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Incorporation and Clearance of Omega-3 Fatty Acids in Erythrocyte Membranes and Plasma Phospholipids

Jing Cao,[1] Kerry A. Schwichtenberg,[1] Naomi Q. Hanson,[1] and Michael Y. Tsai[1*]

Background: **The sum of eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3) in erythrocyte membranes, termed the omega-3 index, can indicate suboptimal intake of omega-3 fatty acids, a risk factor for cardiovascular disease (CVD). To study the effects of fatty acid supplementation, we investigated the rate of incorporation and clearance of these fatty acids in erythrocyte membranes and plasma after intake of supplements.**

Methods: **Twenty study participants received supplementation with either fish oil (1296 mg EPA + 864 mg DHA/day) or flaxseed oil (3510 mg alpha-linolenic acid + 900 mg linoleic acid/day) for 8 weeks. We obtained erythrocyte membrane and plasma samples at weeks 0, 4, 8, 10, 12, 14, 16, and 24 and extracted and analyzed fatty acids by gas chromatography.**

Results: **After 8 weeks of fish oil supplementation, erythrocyte membrane EPA and DHA increased 300% (P <0.001) and 42% (P <0.001), respectively. The mean erythrocyte omega-3 index reached a near optimal value of 7.8%, and remained relatively high until week 12. EPA and DHA showed greater increases and more rapid washout period decreases in plasma phospholipids than in erythrocyte membranes. Flaxseed oil supplementation increased erythrocyte membrane EPA to 133% (P <0.05) and docosapentaenoic acid (DPA, 22:5 ω3) to 120% (P <0.01) of baseline, but DHA was unchanged. In plasma phospholipids, EPA, DPA, and DHA showed a slight but statistically insignificant increase.**

Conclusions: **Erythrocyte membrane EPA+DHA increases during relatively short intervals in response to supplementation at rates related to amount of supplementation. These results may be useful to establish appropriate dosage for omega-3 fatty acid supplementation.**

Measurement of eicosapentaenoic acid (EPA, 20:5 ω3)[2] and docosahexaenoic acid (DHA, 22:6 ω3) in erythrocyte membranes, termed the omega-3 index, can be used by clinical laboratories to assess for suboptimal intake of omega-3 fatty acids, which is a risk factor assessment for cardiovascular disease (CVD) *(1)*.

Investigations have shown that EPA and DHA, the major biological functional components of fish oil, are associated with reduced risk for primary cardiac arrest *(2)*, sudden cardiac death *(3)*, and fatal ischemic heart disease *(4)*. Studies using animal models and isolated cardiac myocytes have suggested that EPA and DHA have a direct protective effect on the heart *(5–10)* attributable in part to the antiarrhythmic effect of EPA and DHA on ion channels *(11)*. Other beneficial effects of fish oil include decreased plasma triglycerides *(12)* and blood pressure *(13, 14)* and decreased expression of inflammatory markers *(15)*.

The evidence that long-chain polyunsaturated omega-3 fatty acids can reduce risk of CVD is sufficiently strong that both the American Heart Association and European Cardiology Society now recommend increased intake of fish or fish oil supplementation *(16, 17)*. Compared with plasma and other tissues, erythrocytes have the strongest association with cardiac tissue omega-3 fatty acid concentrations *(18)* and thus are promising candidates as risk markers for CVD.

Alpha-linolenic acid (ALA, 18:3 ω3) is a natural precursor of EPA and DHA and can be further elongated and unsaturated in vivo. Prospective cohort studies have

[1] University of Minnesota, Minneapolis, MN 55455.
* Address correspondence to this author at: 420 Delaware St. SE, Mayo Mail Code 609, Minneapolis, MN 55455-0392. Fax 612-625-1121; e-mail tsaix001@tc.umn.edu.

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[2] Nonstandard abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CVD, cardiovascular disease; ALA, alpha-linolenic acid; BMI, body mass index; LA, linoleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.

suggested that dietary ALA is beneficial to cardiovascular health, although this evidence may be confounded by other dietary variables associated with high intakes of ALA *(19, 20)*. Human studies were inconsistent as to whether ALA can be converted to EPA and DHA efficiently and thus exert beneficial effects similar to those of the long chain omega-3 fatty acids *(21–24)*.

In spite of the importance of the omega-3 fatty acids, relatively few studies have monitored the correlation of supplement intake and subsequent enrichment of these fatty acids in erythrocyte membranes and plasma. One study has addressed the rate at which fatty acid concentrations in erythrocyte membranes and plasma decrease after discontinuation of supplementation *(25)*, and few data address the effect of ALA intake on plasma and erythrocyte membrane omega-3 fatty acid composition *(21, 23)*. We investigated the incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipid fractions after fish oil or flaxseed oil supplementation.

Materials and Methods

STUDY PARTICIPANTS

We recruited 20 apparently healthy participants [12 women and 8 men; mean (SD) age 48.8 (8) years] for this study. Medical history, family medical history, and demographic information were gathered at recruitment. Exclusion criteria included history of cancer, rheumatoid arthritis, heart attack, liver and kidney disease, neurological/psychological disease, bleeding disorders, history of taking fish oil or flaxseed oil in the past 6 months, and allergies to seafood. People with increased cholesterol or history of diabetes were not excluded from the study. The study protocol was approved by University of Minnesota Institutional Review Board committee. Informed consent and Health Insurance Portability and Accountability Act authorization forms were signed by all participants.

SUPPLEMENTATION AND STUDY DESIGN

We used a randomized, single-blinded parallel study design to compare the effects of 2 supplementation methods in study participants, who were matched for age, sex, and body mass index (BMI) and randomly assigned to receive 8 weeks of supplementation with either fish oil or flaxseed oil. Participants were instructed to orally ingest 6 softgel capsules of fish oil (EPA 1296 mg, DHA 864 mg) plus 1 capsule of vitamin E (400 IU) per day or 6 softgel capsules of flaxseed oil [ALA 3510 mg, linoleic acid (LA) 900 mg] plus 1 capsule of vitamin E (400 IU) per day. Vitamin E capsules were included as an antioxidant to protect the polyunsaturated fatty acids from being oxidized. Fish oil, flaxseed oil, and vitamin E supplements were purchased from Vitamin World™. The nature of the oil supplementation was blinded to participants and coded by A or B, respectively.

Blood collection was performed on the day before supplementation started (week 0), and the last day of weeks 4, 8 (end of supplementation), 10, 12, 14, 16, and 24 (end of wash-out period). The length of the study was 24 weeks. At each visit, all participants were asked to report any illness, use of medication, and omission of softgel capsule intake.

BLOOD SAMPLING AND STORAGE

After study participants fasted overnight, we collected venous blood into 2 10-mL Vacutainer Tubes with K.2.EDTA spray. Samples were put on ice and sent immediately to the laboratory, where erythrocytes were separated from plasma by centrifugation at 2000*g* at 4 °C for 10 min. The plasma was stored at −70 °C, and an equal volume of acid-citrate-dextrose solution was added to the packed cells. The acid-citrate-dextrose/packed cells mixture was stored at 4 °C. Analyses for fatty acids were performed within 1 week.

FATTY ACIDS EXTRACTION AND ANALYSES

Erythrocytes were washed 3 times with ice-cold isotonic saline to remove the buffy coat. The packed cells (2 mL) were lysed with 30 mL of cold distilled water and centrifuged at 20 000*g* at 10 °C for 20 min to form a tight pellet. Supernatant was removed, and the above procedure was repeated 2 times or until supernatant was clear. Erythrocyte ghosts were brought to a volume of 2 mL with distilled water and gently sonicated on ice. Lipids were extracted from the erythrocyte membranes with a mixture of chloroform:methanol (2:1, by volume) *(26)* and 25 μL of 2 g/L 21:0 standard (diheneicosanoyl phosphatidylcholine) was added to the filtered chloroform to monitor the extraction efficiency. Samples with <60% recovery were considered unacceptable. Tubes of chloroform were evaporated to dryness under nitrogen for the formation of methyl esters. For the extraction of plasma phospholipid fatty acids, 0.3 mL of plasma was mixed with 0.7 mL of 0.9% saline. We added 50 μL of 2 g/L 17:0 standard (diheptadecanoyl phosphatidylcholine) to monitor the extraction efficiency. Lipids were extracted from the plasma with a mixture of chloroform:methanol (2:1, by volume), and cholesterol, triglycerides and phospholipid subclasses were separated on a silica thin-layer chromatography plate in a solvent mixture of petroleum ether, diethyl ether, and glacial acetic acid (80:20:1, by volume). The band of phospholipids was harvested for the formation of methyl esters. For both plasma and erythrocytes, fatty acid methyl esters were prepared with 1.5 mL of 14% boron trifluoride in methanol, incubated at 80 °C for 90 min, and extracted with petroleum ether *(27)*. The final product was dissolved in heptane and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph equipped with a HP6890A autosampler *(28)*. The gas chromatograph was configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation

software. Adequate separation of fatty acid methyl esters was obtained over a 50-min period with an initial temperature of 190 °C for 25 min. The temperature was increased to 240 °C at a rate of 2 °C/min and held for 5 min. Fatty acid methyl esters from 12:0 through 24:1n9 were separated, identified and expressed as percent of total. The following CVs were obtained on 20 blind duplicates: LA, 2.6%; ALA, 2.4%; arachidonic acid (AA), 2.4%; EPA, 3.3%; docosapentaenoic acid (DPA), 2.9% and DHA, 2.7%.

STATISTICS

The gaussian pattern of data distribution was assessed by gaussian probability plots and Shapiro–Wilk test. Within-group differences were examined by paired *t* tests. Repeated measures one-way ANOVA was used for multiple comparisons. Data analysis was performed with a Microsoft Excel 2003 Data Analysis Package. Means, SDs, and SEs are included. *P* values <0.05 were considered significant.

Results

COMPLIANCE AND SUPPLEMENTATION INTAKE

One participant in the fish oil group discontinued the study at the end of week 1. Softgel capsules counted at weeks 4 and 8 verified that the remaining 19 participants adhered to the protocol. Plasma and erythrocyte membrane fatty acids were measured as an indicator of dietary intake.

ERYTHROCYTE MEMBRANE FATTY ACIDS

We compared the concentrations of major omega-3 and omega-6 fatty acids in erythrocyte membranes from participants after ingestion of fish oil or flaxseed oil (Fig. 1). After supplementation with fish oil, EPA and DHA increased significantly in erythrocyte membranes ($P < 0.0001$ and $P < 0.001$ respectively). The mean percent EPA increased 300% ($P < 0.001$, Fig. 1A), whereas DHA concentrations increased only 42% ($P < 0.001$, Fig. 1B). The relative amounts of EPA and DHA in erythrocyte membranes increased gradually and significantly in the fish oil

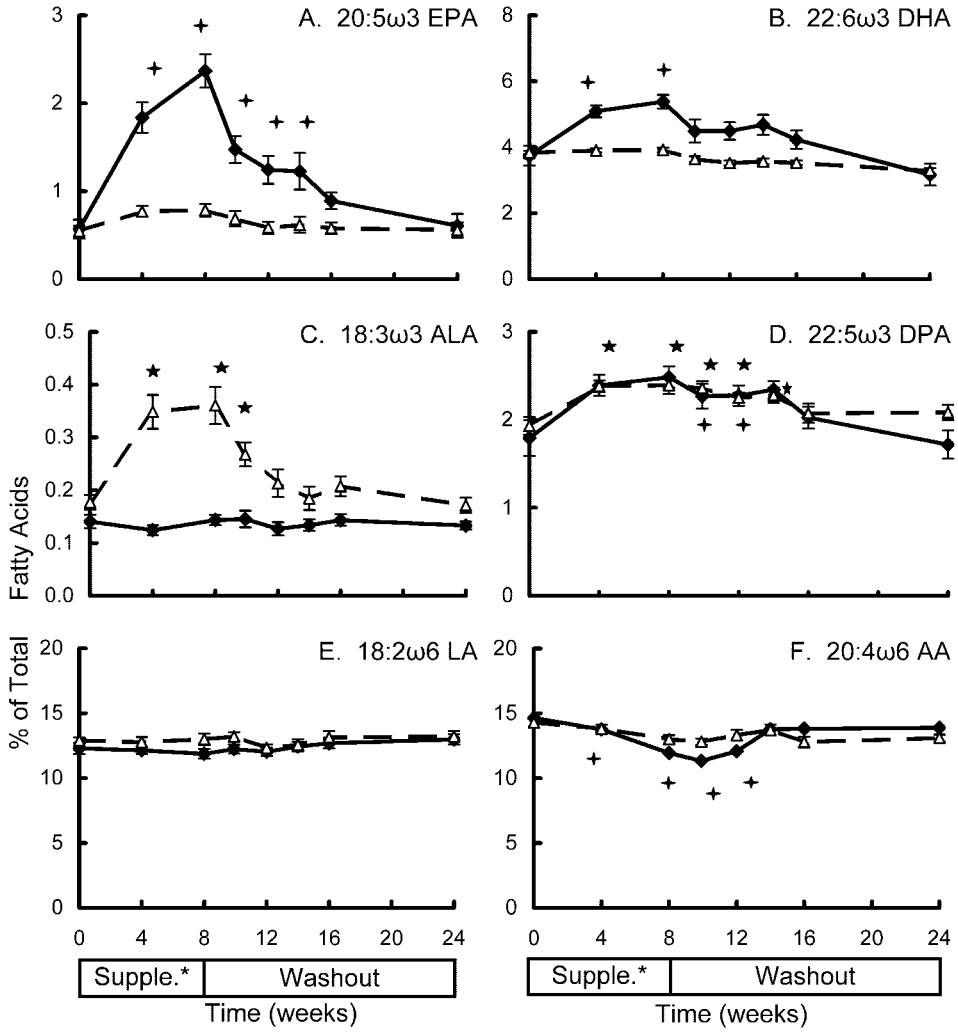

Fig. 1. Changes in the composition of major erythrocyte membrane omega-3 and omega-6 fatty acids over time of study.

Values shown are the mean percentage of total fatty acids (SE) in fish oil group (n = 9) (♦) and flaxseed oil group (n = 10) (△). ✚*P* <0.01 for significant difference from baseline in fish oil group by paired *t*-test. ⋆ *P* <0.01 for significant difference from baseline in flaxseed oil group by paired *t*-test. *Supple., supplementation.

group during supplementation and did not return to baseline concentrations until 16 weeks postsupplementation. The concentration of ALA did not change significantly during the 24 weeks (Fig. 1C). The average concentration of DPA (22:5 ω3), an elongation product of EPA in erythrocyte membranes, increased 39% ($P < 0.01$, Fig. 1D) and decreased to baseline concentration after washout. The rise in EPA and DHA was compensated for by a decrease in LA (18:2 ω6) (Fig. 1E) and AA (20:4 ω6) (Fig. 1F). The mean AA concentration decreased gradually during supplementation, reaching the lowest concentration (23% decrease, $P < 0.001$) at week 10, and returning to the baseline at the end of 24 weeks.

In the flaxseed group, the 33% increase ($P < 0.05$) in mean EPA from the baseline concentration was less than that in the fish oil group (Fig. 1A). DHA showed no statistically significant increase after supplementation (Fig. 1B). The mean ALA concentration increased 100% at week 8 ($P < 0.001$) and remained above baseline until week 10 (Fig. 1C). DPA in erythrocyte membranes showed a significant trend of increase after supplementation ($P < 0.01$) with a mean concentration increase of 20% at week 8 ($P < 0.01$; Fig. 1D). No significant change was detected in the LA concentrations in the flaxseed oil group (Fig. 1E). The 9% decrease in mean AA concentrations was less than that in the fish oil group (Fig. 1F).

RATE OF INCORPORATION OF EPA AND DHA IN ERYTHROCYTE MEMBRANES

The variation in the rate of changes in erythrocyte membranes after fish oil supplementation was less in EPA than DHA (Fig. 2). The EPA concentrations at baseline were 0.2%–1.3%, and after supplementation an increase in EPA of 1.3%–2.4% was observed (Fig. 2A). For 5 of the 9 participants, the incorporation rates (percentage increase per gram of EPA) were within 1 SD of the mean of 1.4. Baseline DHA concentrations were 2.3%–5.0%, and after supplementation DHA concentrations increased to 4.2%–6.4% (Fig. 2B). Compared with EPA, the incorporation rates for DHA had a larger range, 0.8%–2.9%, with a mean of 1.9. Two participants who had relatively high baseline concentrations of DHA (4.8% and 4.5%, respectively) had the lowest incorporation rates (0.8% and 1.1%, respectively), and 2 participants with relatively low baseline concentrations (2.8% and 3.2%, respectively) had the highest incorporation rates (2.8% and 2.9%, respectively).

PLASMA PHOSPHOLIPIDS

Concentrations of the major omega-3 and omega-6 fatty acids in plasma phospholipids from participants after ingestion of fish oil or flaxseed oil are compared in Fig. 3. The concentrations of EPA and DHA in plasma phospholipids increased rapidly and significantly in the fish oil group after supplementation ($P < 0.0001$ for both EPA and

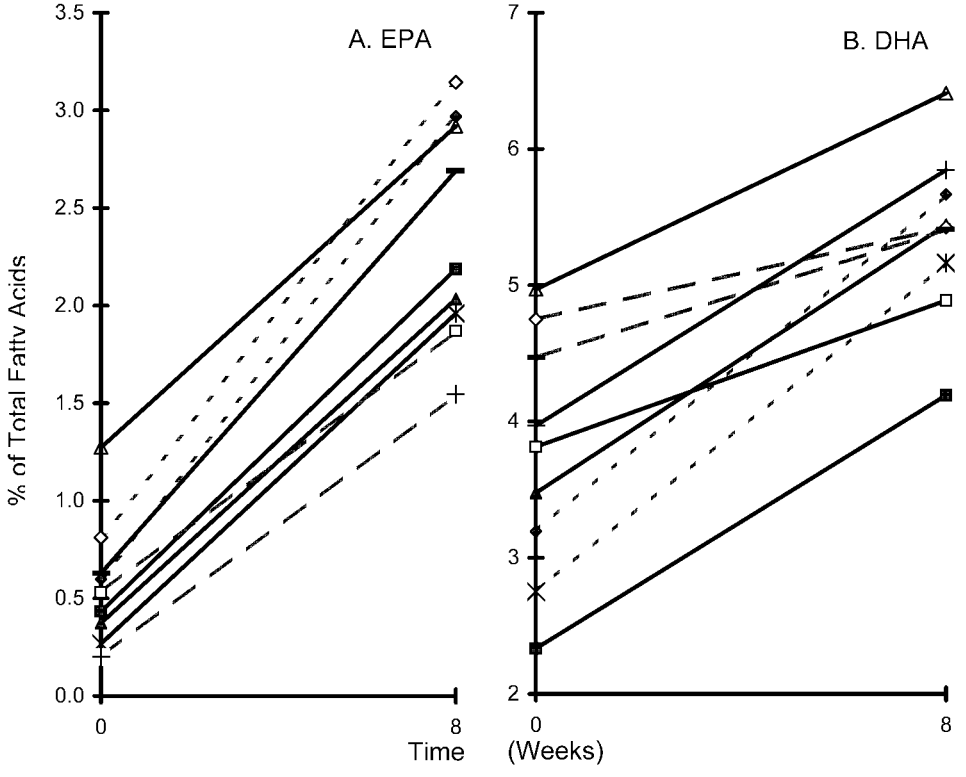

Fig. 2. Erythrocyte membrane EPA and DHA concentrations from participants in the fish oil group (n = 9) at weeks 0 and 8.

The incorporation rates (percent increase per gram of EPA or DHA supplemented) were categorized into 3 groups. Mean (1SD) (*solid line*), < mean − 1SD (*dashed line*), and > mean + 1SD (*dotted line*). Mean (1SD) are 1.4 (0.3) for EPA and 1.9 (0.7) for DHA. Each participant is denoted by the same symbol in Figs. 2A and 2B. ◇, participant 1; ◆, participant 2;△, participant 3;⁻, participant 4; ■, participant 5; ▲, participant 6; *, participant 7; □, participant 8; +, participant 9.

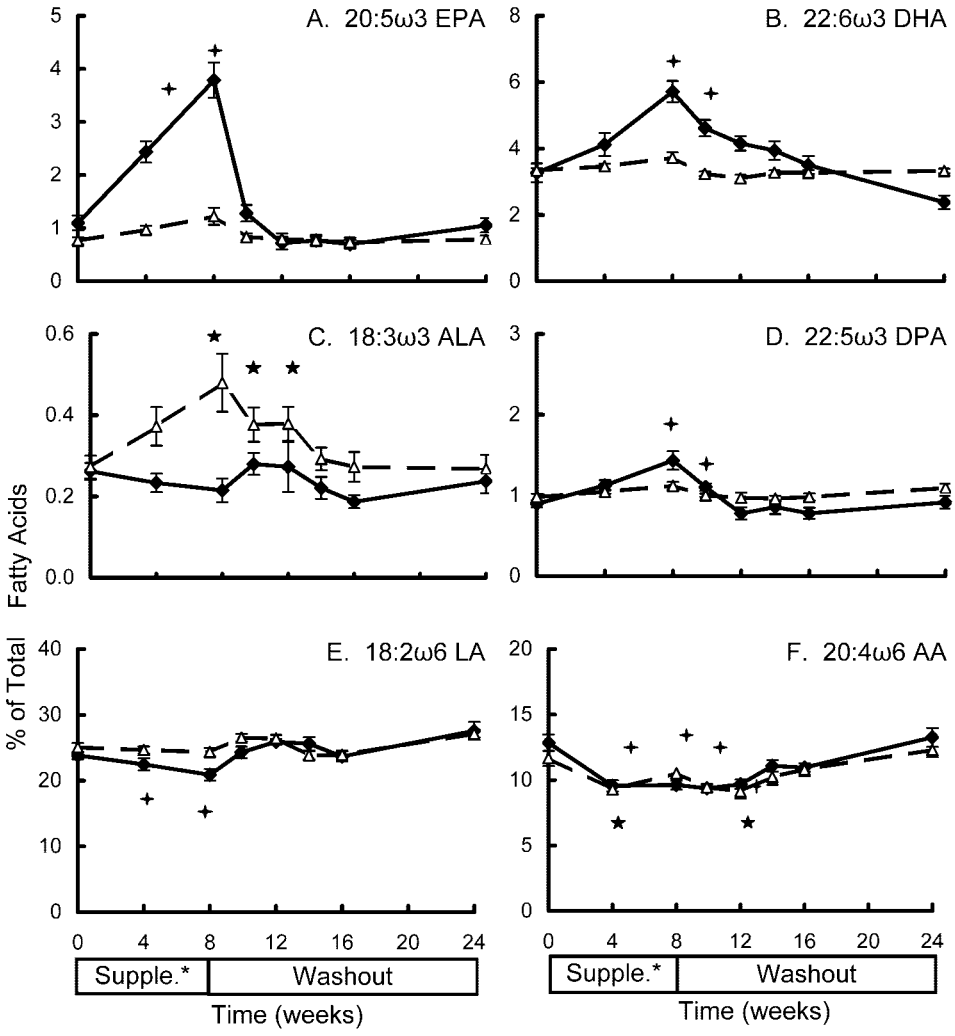

Fig. 3. Changes in the composition of major plasma phospholipid omega-3 and omega-6 fatty acids over time of study.

Values shown are the mean percentage of total fatty acids (SE) in fish oil group (n = 9) (♦) and flaxseed oil group (n = 10) (△). ✚ $P < 0.01$ for significant difference from baseline in fish oil group by paired *t*-test. ⋆ $P < 0.01$ for significant difference from baseline in flaxseed oil group by paired *t*-test. *Supple., supplementation.

DHA). Mean EPA concentration increased 245% ($P < 0.01$, Fig. 3A), and mean DHA concentration increased 73% over baseline ($P < 0.01$, Fig. 3B). Unlike increases observed in erythrocyte membranes, these increases did not persist. Mean EPA concentrations decreased to 34% of the peak concentration at week 10 and to 18% of peak concentration at week 12. Decrease in DHA concentration was less (81% and 74% of peak concentration at weeks 10 and 12, respectively). ALA concentration did not change significantly during the 24-week period (Fig. 3C). Changes in concentration of DPA were less than changes in EPA and DHA concentrations. Mean DPA percentage increased 59% ($P < 0.01$) at week 8 and gradually returned to baseline at the end of the washout period (Fig. 3D). AA concentration showed a 26% decrease ($P < 0.001$) after supplementation and returned to baseline at the end of 24 weeks (Fig. 3E). A 10% decrease in the mean LA concentration was also observed after supplementation (Fig. 3F).

Regarding participants who ingested flaxseed oil, concentrations of EPA, DHA, DPA, and LA showed no statistically significant increase after supplementation (Fig. 3A, 3B, 3D, and 3E, respectively). Only ALA increased 67% ($P < 0.001$) and gradually returned to baseline after supplementation (Fig. 3C). Concentration of AA tended to decrease at week 4, but it fluctuated in the following 12 weeks and returned to baseline at week 24 (Fig. 3F).

OMEGA-3 INDEX

The sums of EPA and DHA and the total omega-3 long chain fatty acids (EPA+DPA+DHA) in erythrocyte membranes and plasma phospholipids from participants at weeks 0, 8, 12, and 24 are compared in Table 1. In erythrocyte membranes, average concentrations of EPA+DHA increased from 4.3% at baseline to 7.8% ($P < 0.001$) after fish oil supplementation. Concentrations then gradually decreased to 5.7% at week 12, and to 3.8% at the end of the washout period. Likewise, the average concentrations of total omega-3 long-chain fatty acids increased significantly from baseline after 8 weeks ($P < 0.001$), and gradually returned to baseline postsupplementation. In plasma phospholipids, the average increase

Table 1. Proportion of key omega-3 fatty acids in erythrocyte membranes from participants supplemented with fish oil (FSO) or flaxseed oil (FXO) over time of study.

		% of Total Fatty Acids[a]			
Fatty acids	Group[b]	Week 0[c]	Week 8[c]	Week 12[c]	Week 24[c]
Erythrocyte membrane EPA+DHA	FSO	4.32 (0.38)	7.75 (0.32)[e]	5.74 (0.35)[f]	3.78 (0.41)
	FXO	4.38 (0.29)	4.70 (0.41)	4.11 (0.43)	3.85 (0.43)
Erythrocyte membrane total omega-3[d]	FSO	6.11 (0.50)	10.24 (0.39)[e]	8.01 (0.42)[f]	5.50 (0.50)
	FXO	6.31 (0.34)	7.10 (0.44)[g]	6.36 (0.46)	5.93 (0.50)
Plasma EPA+DHA	FSO	4.37 (0.38)	9.50 (0.63)[e]	4.87 (0.22)	3.44 (0.21)
	FXO	4.11 (0.21)	4.95 (0.55)[g]	3.90 (0.35)	4.11 (0.25)
Plasma total omega-3[d]	FSO	5.26 (0.38)	10.93 (0.71)[e]	5.65 (0.18)	4.35 (0.21)
	FXO	5.08 (0.22)	6.07 (0.54)[g]	4.87 (0.39)	5.20 (0.26)

[a] Values for fatty acids are expressed as percentage of total fatty acids [mean (SE)].
[b] FSO (n = 9); FXO (n = 10).
[c] Week 0–8, supplementation period; week 8–24, wash out period.
[d] Sum EPA+DPA+DHA.
[e] $P < 0.001$ for significant difference from baseline.
[f] $P < 0.01$ for significant difference from baseline.
[g] $P < 0.05$ for significant difference from baseline.

in concentrations of EPA+DHA was greater than that in the erythrocyte membranes. Concentrations did not persist as they did in erythrocyte membranes, however. The concentration at week 12 of 4.9% did not differ significantly compared with the baseline concentration. Similarly, the average concentrations of total omega-3 long chain fatty acids showed a 106% increase after supplementation, and rapidly returned to near baseline concentrations at week 12.

Supplementation with flaxseed oil failed to produce an effect equivalent to that seen after fish oil supplementation. In both erythrocyte membranes and plasma phospholipids, the increase in mean EPA+DHA and total omega-3 long-chain fatty acid concentrations was less than that in the fish oil group.

Discussion

An overwhelming amount of evidence has demonstrated the beneficial effect of a diet rich in marine fish *(16, 17, 29)*. Because of concerns that fish may be contaminated with environmental toxins *(30, 31)*, supplementation with the active ingredients of fish oil has also been advocated. Because omega-3 fatty acid supplementation has been demonstrated to have cardioprotective effects *(12–15)* and is associated with the reduction of death from CVD *(2–4)*, the American Heart Association has recommended increased intake of oily fish and/or supplementation with EPA and DHA *(16)*. In addition, Harris and von Schacky *(1)* have proposed that the erythrocyte membrane EPA+DHA (omega-3 index) be measured as a new risk factor for death from CVD.

If laboratories are to offer omega-3 indices as a risk factor for CVD, more studies are needed to establish the quantitative and temporal relationship between intake of omega-3 fatty acids, incorporation into erythrocyte membranes, and the rate at which these fatty acids diminish from erythrocyte membranes over time when supplementation is discontinued or interrupted. In the current study, we measured erythrocyte membrane and plasma phospholipid fatty acids in 9 individuals before and after supplementation. Mean baseline erythrocyte long chain omega-3 fatty acid concentrations of 0.6% EPA, 3.8% DHA, and 1.8% DPA are similar to those reported by Harris et al. *(1, 18)*, and Katan et al. *(25)*. The mean omega-3 index in the 9 participants increased from 4.3% to 7.8% in erythrocyte membranes, which approximates the 8% recommended by Harris and von Schacky as cardioprotective *(1)*. In comparison, Katan et al. *(25)* showed that supplementation with 6 g/day of fish oil with ~1.62 g/day of EPA and 0.33 g/day of DHA for a year resulted in a mean omega-3 index value of 9.1, and Harris and von Schacky demonstrated that giving individuals 0.77 g of EPA and 1.26 g of DHA for 20 weeks resulted in an omega-3 index of 11.6 *(1)*. Taken together, these results seem to indicate that a value of ~8% is achievable with ingestion of 2 g of EPA+DHA over a period of 8–20 weeks.

Our study shows that for each gram of EPA ingested, there was a mean increase of 1.4% after 8 weeks (range 1.0%–1.8%). This compares with an increase of 1.7% after 8 weeks and 2.3% after 1 year reported by Katan et al. *(25)*, 1.8% in 6 months by Harris et al. *(18)*, and 1.5% in 6 weeks by Brown et al. *(32)*. With regard to DHA, the mean increase of 1.9% per gram of DHA in 8 weeks in our participants compares with 1.7% observed by Brown et al. *(32)* after 6 weeks. As shown in Fig. 1B, the increase in mean DHA concentration after 4 weeks of supplementation accounted for 82% of the total increase. We observed somewhat larger variations of percentage increases per gram of DHA among our participants, in general agreement with Katan et al. *(25)*, who reported that the incorporation of DHA in erythrocyte membranes was erratic. However, the results from our study indicate that individual variations are to a large extent a factor of the

baseline DHA concentration. Thus individuals with higher DHA concentrations tend to take up additional DHA at a slower rate than those with lower baseline concentrations, suggesting that erythrocyte membrane DHA concentrations are regulated to some degree. In our study, the DHA concentration seems to plateau at ~6%. Although increases >6% DHA in erythrocyte membranes are possible with further augmentation, the rate of increase is more gradual compared to that seen in the initial phase of supplementation.

In the current study, we also monitored the changes in erythrocyte membrane fatty acid compositions at 2-week intervals postsupplementation. Our results seem to indicate a biphasic decrease of EPA and DHA with a steeper slope in the first 2 weeks postsupplementation, followed by a more gradual decline in the next 6 weeks. After 8 weeks of supplementation, the EPA and DHA concentrations were still marginally higher than baseline, but concentrations returned to baseline when measured 16 weeks postsupplementation. These data indicate that for the purposes of designing studies involving the use of omega-3 fatty acid supplementation, a washout period of 8 to 16 weeks may be required.

We also measured composition of fatty acids in the plasma phospholipids fraction. The increases in EPA and DHA mirrored the finding in the erythrocytes, and the sum reached a mean concentration of 9.5% after supplementation. In contrast, EPA fell to presupplementation concentrations 2 weeks postsupplementation. The decline in DHA concentration was more gradual, such that 54% of the increase in mean DHA concentration was retained 2 weeks postsupplementation, and 25% was retained 6 weeks postsupplementation. Thus erythrocyte membrane is a better index for monitoring long-term intake of omega-3 fatty acids, whereas plasma phospholipids are more sensitive to short-term changes in the intake of omega-3 fatty acids and may thus be more useful in monitoring the compliance of individuals in intervention studies with fish oil supplementation.

The current study shows that intake of ALA had little effect on EPA and DHA concentrations in the blood. For each gram of ALA supplement, the mean erythrocyte membrane EPA and DHA concentrations increased 0.1% and <0.1% (0.03%) respectively. Wilkinson et al. *(33)* reported similar findings in a study involving flaxseed oil supplementation for 12 weeks. At a dosage of 25.7 g of ALA per day, the mean increase of EPA was 0.1% per gram of ALA supplemented, whereas no detectable increase was seen for DHA. Our results and those of Wilkinson et al. suggest that conversion of ALA to EPA and DHA in vivo proceeds at a slow rate.

Supplementation with flaxseed oil resulted in a significant increase of DPA concentrations in erythrocyte membranes, comparable to that in the fish oil group. Previous studies have demonstrated the formation of DPA from EPA and DHA after dietary supplementation *(34–36)*. The rapid conversion between EPA and DPA indicates the possibility that DPA can be a potential storage form for EPA. Thus, we speculate that the mechanism by which flaxseed oil exerts its cardioprotective effects may be 2-fold. First, ALA in flaxseed oil may have protective effects on cardiac arrhythmia, inflammation, and thrombosis. In addition, ALA supplementation enriches EPA and DPA composition in erythrocyte membranes. Although EPA concentration is only mildly increased, the increase in DPA in erythrocyte membranes may act to sustain a constant supply of EPA and its beneficial effects. In summary, we demonstrated that supplementation with ~2.1 g of long-chain omega-3 fatty acids for 8 weeks in a Midwestern population with relatively low baseline omega-3 fatty acid concentrations increased the mean omega-3 index concentration close to the desirable concentration of 8%. On the basis of this limited data, we speculate that for most individuals, the desired concentration can be achieved in 8–12 weeks with this dosage of supplementation. The rate of incorporation per gram of supplementation for both EPA and DHA was fairly similar in several studies during a 6–12 week period of time. Thus, with the use of this type of data and an individual's baseline erythrocyte membrane fatty acid profile, it is theoretically possible to determine the appropriate dosage of omega-3 fatty acid supplementation.

References

1. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 2004;39:212–20.
2. Siscovick DS, Raghunathan TE, King I. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA 1995;274:1363–7.
3. Albert CM, Campos H, Stampfer MJ. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med 2002;346:1113–8.
4. Lemaitre RN, King IB, Mozaffarian D, Kuller LH, Tracy RP, Siscovick DS. n-3 Polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the Cardiovascular Health Study. Am J Clin Nutr 2003;77:319–25.
5. McLennan PL, Bridle TM, Abeywardena MY, Charnock JS. Comparative efficacy of n-3 and n-6 polyunsaturated fatty acids in modulating ventricular fibrillation threshold in marmoset monkeys. Am J Clin Nutr 1993;58:666–9.
6. McLennan PL, Abeywardena MY, Charnock JS. Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. Am Heart J 1988;116:709–17.
7. McLennan PL. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. Am J Clin Nutr 1993;57:207–12.
8. Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. Circulation 1999;99:2452–7.
9. Kang JX, Xiao YF, Leaf A. Free, long-chain, polyunsaturated fatty acids reduce membrane electrical excitability in neonatal rat cardiac myocytes. Proc Natl Acad Sci 1995;92:3997–4001.
10. Leaf A, Weber PC. Cardiovascular effects of n-3 fatty acids. N Engl J Med 1988;318:549–57.
11. Leaf A. The electrophysiologic basis for the antiarrhythmic and

anticonvulsant effects of n-3 polyunsaturated fatty acids: heart and brain. Lipids 2001;36:S107–10.
12. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65(Suppl 5):1645S–54S.
13. Appel LJ, Miller ER III, Seidler AJ, Whelton PK. Does supplementation of diet with "fish oil" reduce blood pressure? A meta-analysis of controlled clinical trials. Arch Intern Med 1993;153: 1429–38.
14. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation 1993;88:523–33.
15. Trebble T, Arden NK, Stroud MA, Wootton SA, Burdge GC. Inhibition of tumour necrosis factor-α and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. Br J Nutr 2003;90:405–12.
16. Kris-Etherton PM, Harris WS, Appel LJ; AHA Nutrition Committee. American Heart Association. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. Arterioscler Thromb Vasc Biol 2003;23:151–2.
17. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, et al. European Society of Cardiology Committee for Practice Guidelines. European guidelines on cardiovascular disease prevention in clinical practice: third joint task force of European and other societies on cardiovascular disease prevention in clinical practice. Eur J Cardiovasc Prev Rehabil 2003;10: S1–10.
18. Harris WS, Sands SA, Windsor SL, Ali HA, Stevens TL, Magalski A, et al. Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. Circulation 2004;110:1645–9.
19. Hu FB, Stamfer MJ, Manson JE. Dietary intake of α-linolenic acid and risk of fatal ischemic heart disease among women. Am J Clin Nutr 1999;69:890–7.
20. de Longeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction. final report of the Lyon Heart Study. Circulation 1999;99:779–85.
21. Wilkinson P, Leach C, Ah-Sing EE, Hussain N, Miller GJ, Millward DJ, et al. Influence of α-linolenic acid and fish-oil on markers of cardiovascular risk in subjects with an atherogenic lipoprotein phenotype. Atherosclerosis 2005;181:115–24.
22. Finnegan YE, Minihane AM, Leigh-Firbank EC, Kew S, Meijer GW, Muggli R. Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. Am J Clin Nutr 2003;77:783–95.
23. de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, et al. Mediterranean α-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.
24. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A. Dietary α-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. Atherosclerosis 2003;167:237–42.
25. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. J Lipid Res 1997;38:2012–22.
26. Reed CF, Swisher SN, Marinetti GV, Eden EG. Studies of the lipids of the erythrocyte. I. Quantitative analysis of the lipids of normal human red blood cells. J Lab Clin Med 1960;56:281–9.
27. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 1964;5:600–8.
28. Salem N Jr., Reyzer M, Karanian J. Losses of arachidonic acid in rat liver after alcohol inhalation. Lipids 1996;31:S153–56.
29. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis 2006 Mar 9 [Isqb[Epub ahead of print].
30. Guallar E, Sanz-Gallardo MI, van't Veer P, Bode P, Aro A, Gomez-Aracena J et al.; Heavy Metals and Myocardial Infarction Study Group. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 2002;347:1747–54.
31. Yamaguchi N, Gazzard D, Scholey G, Macdonald DW. Concentrations and hazard assessment of PCBs, organochlorine pesticides and mercury in fish species from the Upper Thames: river pollution and its potential effects on top predators. Chemosphere 2003; 50:265–73.
32. Brown AJ, Pang E, Roberts DC. Persistent changes in the fatty acid composition of erythrocyte membranes after moderate intake of n-3 polyunsaturated fatty acids: study design implications. Am J Clin Nutr 1991;54:668–73.
33. Wijendran V, Hayes KC. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annu Rev Nutr 2004;24:597–615.
34. von Schacky C, Weber PC. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. J Clin Invest 1985;76:2446–50.
35. Croset M, Lagarde M. In vitro incorporation and metabolism of eicosapentaenoic and docosahexaenoic acids in human platelets–effect on aggregation. Thromb Haemost 1986;56:57–62.
36. Fischer S, Vischer A, Preac-Mursic V, Weber PC. Dietary docosahexaenoic acid is retroconverted in man to eicosapentaenoic acid, which can be quickly transformed to prostaglandin I_3. Prostaglandins 1987;34:367–75.