatty acid profiles in healthy women. On the basis of calculated PROCAM values, the 4:2 group was estimated to have a 43% reduction in the 10-y risk of myocardial infarction.   *Am J Clin Nutr* 2003;77:37–42.

KEY WORDS   Eicosapentaenoic acid, EPA, n−3 fatty acids, docosahexaenoic acid, DHA, arachidonic acid, γ-linolenic acid, dihomo-γ-linolenic acid, triacylglycerols, LDL cholesterol, HDL cholesterol, women

INTRODUCTION

The role of elevated blood lipids in the etiology of atherosclerosis and cardiovascular disease (CVD) is well established, although there are conflicting opinions on the relative importance of various lipids, lipoproteins, and their calculated ratios in predicting the CVD risk for women (1–4). Moderately elevated triacylglycerol concentrations are recognized as a particularly important risk factor for CVD and myocardial infarction (MI) in women (3, 5, 6). Currently, there is also increasing interest in the role of inflammation in CVD in the early cellular events of atherosclerosis development (7–9). Inflammation also appears to be important in the later stages of CVD, when the tendency of established plaque to rupture depends on the degree of inflammation present (9).

Proinflammatory eicosanoids from various cellular sources (eg, leukocytes and platelets), including prostaglandin E_2 (PGE_2), thromboxane A_2 (TXA_2), and leukotriene B_4 (LTB_4) are synthesized from arachidonic acid (AA, 20:4 n−6) on phospholipase-mediated release from cellular phospholipids. Antiinflammatory products generated from the n−6 precursor fatty acid dihomo-γ-linolenic acid (DGLA), the product of γ-linolenic acid (GLA) elongation, include PGE_1, 15-hydroxy-DGLA, and 15-(S)-hydroxy-8,11,13-eicosatrienoic acid. The eicosanoids derived from eicosapentaenoic acid (EPA, 20:4n−3), such as LTB_5, have little or no inflammatory potential (10–12).

Supplementation with GLA has been observed to increase serum lipid concentrations of DGLA and its eicosanoids, to reduce concentrations of EPA and docosahexaenoic acid (DHA, 22:6 n−3), and, in some studies, to increase AA concentrations (13–16). Supplementation with EPA+DHA results in an increase in the physiologic concentrations of EPA and DHA, a decrease in DGLA, and a decrease in the conversion of DGLA to AA (14, 17). In animal models, supplementation with both GLA and EPA+DHA increased DGLA and EPA concentrations and decreased AA concentrations in tissues and cells (18, 19). In a recently published study in humans, dietary supplementation with 6 g of a mixture of GLA and EPA (1:1) increased serum lipid concentrations of DGLA and EPA but not those of AA (20).

Given the role of blood lipids in atherosclerosis and CVD development, it is important to assess the effects of mixed fatty acid supplementation on blood lipids and lipoproteins. In many studies, dietary supplementation with fish oils enriched in n−3 fatty acids reduced triacylglycerol concentrations, usually increased HDL-cholesterol concentrations, but had mixed effects on LDL-cholesterol concentrations (21–24). Dietary supplementation with GLA-containing oils has had variable effects on circulating lipids (15, 25, 26). The effects of GLA added to EPA+DHA supplements on blood lipids and lipoproteins have received little attention in human trials.

The purpose of the present study was to determine the effects of varied levels of GLA supplementation in combination with a constant intake of EPA+DHA on the triacylglycerol-lowering effect of EPA+DHA alone and on the fatty acid patterns of serum phospholipids in women.

SUBJECTS AND METHODS

Subjects

After prescreening and exclusion for consumption of fish, borage, or evening primrose oil, 32 women aged 36–68 y were recruited for the study through a newspaper article. The fasting triacylglycerol concentrations of the subjects were measured with a compact instant blood lipid analyzer (Cholestech LDX System; Cholestech, Hayward, CA) to facilitate assignment to groups by random block design. The Human Ethics Committee of the University of Guelph approved the study, and all subjects gave written informed consent.

Supplements

Supplements were given in the form of gelatin capsules (encapsulation by Bioriginal Food and Science Corp, Saskatoon, Canada) and contained 1100 mg of a fish oil (EPA+DHA) concentrate or a GLA-enriched concentrate derived from borage seed oil (Croda Chemicals Ltd, Goole, United Kingdom). The fish oil concentrate contained a total of 70% n−3 fatty acids (35% EPA and 25% DHA); thus, each capsule contained 660 mg EPA+DHA. The borage seed oil was concentrated to contain 40% GLA, such that each capsule provided 440 mg GLA. Daily capsule supplementation was adjusted to provide the intended average daily intake of 4 g EPA+DHA alone or concomitant with the fixed amounts of GLA (*see* below). This dose of EPA+DHA was used previously to yield a substantial reduction in circulating triacylglycerol concentrations (27). The dose of GLA was within the range of that used in previous clinical studies (28, 29). The clinical coordinator and subjects were blinded to the type of supplementation.

Study design

The subjects provided 12-h fasting blood samples at the beginning and end of the 28-d study period. Duplicate measures of sitting blood pressures and resting heart rate were measured with an automated digital blood pressure monitor. Height, weight, and waist circumference were measured in duplicate. Using the fasting triacylglycerol concentrations to balance the group means in a random block design, we assigned the subjects to 1 of 4 groups: daily supplementation with 4 g EPA+DHA and no GLA (4:0, or control group) or 4 g EPA+DHA plus 1 g GLA (4:1 group), 2 g GLA (4:2 group), or 4 g GLA (4:4 group) for 28 d. The subjects provided a 3-d diet record (completed on 2 weekdays and 1 weekend day) once during the 28-d period of the study; the data were analyzed by using FOOD PROCESSOR NUTRITION ANALYSIS software (version 7.1; ESHA Research, Salem, OR). The subjects were asked to report any side effects of supplement consumption as they occurred or at the end of the study.

Laboratory analyses

After the subjects had fasted overnight, blood was collected by venipuncture into evacuated tubes that were free of anticoagulant or that contained sodium heparin as anticoagulant (Vacutainer; Becton Dickinson, Rutherford, NJ). After time was allowed for the blood in the tubes without anticoagulant to clot, samples were centrifuged at $2.7 \times g$ for 15 min at 25 °C. The recovered serum or plasma was portioned and stored at −80 °C until analyzed. Total plasma cholesterol, HDL-cholesterol, and triacylglycerol concentrations were quantified enzymatically with an autoanalyzer in a provincially certified hospital laboratory subject to the provincial Laboratory Proficiency Testing Program. LDL cholesterol was calculated by using the Friedewald equation (30). The fatty acid compositions of total serum phospholipid, a commonly used fatty acid biomarker [EPA and DHA elevations reflect fish oil intake (source of EPA and DHA) and DGLA elevation reflects consumption of GLA concentrate] (31, 32), were measured after extraction and derivatization of the fatty acid methyl esters, followed by capillary gas–liquid chromatography (33).

Statistical analyses

Statistical analyses were performed with SAS software (version 6.12; SAS Institute, Cary, NC). Because of the nonlinearity of the triacylglycerol data, all statistical comparisons used log triacylglycerol conversions. Baseline characteristics for anthropometric, dietary, lipid, and fatty acid data, across all 4 groups, were compared by analysis of variance. Paired *t* tests were used to compare differences between the lipid and fatty acid indexes on day 0 and those on day 28 within each of the 4 groups. For the fatty acids, the general linear model procedures (one-factor analysis of variance on the changes, least-squares means, and contrast statements, with Bonferroni corrections) were used to compare magnitudes of change across the groups. Unless otherwise indicated, all significance levels were at $P \leq 0.05$. The data for one subject was removed from the data set because of a lack of compliance (as measured by the aforementioned fatty acid biomarkers), possibly because of an underlying bipolar depression.

RESULTS

The baseline characteristics of the subjects are listed in **Table 1**. No significant differences were noted across the 4 groups. Dietary intakes for the 30 subjects who completed 3-d dietary records are shown in **Table 2**. No significant differences were observed across the 4 groups for any of the dietary components.

No significant differences were observed in fasting blood glucose concentrations, body weights, BMIs, waist circumferences, blood pressures, or heart rates during the study (data not shown), except for a small but significant group mean increase in weight and BMI between days 0 and 28 for 2 of the groups. The increases in average weights for the 4:0 and 4:4 groups were 0.63 kg (0.97%) and 0.53 kg (0.78%), respectively. A weight loss or gain of < 1 kg would not be expected to significantly alter blood lipid variables (34).

TABLE 1

Baseline characteristics of the subjects by treatment group[1]

Characteristic	All subjects (n = 31)	4:0 Group (control) (n = 8)	4:1 Group (n = 8)	4:2 Group (n = 7)	4:4 Group (n = 8)
Age (y)	48.0 ± 1.9	49.3 ± 3.4	47.9 ± 4.3	49.3 ± 3.3	44.9 ± 4.2
Weight (kg)	68.2 ± 1.9	65.0 ± 4.0	68.2 ± 5.6	72.3 ± 2.4	67.8 ± 1.8
Systolic BP (mm Hg)	119 ± 2	120 ± 4	118 ± 4	120 ± 5	118 ± 5
Diastolic BP (mm Hg)	78 ± 2	79 ± 1	81 ± 4	76 ± 4	77 ± 4
BMI (kg/m^2)	25.5 ± 0.7	24.5 ± 1.3	25.5 ± 2.3	26.7 ± 1.0	25.4 ± 0.7
Waist circumference (cm)	80.5 ± 1.8	79.1 ± 4.2	80.5 ± 5.3	83.1 ± 2.2	79.9 ± 2.3

[1] $\bar{x}$ ± SEM. BP, blood pressure. The 4:0 (control) group consumed 4 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) daily, the 4:1 group consumed 4 g EPA+DHA and 1 g γ-linolenic acid (GLA) daily, the 4:2 group consumed 4 g EPA+DHA and 2 g GLA daily, and the 4:4 group consumed 4 g EPA+DHA and 4 g GLA daily. There were no significant differences between the 4 groups in any variable (P > 0.05).

Plasma lipid values and calculated lipid ratios for days 0 and 28 are presented in **Table 3**. Mean total cholesterol (TC) values in all groups were unchanged from days 0 to 28, whereas mean LDL-cholesterol concentrations were significantly lower on day 28 than on day 0 in the 4:2 group (by 11.3%). In the 4:4 group, this reduction was 12.9% (P = 0.09). For both the 4:0 and the 4:1 groups, mean LDL-cholesterol values on day 28 were within 2% of the day 0 values. Mean HDL-cholesterol concentrations were significantly higher on day 28 than on day 0 in the 4:0 group (by 7.0%) and the 4:1 group (by 10.0%). Mean non-HDL-cholesterol concentrations were significantly lower on day 28 than on day 0 in the 4:1 and 4:2 groups, by 5.3% and 14.4%, respectively, and by 7.8% (P = 0.10) and 12.0% (P = 0.07) in the 4:0 and 4:4 groups, respectively.

The ratio of total to HDL cholesterol was significantly reduced in all 4 groups, by 11.0%, 9.6%, 14.0%, and 14.7% (group means) in the 4:0, 4:1, 4:2, and 4:4 groups, respectively. The mean group reductions from days 0 to 28 in the ratio of LDL to HDL cholesterol were statistically significant in the 4:1 group (6.0% reduction), the 4:2 group

(14.8% reduction), and the 4:4 group (19.9% reduction). Mean group triacylglycerol concentrations were significantly lower on day 28 than on day 0 in the 4:0, 4:1, and 4:2 groups, by 39.7%, 39.2%, and 35.4%, respectively. In the 4:4 group, no significant reductions in triacylglycerol concentrations were observed from days 0 to 28.

The fatty acid compositions of serum phospholipids are shown in **Table 4**. Fatty acid ratios of particular interest are presented graphically in **Figure 1**. All 4 groups had significantly higher mean percentages of the n−3 fatty acids and higher ratios of n−3 to n−6 fatty acids on day 28 than on day 0. Marked effects of supplementation on specific fatty acids, which are eicosanoid precursors (Table 4), were observed. In the 4:0 group, DGLA decreased significantly between days 0 and 28 (group mean reduction of 47%). In the 4:1 group, no significant change between days 0 and 28 was observed. In the 4:2 and 4:4 groups, DGLA increased significantly (31% and 82%, respectively) between days 0 and 28. These findings were also reflected in the calculated DGLA-AA ratios (Figure 1), except that the change in this ratio (35% increase in the

TABLE 2

Daily dietary intakes of the subjects by treatment group[1]

Dietary component	All subjects (n = 30)	4:0 Group (control) (n = 8)	4:1 Group (n = 7)	4:2 Group (n = 7)	4:4 Group (n = 8)
Energy					
(kcal)	2031 ± 100	2001 ± 191	2125 ± 208	1995 ± 237	2011 ± 210
(kJ)	8499 ± 420	8373 ± 801	8891 ± 871	8349 ± 991	8413 ± 878
Protein					
(% of energy)	17.1 ± 0.8	18.1 ± 1.5	16.7 ± 1.7	16.6 ± 1.3	17.0 ± 2.1
(g)	85.5 ± 3.7	88.2 ± 5.3	88.5 ± 9.4	85.0 ± 11.2	80.8 ± 5.0
Carbohydrate					
(% of energy)	46.7 ± 2.0	46.5 ± 2.1	49.0 ± 6.4	48.9 ± 4.0	42.9 ± 3.3
(g)	241 ± 15	238 ± 25	271 ± 50	238 ± 23	220 ± 26
Total fat					
(% of energy)	34.5 ± 1.7	33.5 ± 2.5	33.3 ± 5.0	32.7 ± 3.4	38.1 ± 2.8
(g)	80.3 ± 5.8	77.4 ± 9.8	77.3 ± 11.3	78.5 ± 14.1	87.5 ± 13.3
Alcohol					
(% of energy)	1.8 ± 0.4	1.9 ± 0.6	1.4 ± 0.9	1.9 ± 0.8	1.9 ± 0.9
(g)	5.6 ± 1.3	5.6 ± 2.4	5.2 ± 3.7	6.1 ± 2.4	5.6 ± 2.6
Saturated fat (g)	30.2 ± 2.6	30.6 ± 5.8	29.2 ± 5.9	28.2 ± 4.6	32.4 ± 4.9
Monounsaturated fat (g)	27.1 ± 2.4	24.2 ± 4.0	26.1 ± 4.6	27.7 ± 5.1	30.3 ± 5.8
Polyunsaturated fat (g)	11.6 ± 1.1	10.4 ± 1.6	11.3 ± 1.1	13.9 ± 3.7	11.0 ± 1.9
Dietary fiber (g)	19.9 ± 1.6	19.1 ± 3.7	19.5 ± 3.3	21.2 ± 2.9	19.8 ± 3.0
Cholesterol (mg)	305 ± 30	342 ± 38	297 ± 102	269 ± 35	307 ± 60
Sodium (mg)	2951 ± 154	2915 ± 335	2633 ± 224	3147 ± 398	3092 ± 285

[1] $\bar{x}$ ± SEM. The 4:0 (control) group consumed 4 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) daily, the 4:1 group consumed 4 g EPA+DHA and 1 g γ-linolenic acid (GLA) daily, the 4:2 group consumed 4 g EPA+DHA and 2 g GLA daily, and the 4:4 group consumed 4 g EPA+DHA and 4 g GLA daily. There were no significant differences between the 4 groups in any variable (P > 0.05).

TABLE 3
Effects of supplementation with a fish oil concentrate alone or in combination with a GLA-enriched borage seed oil concentrate on plasma lipid profiles by treatment group[1]

Lipid or lipoprotein ratio	4:0 Group (control) ($n = 8$)		4:1 Group ($n = 8$)		4:2 Group ($n = 7$)		4:4 Group ($n = 8$)	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Total cholesterol (mmol/L)	5.51 ± 0.27	5.29 ± 0.25	5.35 ± 0.36	5.27 ± 0.34	5.68 ± 0.38	5.18 ± 0.36	5.47 ± 0.40	5.08 ± 0.40
LDL cholesterol (mmol/L)	3.47 ± 0.25	3.39 ± 0.30	3.28 ± 0.33	3.33 ± 0.34	3.64 ± 0.25	3.23 ± 0.20[2]	3.49 ± 0.42	3.04 ± 0.38
HDL cholesterol (mmol/L)	1.42 ± 0.13	1.52 ± 0.14[3]	1.41 ± 0.07	1.55 ± 0.07[2]	1.51 ± 0.19	1.61 ± 0.21	1.47 ± 0.15	1.56 ± 0.15
Non–HDL cholesterol (mmol/L)	4.09 ± 0.35	3.77 ± 0.32	3.94 ± 0.36	3.73 ± 0.36[2]	4.18 ± 0.24	3.58 ± 0.18[2]	4.00 ± 0.48	3.52 ± 0.43
Triacylglycerols (mmol/L)	1.36 ± 0.33	0.82 ± 0.12[3]	1.43 ± 0.34	0.87 ± 0.15[4]	1.16 ± 0.35	0.75 ± 0.16[2]	1.11 ± 0.17	1.03 ± 0.19
Total:HDL cholesterol	4.18 ± 0.53	3.72 ± 0.40[2]	3.84 ± 0.30	3.47 ± 0.30[2]	4.06 ± 0.48	3.49 ± 0.39[3]	4.07 ± 0.57	3.47 ± 0.41[2]
LDL:HDL cholesterol	2.65 ± 0.37	2.44 ± 0.35	2.35 ± 0.24	2.21 ± 0.28[2]	2.57 ± 0.24	2.19 ± 0.26[5]	2.66 ± 0.48	2.13 ± 0.35[2]

[1] $\overline{x}$ ± SEM. The 4:0 (control) group consumed 4 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) daily, the 4:1 group consumed 4 g EPA+DHA and 1 g γ-linolenic acid (GLA) daily, the 4:2 group consumed 4 g EPA+DHA and 2 g GLA daily, and the 4:4 group consumed 4 g EPA+DHA and 4 g GLA daily.
[2–5] Significantly different from day 0 (paired t test): [2] $P \le 0.05$, [3] $P < 0.01$, [4] $P < 0.001$, [5] $P < 0.0001$.

group mean) in the 4:1 group from days 0 to 28 was significant and was mainly due to a significant reduction in the group mean AA value. From days 0 to 28, a significant reduction was observed in AA in all 4 groups: 23%, 22%, 11%, and 18% in groups 4:0, 4:1, 4:2, and 4:4, respectively. All groups had significant mean increases in EPA and DHA: 468%, 369%, 359%, and 317% for EPA and 87%, 100%, 103%, and 97% for DHA in the 4:0, 4:1, 4:2, and 4:4 groups, respectively. The EPA-AA ratios were also significantly higher in all groups after 28 d of supplementation (Figure 1).

For group pairwise comparisons, as reflected by the different superscript letters in Table 4, there were no significant differences in the group mean reductions in AA from days 0 to 28. Significant differences were observed in all pairwise comparisons of group mean changes in DGLA and in all group mean DGLA-AA ratios from days 0 to 28, except for the comparison between the 4:1 and 4:2 groups (Figure 1). Both the increases in EPA (as a percentage of total fatty

acids) and the increases in the EPA-AA ratio were significantly different between the 4:0 and 4:4 groups (Table 4 and Figure 1).

DISCUSSION

We evaluated the effects of a fixed amount of EPA+DHA supplementation, with or without different levels of GLA supplementation, on plasma lipids, lipid ratios, and fatty acid profiles of serum phospholipids. EPA+DHA supplementation alone (4 g) or in conjunction with GLA supplementation (1 or 2 g) reduced serum triacylglycerol concentrations by 40%, 39%, and 35% in the 4:0, 4:1, and 4:4 groups, respectively, whereas triacylglycerol concentrations did not change significantly when GLA supplementation increased to 4 g/d. Others have reported that marine oil supplements significantly lowered plasma triacylglycerol concentrations (24, 27), whereas GLA supplementation was without effect (25, 35). The triacylglycerol

TABLE 4
Fatty acid compositions of serum phospholipids before and after supplementation by treatment group[1]

Fatty acid	4:0 Group (control) ($n = 8$)		4:1 Group ($n = 8$)		4:2 Group ($n = 7$)		4:4 Group ($n = 8$)	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
	% by wt of total fatty acids							
18:1	11.72 ± 0.35	10.16 ± 0.42[2]	11.27 ± 0.48	9.19 ± 0.47[3]	11.75 ± 0.49	9.99 ± 0.28[3]	11.06 ± 0.68	9.20 ± 0.37[3]
18:2n−6	18.55 ± 0.62	14.98 ± 0.70[3]	19.10 ± 0.49	14.56 ± 0.88[3]	19.04 ± 1.28	15.70 ± 1.29	20.29 ± 1.04	14.38 ± 0.82[4]
18:3n−6, GLA	0.06 ± 0.02	0.00 ± 0.00[a,2]	0.09 ± 0.01	0.08 ± 0.02[a]	0.06 ± 0.03	0.15 ± 0.01[b,3]	0.07 ± 0.02	0.23 ± 0.02[b,4]
18:3n−3	0.21 ± 0.03	0.16 ± 0.02	0.22 ± 0.03	0.16 ± 0.01	0.24 ± 0.04	0.15 ± 0.03	0.23 ± 0.03	0.13 ± 0.01[2]
20:3n−6, DGLA	3.30 ± 0.30	1.74 ± 0.16[a,4]	2.54 ± 0.34	2.58 ± 0.26[b]	3.02 ± 0.24	3.95 ± 0.33[b,2]	2.79 ± 0.22	5.08 ± 0.56[c,4]
20:4n−6, AA	9.78 ± 0.66	7.51 ± 0.30[3]	10.00 ± 0.64	7.85 ± 0.52[4]	9.18 ± 0.69	8.20 ± 0.56[3]	10.21 ± 0.74	8.41 ± 0.57[3]
20:5n−3, EPA	1.12 ± 0.13	6.36 ± 0.52[a,5]	1.22 ± 0.37	5.72 ± 0.76[a,b,5]	1.11 ± 0.20	5.09 ± 0.27[a,b,5]	0.96 ± 0.26	4.00 ± 0.34[b,5]
22:5n−3	0.85 ± 0.04	1.46 ± 0.11[3]	0.94 ± 0.094	1.69 ± 0.17[4]	0.83 ± 0.03	1.34 ± 0.09[4]	0.77 ± 0.06	1.39 ± 0.09[4]
22:6n−3, DHA	3.41 ± 0.25	6.37 ± 0.22[5]	3.35 ± 0.46	6.69 ± 0.55[5]	2.94 ± 0.257	5.97 ± 0.23[5]	3.11 ± 0.41	6.14 ± 0.41[5]
ΣSFA	45.72 ± 0.80	46.44 ± 0.87[2]	45.73 ± 0.80	46.13 ± 1.14	45.39 ± 0.91	45.12 ± 0.96	45.34 ±1.60	45.34 ± 0.61
Σn −6	31.67 ± 0.57	24.24 ± 0.94[5]	31.73 ± 0.98	25.06 ± 1.54[4]	31.30 ± 1.03	28.00 ± 1.14	33.36 ± 0.63	28.09 ± 0.66[5]
Σn −3	5.60 ± 0.23	14.35 ± 0.81[5]	5.73 ± 0.86	14.27 ± 1.38[5]	5.13 ± 0.39	12.55 ± 0.46[5]	5.07 ± 0.67	11.66 ± 0.77[5]
Σn−3:Σn−6	0.18 ± 0.01	0.61 ± 0.06[5]	0.19 ± 0.03	0.61 ± 0.09[4]	0.17 ± 0.01	0.46 ± 0.03[5]	0.15 ± 0.02	0.42 ± 0.04[5]
DGLA:AA	0.35 ± 0.04	0.23 ± 0.2[a,2]	0.26 ± 0.04	0.35 ± 0.05[b,2]	0.34 ± 0.04	0.50 ± 0.06[b,3]	0.29 ± 0.04	0.64 ± 0.05[c,3]
EPA:AA	0.12 ± 0.02	0.88 ± 0.11[a,5]	0.14 ± 0.05	0.79 ± 0.15[a,b,4]	0.13 ± 0.03	0.64 ± 0.05[a,b,5]	0.09 ± 0.02	0.49 ± 0.04[b,5]
DGLA+EPA:AA	0.48± 0.05	1.10 ± 0.10[4]	0.40 ± 0.07	1.14 ± 0.18[4]	0.47 ± 0.05	1.14 ± 0.10[5]	0.38 ± 0.04	1.13 ± 0.13[4]

[1] $\overline{x}$ ± SEM. DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; SFA, saturated fatty acids (various minor SFAs were omitted). The 4:0 (control) group consumed 4 g eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) daily, the 4:1 group consumed 4 g EPA+DHA and 1 g γ-linolenic acid (GLA) daily, the 4:2 group consumed 4 g EPA+DHA and 2 g GLA daily, and the 4:4 group consumed 4 g EPA+DHA and 4 g GLA daily. Values within a row with different superscript letters indicate significant pairwise differences between groups in the magnitude of the change from day 0 to day 28.
[2–5] Significantly different from day 0 (paired t test): [2] $P \le 0.05$, [3] $P < 0.01$, [4] $P < 0.001$, [5] $P < 0.0001$.
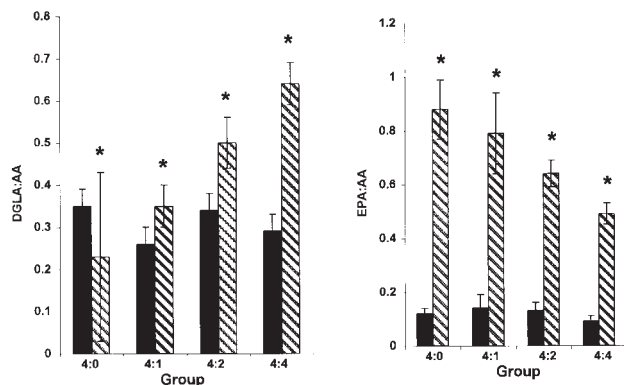
FIGURE 1. Mean (±SEM) ratios of dihomo-γ-linolenic acid (DGLA) to arachidonic acid (AA) and of eicosapentaenoic acid (EPA) to AA in serum phospholipids on day 0 (■) and day 28 (▨) after supplementation with EPA + docosahexaenoic acid (DHA) or EPA+DHA and γ-linolenic acid (GLA). The 4:0 (control) group consumed 4 g EPA + DHA daily, the 4:1 group consumed 4 g EPA+DHA and 1 g GLA daily, the 4:2 group consumed 4 g EPA+DHA and 2 g GLA daily, and the 4:4 group consumed 4 g EPA+DHA and 4 g GLA daily. *Significantly different from day 0, $P \leq 0.05$ (paired *t* test).

lowering seen with the EPA+DHA supplementation level of 4 g is in the predictable range, based on other studies (24, 27).

Interestingly, the combination of EPA+DHA and GLA in the 4:2 and 4:4 groups resulted in a tendency of LDL cholesterol to decrease by 11.3% and 12.7% (group means), respectively, which were only significant in the former group (Table 3). These results are of considerable interest because fish oil alone (EPA+DHA) typically has no effect or a modest elevating effect on LDL-cholesterol concentrations (22). Furthermore, the 4:2 group had the largest mean reduction (14.4%) in non-HDL-cholesterol concentrations.

The international task force for the prevention of CVD uses the variables age, systolic blood pressure, cigarette smoking, family history of diabetes and MI, and LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations with the PROCAM risk calculator to predict the risk of MI within the next 10 y (36). On the basis of this risk calculator, the lipid changes observed (before and after supplementation) in the 4 groups studied yielded estimated reductions in the risk of MI over a 10-y period of 37%, 33%, 43%, and 24% (group means) in the 4:0, 4:1, 4:2, and 4:4 groups, respectively. Of all the lipid variables we assessed, 5 were beneficially modified in the 4:1 and the 4:2 groups compared with 3 and 2 in the 4:0 and 4:4 groups, respectively. Furthermore, considering the recent publication on the importance of non-HDL cholesterol as a predictor of CVD mortality (4), it is of interest to note that non-HDL-cholesterol concentrations decreased significantly only in the 2 intermediate groups (4:1 and 4:2).

The fatty acid profiles of serum phospholipids followed closely the degree of supplementation in each of the groups and illustrate the competitive interactions between fatty acid (n−3 compared with n−6) metabolic pathways (14, 20). For example, although all groups were supplemented with 4 g EPA+DHA, fatty acid concentrations of EPA decreased linearly with increasing amounts of GLA supplements (Table 4). This was reflected by a corresponding progressive increase in DGLA values, from a group mean low of 1.74% by weight in the 4:0 group to a group mean high of

5.08% by weight in the 4:4 group. All 4 groups showed similar and significant group mean reductions in AA concentrations in serum phospholipids on supplementation. It is of interest to note that the expected dietary GLA-induced increase in AA in serum phospholipids (20) was attenuated in this study by the presence of EPA+DHA. The multiple factors that likely account for an actual reduction in AA concentrations include competition by EPA and DGLA for esterification into cellular phospholipids and the attenuating effect of n−3 fatty acids on Δ^5-desaturase (EC 1.14.99.25), which affects the conversion of DGLA to AA (6, 14, 16).

Many diseases and conditions such as atherosclerosis, CVD, and rheumatoid arthritis have inflammatory components that can contribute to the etiology of a disease or exacerbate its manifestations in the body (7, 28, 37). AA, DGLA, and EPA are eicosanoid precursors whose metabolic products may exert significant influence on cellular functions. For example, eicosanoids synthesized from AA, such as PGE_2, LTB_4, LTC_4, and TXA_2 have proinflammatory properties (38–40). The major eicosanoid synthesized from DGLA, PGE_1, is predominantly seen as antiinflammatory (11, 28, 41–43). In addition, lipoxygenase products of DGLA metabolism—such as 15-hydroxy-DGLA and 15-(*S*)-hydroxy-8, 11,13-eicosatrienoic acid—also have antiinflammatory effects (11, 13, 28). EPA-derived eicosanoids, eg, LTB_5 and TXA_3, are considered to have little or no inflammatory or thrombotic activity in contrast with those produced from AA (12, 40, 43, 44).

In the present study, adding a source of EPA+DHA to the diet, concomitant with incremental amounts of GLA, reduced mean AA concentrations in serum phospholipids and increased concentrations of EPA. However, DGLA increased only when the ratio of EPA+DHA to GLA was 4:2 or 4:4. The amount of EPA and GLA supplements used by Barham et al (20), 3 g of each, increased serum lipid concentrations of both DGLA and EPA but did not affect the reductions in AA concentrations seen in our study.

It is noteworthy that increasing concentrations of DHA in serum phospholipids have been inversely correlated with coronary heart disease (45). In this regard, DHA concentrations reaching 4.8% by weight of fatty acids in serum phospholipids have been associated with an odds risk ratio of 0.66. Of note, all 4 groups in our study attained this DHA concentration, increasing from a range (group means) of 2.94–3.41% by weight at entry to 5.97–6.69% by weight after supplementation.

In summary, the 4:2 group (4 g EPA+DHA and 2 g GLA) had the greatest overall reduction (43%) in MI risk on the basis of the PROCAM program (which takes LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations into account). The 4:2 and the 4:4 groups also had increased concentrations of DGLA and lower AA concentrations while simultaneously maintaining an increase in total n−3 fatty acids.

With recent recommendations by the American Heart Association for EPA+DHA supplementation in CVD patients (46), the possible inclusion of GLA may be of added benefit. Further studies using mixtures of EPA+DHA and GLA in patients with CVD or inflammatory conditions, such as rheumatoid arthritis, are needed for the development of additional treatment strategies for these patients. ❧

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