

Distribution, interconversion, and dose response of n-3 fatty acids in humans¹⁻⁴

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ABSTRACT

n-3 Fatty acids have important visual, mental, and cardiovascular health benefits throughout the life cycle. Biodistribution, interconversion, and dose response data are reviewed herein to provide a basis for more rational n-3 dose selections. Docosahexaenoic acid (DHA) is the principal n-3 fatty acid in tissues and is particularly abundant in neural and retinal tissue. Limited storage of the n-3 fatty acids in adipose tissue suggests that a continued dietary supply is needed. A large proportion of dietary α -linolenic acid (ALA) is oxidized, and because of limited interconversion of n-3 fatty acids in humans, ALA supplementation does not result in appreciable accumulation of long-chain n-3 fatty acids in plasma. Eicosapentaenoic acid (EPA) but not DHA concentrations in plasma increase in response to dietary EPA. Dietary DHA results in a dose-dependent, saturable increase in plasma DHA concentrations and modest increases in EPA concentrations. Plasma DHA concentrations equilibrate in approximately 1 mo and then remain at steady state throughout supplementation. DHA doses of ≈ 2 g/d result in a near maximal plasma response. Both dietary DHA and EPA reduce plasma arachidonic acid concentrations. Tissue contents of DHA and EPA also increase in response to supplementation with these fatty acids. Human milk contents of DHA are dependent on diet, and infant DHA concentrations are determined by their dietary intake of this fatty acid. We conclude that the most predictable way to increase a specific long-chain n-3 fatty acid in plasma, tissues, or human milk is to supplement with the fatty acid of interest. *Am J Clin Nutr* 2006;83(suppl):1467S-76S.

KEY WORDS Docosahexaenoic acid, eicosapentaenoic acid, α -linolenic acid, n-3 fatty acids, human dose response

INTRODUCTION

The n-3 fatty acids make up a family of essential fats that humans are unable to synthesize de novo. The parent 18-carbon fatty acid, α -linolenic acid (ALA; 18:3n-3), is present in various vegetable oils, such as flaxseed, linseed, canola, and soy oils. Americans on average consume ≈ 1.3 g ALA/d (1-3). ALA can be metabolically converted to the various long-chain n-3 fatty acids, including eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). However, enzymatic conversion efficiencies vary considerably among species and appear to be relatively inefficient in humans. The principal long-chain n-3 fatty acids in the diet are DHA and EPA. Algae are the primary producers of DHA and EPA in the ecosystem, and several refined algal oils are rich sources of DHA. Fish consume algae and therefore are rich in DHA and EPA. With limited intake

of marine foods in the United States, combined intake of DHA and EPA is estimated at only ≈ 100 mg/d (1, 2).

Fatty acids of the n-3 family, particularly the long-chain n-3 fatty acids, are important nutrients throughout the life cycle. Infants require DHA for visual and cognitive development (4-6), and in children, cardiovascular benefits have been attributed to long-chain n-3 fatty acids (7, 8). DHA and EPA are important for the prevention of cardiovascular disease and resulted in decreased cardiac mortality in a large secondary prevention study (9). Recent epidemiologic and preclinical studies also suggest that DHA may protect against Alzheimer disease and other types of dementia (10-12), and long-chain n-3 fatty acids may protect against advanced age-related macular degeneration (13), which suggests a continued role of these fats in brain and eye health in adults and the elderly.

The potential health benefits of n-3 fatty acids have been examined in many clinical trials. These studies, however, generally used combinations of n-3 fatty acids, especially DHA plus EPA, which makes it difficult to discern specific roles and health benefits of the individual n-3 fatty acids. A main focus of this symposium is to provide more clarity on the functional role of the individual n-3 fatty acids. The recent availability of pure sources of DHA and EPA has helped to accelerate this research. In addition, many clinical studies used high pharmacologic doses of n-3 fatty acids to ensure maximal therapeutic effects, but failed to provide dose ranging information, particularly with respect to lower intakes compatible with nutrients in foods. The purpose of the present review is to explore the tissue distribution, interconversion, and dose effects of the specific n-3 fatty acids on fatty acid levels in human blood and tissues and thereby provide guidance for developing n-3 dose selection and recommendations.

TISSUE DISTRIBUTION OF n-3 FATTY ACIDS

n-3 Fatty acids are present in cell membranes and are incorporated primarily into phospholipids, as well as sphingolipids and plasmalogens. These fatty acids, particularly the more highly

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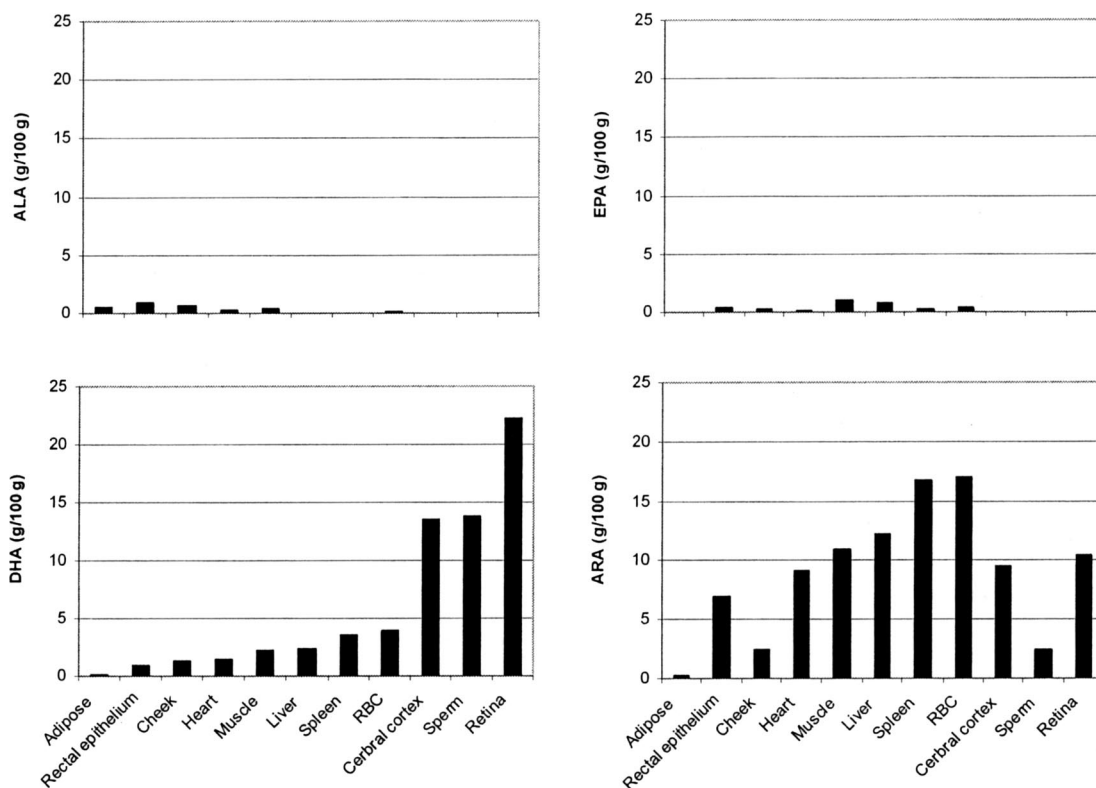


FIGURE 1. Cross-study analysis of fatty acid concentrations (g/100 g of total fatty acids) in tissues from adults from the United States, Canada, Australia, or Europe. References: adipose tissue (21), rectal epithelium (22), muscle (23), liver and spleen (24), heart and cheek (25), red blood cell (26), cerebral cortex (27), sperm (28), and peripheral retina (29). ALA, α -linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RBC, red blood cell.

unsaturated long-chain $n-3$ fatty acids, can influence the biophysical properties of membranes (eg, fluidity, thickness, and deformability) and therefore affect activity of transmembrane proteins (14). DHA, as the most unsaturated fatty acid in membranes, is highly flexible within the membrane and is particularly effective at accommodating transitional changes associated with transmembrane protein activation (15, 16). DHA, EPA, and ALA, as well as the $n-6$ fatty acid arachidonic acid (ARA), compete for the *sn-2* position on membrane phospholipids. The relative proportion of these fatty acids also determines their availability after phospholipase cleavage as substrates for cyclooxygenases and lipoxygenases, and hence the balance of eicosanoids and other antiinflammatory autoids, such as resolvins (17). These fatty acids are also ligands for nuclear receptors such as peroxisome proliferator-activated receptors and retinoid X receptor, and can therefore influence gene regulation (18–20). Thus, the overall membrane fatty acid composition can have a large impact on cell and organ function as well as a wide variety of biological processes.

The proportions of the $n-3$ fatty acids ALA, EPA and DHA, as well as of the $n-6$ fatty acid ARA for comparison, found in various organs of adults from the United States, Canada, Europe, or Australia are depicted in **Figure 1**. DHA is the most abundant $n-3$ fatty acid in membranes and is present in all organs. It is also the most variable among organs and is particularly abundant in neural tissue, such as brain and retina. Only minute quantities of ALA and EPA are generally present in tissues, and DHA generally exceeds EPA 5- to 30-fold in most organs. However, DHA

is several hundred-fold more abundant than EPA in brain and retina. For comparison, ARA is also relatively abundant in most tissues but has a distribution distinct from that of DHA.

In adipose tissue, where fatty acids are stored as triacylglycerol, linoleic acid (LA; $18:2n-6$) is the most abundant polyunsaturated fatty acid at $\approx 12-16\%$ of fatty acids, and ALA is the predominant $n-3$ fatty acid at $\approx 1\%$ (21, 30, 31). Only very small amounts of DHA or EPA are present in adipose tissue (21, 30, 31), which suggests a limited storage capacity of these long-chain $n-3$ fatty acids and implies the need for a continuous supply through the diet.

In healthy North Americans, DHA generally constitutes $\approx 4\%$ of total lipid in red blood cells (26, 32, 33). The mean red blood cell DHA content in healthy persons who do not regularly take DHA supplements as measured in our laboratory ($n = 284$) is 4.0% of total fatty acids, with a normal range of 1.9% to 7.9%. Mean plasma or serum phospholipid DHA contents have been reported in the range of 2.5% to 3.4% of fatty acids in healthy adults in North America (28, 32, 34). The mean plasma phospholipid DHA content in healthy individuals measured in our laboratory is 3.5% of total fatty acids ($n = 294$), with a normal range of 1.5% to 7.5%.

METABOLIC INTERCONVERSION OF $n-3$ FATTY ACIDS

The conversion of ALA to EPA and DHA occurs primarily in the liver in the endoplasmic reticulum and involves a series of elongation enzymes that sequentially add 2-carbon units to the



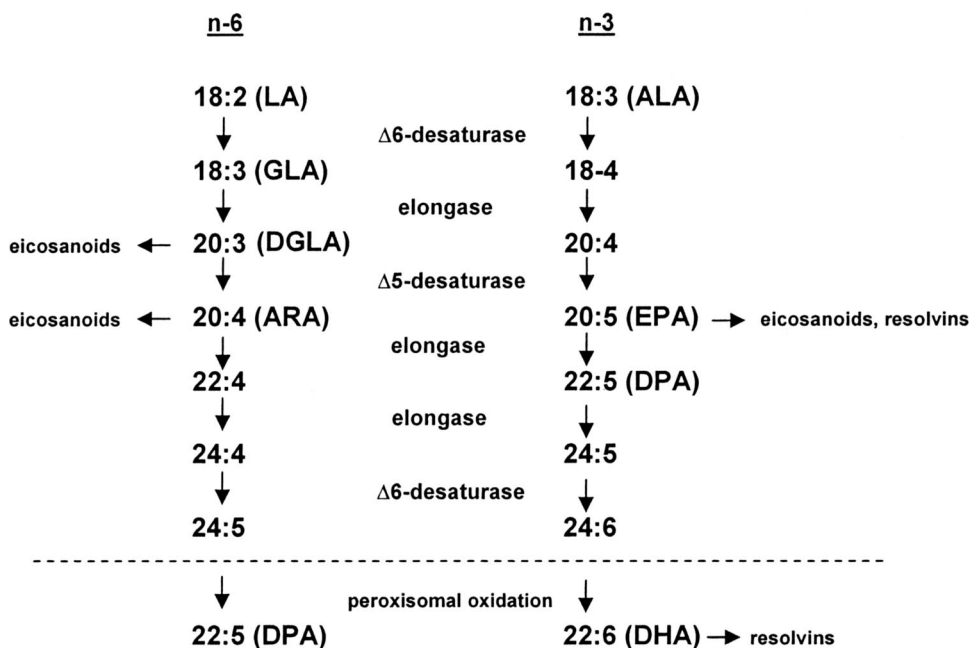


FIGURE 2. Biochemical pathway for the interconversion of n-6 and n-3 fatty acids. ALA, α -linolenic acid; ARA, arachidonic acid; DGLA, dihomo- γ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ -linolenic acid; LA, linoleic acid.

fatty acid backbone and desaturation enzymes that insert double bonds into the molecules (**Figure 2**). The final conversion of ALA to DHA requires a translocation to the peroxisome for a β -oxidation reaction. Because the same enzymes involved in n-3 synthesis are responsible for conversion of the n-6 fatty acid LA to ARA, background diet can influence the conversion of these fatty acids. The intake of LA in the American diet, which is ≈ 15 g/d, is approximately an order of magnitude greater than the intake of ALA (1-3).

Several investigators have performed detailed studies of the *in vivo* conversion of ALA to its long-chain n-3 derivatives EPA, docosapentaenoic acid (DPA), and DHA in humans by using uniformly labeled [^{13}C]- or [^3H]ALA as a tracer (reviewed in references 35 and 36). These studies have consistently shown that ≥ 15 -35% of dietary ALA is rapidly catabolized to carbon dioxide for energy (37-40), and that only a small proportion, estimated by using compartment models to be $< 1\%$, is converted to DHA (41, 42). In fact, ALA has the highest rate of oxidation among all unsaturated fatty acids (43). The fractional conversion of ALA to EPA, estimated by measuring peak or area under the curve plasma contents of the labeled fatty acids, varies between 0.3% and 8% in men, and the conversion of ALA to DHA is $< 4\%$ and often undetectable in males (39, 40, 44, 45). Conversion of ALA to long-chain n-3 fatty acids appears to be more efficient in women: up to 21% is converted to EPA and up to 9% is converted to DHA (38), with a concomitant reduction in the rate of ALA oxidation ($\approx 22\%$ compared with $\approx 33\%$ in men) (38-40). The conversion of DPA to DHA is the rate-limiting step in the conversion of ALA to DHA, and dietary DHA and EPA down-regulate this step by 70% (41). Others have also shown that dietary EPA and DHA reduce the conversion of ALA to long-chain n-3 fatty acids (37, 39). Diets high in LA may influence, through substrate competition and inhibition of Δ^6 desaturase enzyme, the metabolism of the n-3 fatty acids. Emken et al (44) showed that a diet high in LA reduces the conversion of ALA to

its long-chain derivatives by 40%, with a net reduction in long-chain n-3 fatty acid accumulation of 70%. Diets high in ALA appear to increase the rate of ALA oxidation, limiting its accumulation in plasma and reducing its conversion rate to EPA and DHA (37). Considerable variability in the conversion rates among individuals has been reported, even when the subjects have similar background diets (44). This interindividual variability, along with modest ALA intakes and high amounts of LA in the American diet, suggests that ALA cannot reliably replace EPA and particularly DHA in the diet.

DHA itself also serves as a substrate for metabolic retroconversion to EPA and DPA through a β -oxidation reaction. In studies in which ^{13}C -labeled DHA was fed, Brossard et al (46) calculated the retroconversion rate of DHA to EPA in humans receiving normal dietary amounts of DHA to be $\approx 1.4\%$. Human clinical data have suggested rates as high as 12% with high chronic DHA consumption (47, 48). Retroconversion of DHA to EPA is hormonally regulated and decreases in women receiving hormone replacement therapy (49).

EFFECTS OF ALA SUPPLEMENTATION ON PLASMA FATTY ACIDS

Several case studies involving n-3-deficient patients reported that intervention with ALA results in marked increases in plasma concentrations of both EPA and DHA (50, 51). In addition, vegans who consume ALA but not EPA and DHA in their diets have low but stable concentrations of DHA in plasma (52, 53). Together, these findings suggest that humans can convert meaningful quantities of ALA to EPA and DHA, particularly in the presence of a deficiency or a background of low n-6 fatty acids. However, consistent with the isotope-tracer studies, the vast majority of the ALA supplementation studies show a limited conversion of ALA to its long-chain n-3 derivatives. These studies have been performed in healthy individuals consuming typical

Western diets generally high in n-6 fatty acids. Chan et al (54), for example, studied the dose effect of ALA supplementation on plasma phospholipid n-3 fatty acid concentrations and found a small but significant dose-dependent increase in ALA and EPA concentrations in phospholipids, but no increase in DHA. Notably, a high ratio of LA to ALA in the diet reduced the conversion of ALA to EPA. Others have found increased amounts of ALA and EPA but not DHA after supplementation with 2-3 g ALA/d (55, 56).

To explore the ALA dose-response relation across a wider range of doses in a systematic fashion, we conducted a cross-study meta-regression analysis of plasma phospholipid n-3 fatty acid concentrations after ALA supplementation. Changes in plasma phospholipid fatty acid concentrations from various studies involving supplementation of healthy adults with ALA-rich oils are plotted in **Figure 3**. ALA supplementation with up to 14 g/d resulted in dose-dependent but modest increases in plasma ALA concentrations. Some of the observed variability, especially at low ALA doses, is attributed to differences in the amount of LA concurrently administered in the diet, because LA reduces ALA accumulation (44). Nonetheless, the dose response appears linear ($r^2 = 0.79$, $P = 0.008$). There are small increases in EPA after ALA supplementation ($r^2 = 0.49$, $P = 0.052$); however, plasma phospholipid DHA concentrations do not detectably increase after ALA supplementation with doses up to 14 g/d. This analysis suggests that, given the typical Western diet, even large doses of ALA result in only marginal changes in ALA concentrations in plasma, small increases in plasma EPA, and no effect on DHA, which is consistent with a high oxidation rate and the low fractional conversion rates discussed above. These conversion rates may be altered in the presence of a diet containing reduced amounts of n-6 fatty acids.

EFFECT OF DHA SUPPLEMENTATION ON PLASMA FATTY ACIDS

Many studies have shown that supplementation with DHA triacylglycerol or DHA ethyl esters results in increased plasma DHA concentrations (47, 49, 63-70). We have further analyzed the dose-response effect of supplemental DHA on plasma phospholipid fatty acid concentrations by using a cross-study meta-regression analysis. Plasma phospholipid fatty acid concentrations from 12 different studies (16 different supplementation groups) with doses ranging from 0.2 to 6 g DHA/d for 1 to 6 mo are shown in **Figure 4**. This analysis shows that plasma phospholipid DHA concentrations increase in a dose-dependent, saturable manner in response to dietary DHA, which was suggested previously by Vidgren et al (71). Plasma phospholipid DHA concentrations are highly sensitive to dietary intake of this fatty acid at doses up to ≈ 2 g/d. At doses above this amount, plasma DHA concentrations approach saturation and increase only incrementally. DHA supplementation also results in an apparent linear increase in EPA concentrations, presumably through retroconversion, with EPA concentrations increasing by ≈ 0.4 g/100 g fatty acid for each 1 g of DHA intake. There is also a concurrent dose-dependent, saturable reduction in plasma phospholipid ARA concentrations, although the ARA response is more variable among studies.

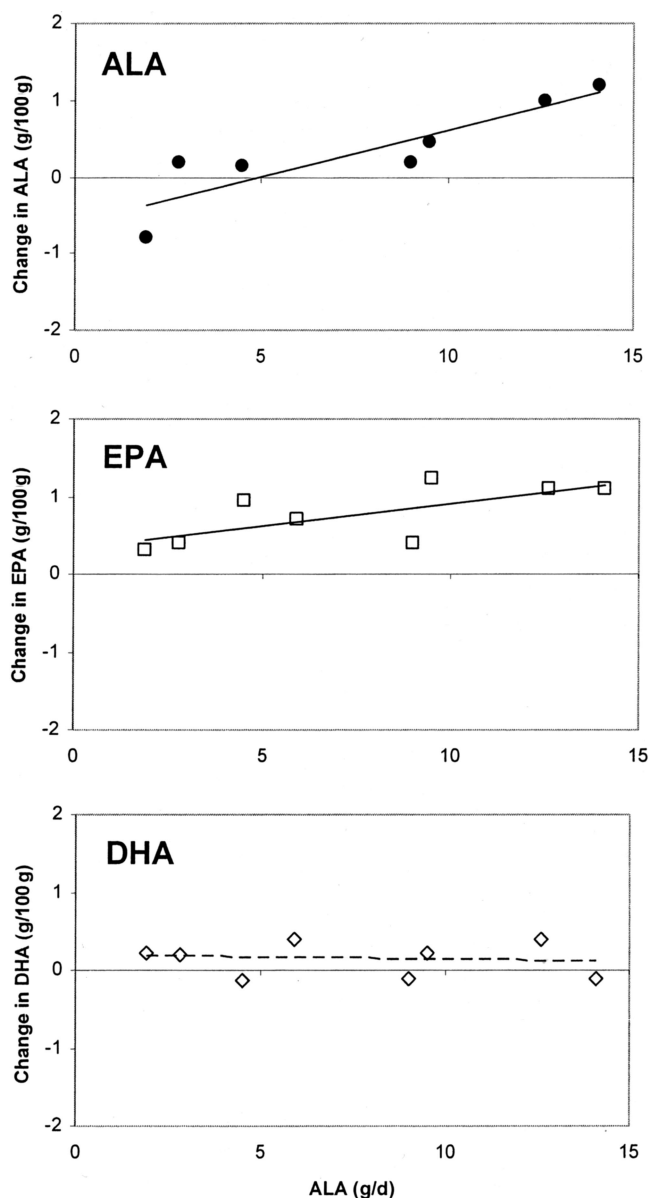


FIGURE 3. Cross-study meta-regression analysis of the effect of α -linolenic acid (ALA) supplementation of adults on plasma phospholipid ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) concentrations. Clinical studies involving supplementation with ALA-rich oils, which were identified through PUBMED (National Library of Medicine, Bethesda, MD), that reported both ALA intakes and plasma phospholipid fatty acid concentrations in g/100 g of fatty acids in supplementation groups of ≥ 10 healthy individuals for ≥ 1 mo were included in the analysis (57-62). Lines represent the least-squares regression analysis of best linear fit (MINITAB Statistical Software, version 13.32; MiniTab Inc, State College, PA). Regression lines for change (Δ) in plasma phospholipid fatty acid concentrations in g/100 g of fatty acids with ALA doses in g/d are as follows: Δ ALA = $-0.60 + 0.12$ [ALA dose], $r^2 = 0.79$, $P = 0.008$; Δ EPA = $0.34 + 0.06$ [ALA dose], $r^2 = 0.49$, $P = 0.052$; Δ DHA: no effect of ALA dose on DHA.

EFFECTS OF EPA SUPPLEMENTATION ON PLASMA FATTY ACIDS

Supplementation of adults with ≈ 4 g/d of pure EPA ethyl ester results in significant increases in EPA concentrations in whole plasma and plasma or serum phospholipids, but no increase in

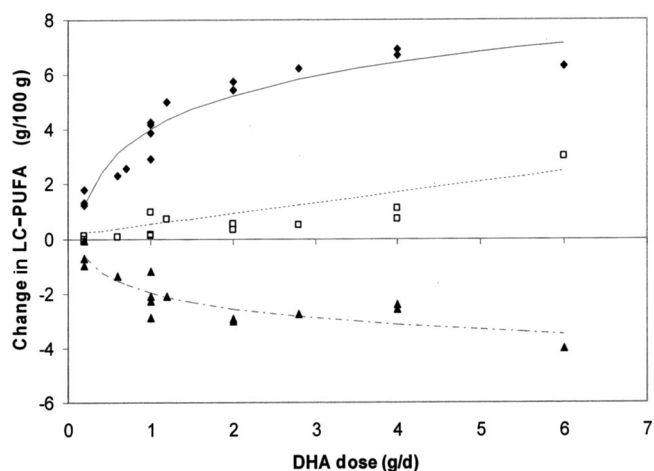


FIGURE 4. Cross-study meta-regression dose-response analyses of the effect of docosahexaenoic acid (DHA) supplementation on plasma phospholipid concentrations of DHA (◆), eicosapentaenoic acid (EPA; □), and arachidonic acid (ARA; ▲). Studies that met the following criteria were included in the analyses: 1) adults ($n \geq 6$ in supplement group) were supplemented daily for 1–6 mo with a DHA source containing little or no EPA [ie, DHA-to-EPA ratio of at least 20:1; included algal DHA triacylglycerol sources (DHASCO or DHASCO-S oils; Martek Biosciences Corporation, Columbia, MD) or pure DHA ethyl esters] and 2) plasma phospholipid fatty acids were reported in g/100 g of fatty acids. Studies involving individuals with metabolic disorders that affect fatty acid metabolism (eg, retinitis pigmentosa or cystic fibrosis) were excluded from the analysis. One study with children was included because the fatty acid results were similar to the results in adults. Published studies identified in PUBMED (National Library of Medicine, Bethesda, MD; 49, 65–70) as well as unpublished data (data on file, Martek Biosciences Corporation) were included in the analysis. DHA supplementation doses ranged between 0.2 and 6 g DHA/d. Changes from baseline fatty acid concentrations for each supplementation group were recorded, and linear (EPA) or logarithmic (DHA and ARA) curve fits were generated. LC-PUFA, long-chain polyunsaturated fatty acids. The equations for the DHA, EPA, and ARA curves, respectively, were as follows: $y = 1.742\ln(x) + 4.023$, $r^2 = 0.91$; $y = 0.150 + 0.389x$, $r^2 = 0.73$; $y = -0.834\ln(x) - 1.977$, $r^2 = 0.77$. Data points were not weighted by sample size of the supplementation group.

DHA concentrations, which is consistent with the poor enzymatic conversion of EPA to DHA (64, 68, 72, 73). A meta-regression dose-response analysis of pure EPA supplementation could not be performed because a sufficient number of studies across a range of EPA doses were not available.

EFFECTS OF SUPPLEMENTATION WITH DHA AND EPA ON PLASMA FATTY ACIDS

Studies with fish oils containing both DHA and EPA have consistently shown increases in both DHA and EPA in plasma (25, 63, 74–77). Blonk et al (74) performed a dose-response analysis of supplementation with marine lipids containing a 2:3 ratio of DHA and EPA at doses up to 6 g total long-chain n-3 fatty acids per day. The results of this study, depicted graphically in **Figure 5**, suggest a near linear increase in plasma EPA concentrations and an apparent saturable increase in DHA concentrations after supplementation with the combination of fatty acids. The apparent DHA saturation dose was 1.2 g/d, which is considerably lower than when pure DHA is provided and which suggests a possible displacement with EPA in plasma phospholipids. ARA concentrations, as expected, also decreased dose-dependently in response to the combined DHA and EPA supplementation.

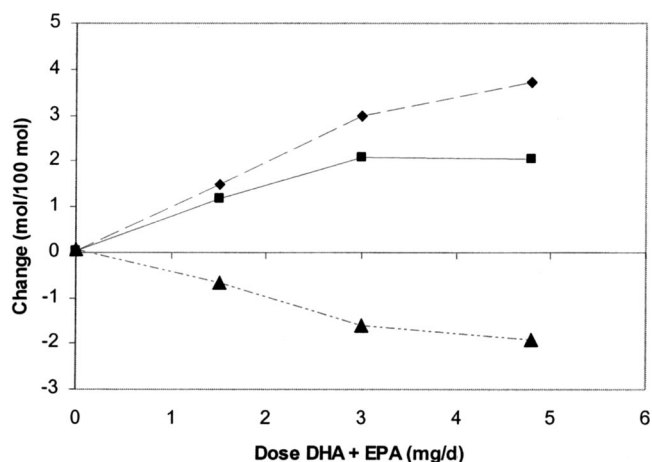


FIGURE 5. Dose-response analysis of the effect of human supplementation with docosahexaenoic acid (DHA) plus eicosapentaenoic acid (EPA) for 12 wk on EPA (◆), DHA (■), and arachidonic acid (▲). Adapted from reference 74 with permission from the American Society for Clinical Nutrition.

COMPARISON OF EFFECTS OF SUPPLEMENTATION WITH ALA, EPA, AND DHA ON PLASMA FATTY ACIDS

The effects of supplementation with similar doses of ALA or pure EPA or DHA ethyl esters on plasma phospholipid fatty acid concentrations are compared across studies in **Figure 6**. As shown in the figure, both DHA and EPA increase their respective fatty acid in plasma to a similar degree, but ALA is a relatively inefficient source for raising any n-3 fatty acids in plasma phospholipids. EPA and DHA result in similar reductions in plasma phospholipid ARA concentrations. This comparison suggests that the most efficient way to increase plasma concentrations of a particular n-3 fatty acid of interest is to supplement with that specific fatty acid.

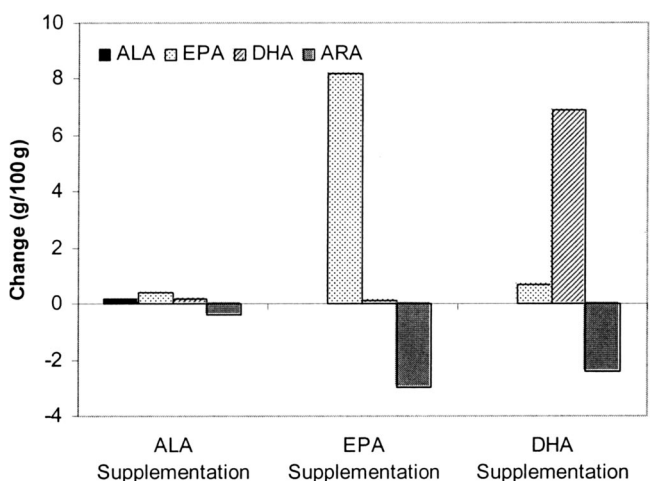


FIGURE 6. Cross-study effects of 3.7 g α -linolenic acid (ALA)/d from canola oil, 4 g pure eicosapentaenoic acid (EPA) ethyl ester, or 4 g pure docosahexaenoic acid (DHA) ethyl ester on the change in plasma phospholipid fatty acid concentrations. ARA, arachidonic acid. Adapted from references 58 and 68 with permission from the American Society for Clinical Nutrition.

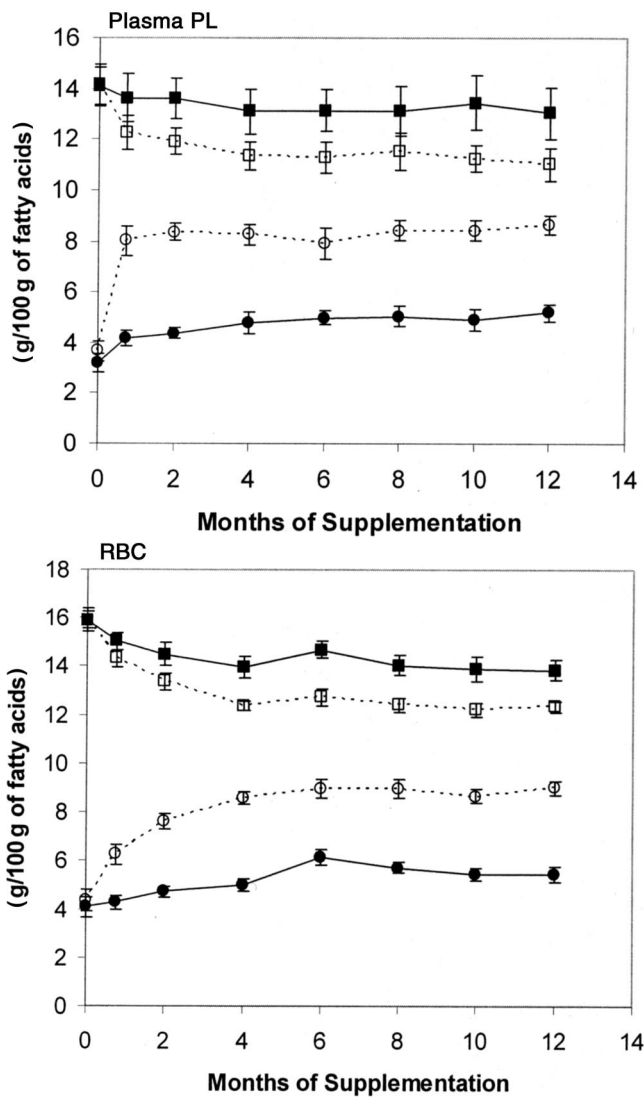


FIGURE 7. Kinetics of docosahexaenoic acid (DHA) supplementation on mean (\pm SEM) plasma phospholipid (PL) and red blood cell (RBC) DHA (○ and ●) and arachidonic acid (ARA; □ and ■) concentrations (data on file, Martek Biosciences Corporation, Columbia, MD). Twenty hyperlipidemic adults receiving statin therapy were randomly assigned to receive either 200 mg (solid lines and symbols) or 1000 mg (dashed lines and open symbols) DHA from DHASCO oil daily for 12 mo ($n = 10$ per group). Fasting blood samples were collected at baseline, 3 wk, and 2, 4, 6, 8, 10, and 12 mo and were separated into plasma and RBCs and stored under nitrogen gas at -80°C until analyzed. Plasma lipids were extracted, and phospholipids were isolated by thin-layer chromatography as described (66). RBC total lipids were extracted by the method of Bligh and Dyer (78). Fatty acids from plasma phospholipids and RBC lipids were saponified and methylated with boron trifluoride, and fatty acid methyl esters were identified by gas-liquid chromatography and flame ionization detection as described (66). The protocol was approved by The New England Institutional Review Board (Wellesley, MA).

KINETICS OF LONG-CHAIN $n-3$ SUPPLEMENTATION IN PLASMA AND RED BLOOD CELLS

We have studied the kinetics of DHA supplementation in plasma and red blood cells. As shown in **Figure 7**, plasma phospholipid concentrations of DHA increased rapidly in a dose-dependent manner after daily supplementation, and, in agreement with the findings of others (79, 80), reached equilibrium

within 1 mo of the start of a new high-dose supplementation regimen. The kinetics of the response was slightly slower with low-dose supplementation. Once new equilibrium concentrations were attained, steady state concentrations were maintained throughout the supplementation period. A similar phenomenon was also reported by Wheaton et al (81) in whole plasma, who showed elevated DHA throughout a 4-y supplementation period. ARA concentrations decreased in a more gradual dose-dependent manner, but eventually resulted in new equilibrium concentrations in plasma phospholipids (**Figure 7**). Red blood cell kinetics followed a similar pattern; however, it took 4–6 mo after the start of DHA supplementation to reach new steady state concentrations, which is consistent with the slower turnover of these cells. Red blood cell DHA concentrations were maintained thereafter throughout the supplementation period. Other studies with combinations of long-chain $n-3$ supplementation have shown that EPA accumulates more rapidly in plasma and red blood cells than does DHA (75, 79).

The kinetics of DHA and EPA washout after $n-3$ supplementation have been studied by other investigators, who have consistently shown that DHA is more slowly cleared from plasma than is EPA. Marangoni et al (75) reported that whole plasma DHA concentrations decreased slowly once long-chain $n-3$ supplementation had stopped and even after 24 wk were not entirely back to baseline levels. EPA concentrations, on the other hand, decreased rapidly after supplementation was stopped and approached baseline within 4 wk. Others have shown a more rapid washout of EPA and slower reductions in DHA in plasma phospholipids after the completion of fish oil supplementation, and both DHA and EPA decrease more rapidly in plasma cholesterol esters than in phospholipids (76, 82). Katan et al (77) similarly found faster washout of EPA than of DHA from red blood cells.

Differences in the accumulation and retention of DHA and EPA may be related to the lipid moieties in which these fatty acids are stored. DHA is carried predominantly in phospholipids, a more stable lipid fraction in plasma, with lesser portions in triacylglycerol and sterol esters, whereas EPA is more equally distributed between neutral lipids (sterol esters and triacylglycerol) and phospholipids (71, 76, 79). Only small amounts of each of these fatty acids are present in their nonesterified free fatty acid form (80). The differential distribution of DHA and EPA may be linked to differences in the kinetics of washout as well as their saturation dynamics in plasma and availability to tissues.

EFFECTS OF DHA AND EPA SUPPLEMENTATION ON TISSUES

DHA supplementation leads to a dose-dependent increase in plasma phospholipid and red blood cell DHA contents (*see* **Figures 4 and 7**). A strong correlation exists between plasma phospholipid and red blood cell DHA contents ($r^2 = 0.72$, $P < 0.001$), and likewise between EPA contents in these 2 compartments in blood ($r^2 = 0.60$, $P < 0.001$) (**Figure 8**). Others have reported a strong correlation between the combined concentrations of DHA and EPA in red blood cells and plasma (25). Others have also reported a small but significant correlation of cerebral cortex and red blood cell DHA contents in adults, and cheek cell DHA and EPA are correlated with plasma and red blood cell contents of these fatty acids (25, 27). Because plasma and red blood cell contents of DHA and EPA are responsive to dietary intake of



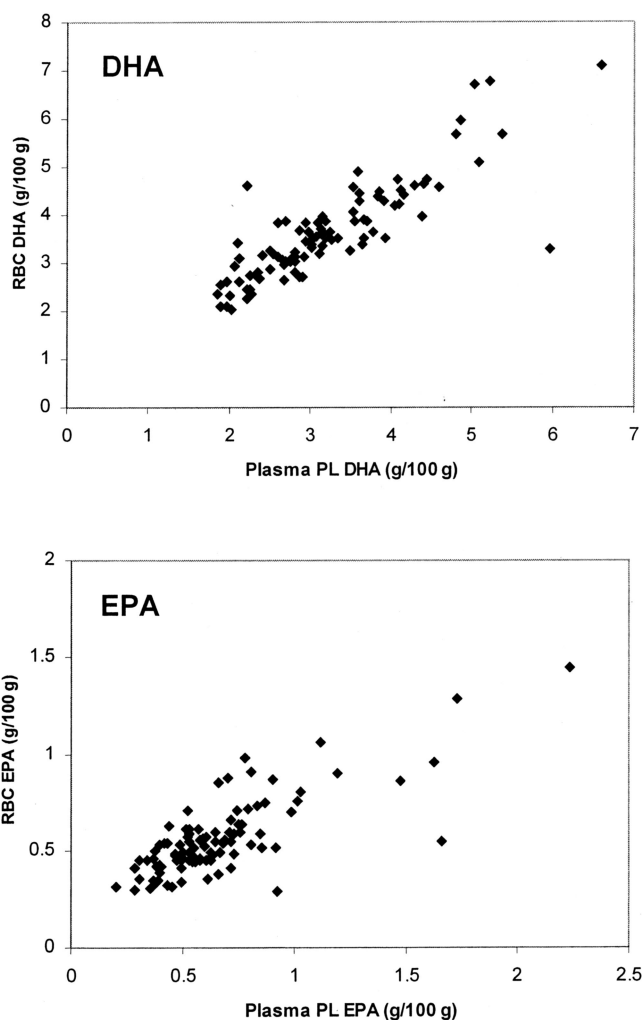


FIGURE 8. Correlation between plasma phospholipid (PL) and red blood cell (RBC) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) concentrations in 96 healthy individuals (data on file, Martek Biosciences Corporation, Columbia, MD). Plasma and RBCs were processed and fatty acids analyzed as described in the legend to Figure 7. The study protocol was approved by The New England Institutional Review Board (Wellesley, MA). Correlation coefficients are as follows: DHA, $r^2 = 0.72$, $P < 0.001$; EPA, $r^2 = 0.60$, $P < 0.001$.

these fatty acids, these correlations suggest plasticity in the fatty acid contents of these organs and that plasma phospholipids or red blood cell DHA contents are generally reasonable markers for tissue DHA concentrations.

Limited data are available on the direct effect of long-chain n-3 supplementation on tissue n-3 contents in humans because of the limited accessibility of human tissues for biopsy. Nonetheless, increases in heart myocardium and skeletal muscle contents of both DHA and EPA in humans have been shown after supplementation with fish oil (23, 25). Others have found increases in adipose tissue and rectal epithelium contents of DHA after 3–6 mo of treatment with DHA (22; J Breslow, personal communication, 2005). Animal studies have also augmented our knowledge about how dietary supplementation with long-chain n-3 fatty acids can affect tissue contents of these fatty acids. Brain, heart, and liver contents of DHA and EPA increased, whereas ARA contents decreased, in a dose-dependent manner in mature rats after 3 mo of supplementation with DHA. Skeletal

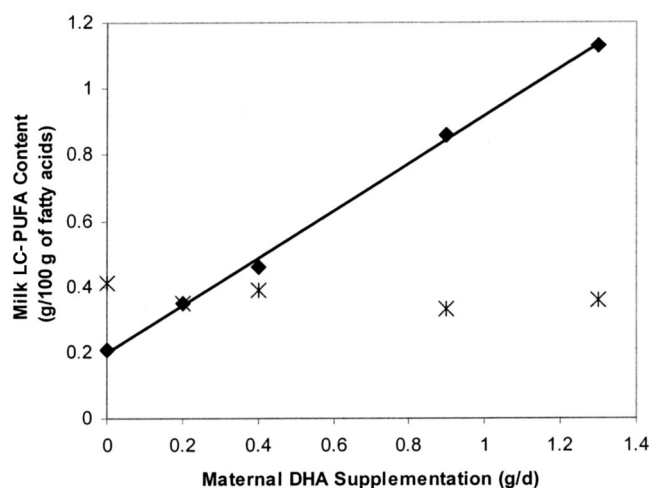


FIGURE 9. Human milk dose response to maternal docosahexaenoic acid (DHA) supplementation. LC-PUFA, long-chain polyunsaturated fatty acids. DHA, \blacklozenge ; ARA, $*$. DHA regression line: $r^2 = 0.998$, $P < 0.001$. Adapted from reference 93 with permission from Macmillian Publishers Ltd.

muscle, heart, liver, red blood cell, and bone marrow contents of DHA and EPA increased, with concomitant reductions in ARA, in weanling rats after DHA supplementation for 2 mo (83, 84). Others have shown repletion of brain and retinal DHA after oral supplementation of n-3-deficient animals (85). Together, these studies indicate that in addition to plasma concentrations, tissue concentrations of DHA and EPA can be elevated through dietary supplementation.

MATERNAL SUPPLEMENTATION

DHA availability to the growing fetus and infant is important because this is the period of most rapid brain growth and development. DHA is transferred from mother to the fetus through the placenta and then to the infant postpartum through breast milk. Relative maternal DHA levels decline during pregnancy (86, 87), but maternal plasma DHA concentrations are responsive to DHA supplementation and increase in a dose-dependent manner (88). Infant n-3 concentrations at birth are correlated with maternal n-3 status (89). Infant red blood cell and plasma DHA concentrations after birth are determined largely by diet. DHA contents rapidly decrease by $\approx 50\%$ in plasma phospholipids and red blood cells within 4 mo after birth without an exogenous source of DHA, but are maintained by human milk or DHA-fortified formula feeding (90–92). Human milk DHA content is exquisitely sensitive to maternal diet and increase in a linear, dose-dependent manner (Figure 9) (93). Recent reports of breast milk contents of EPA and DHA in a variety of countries are depicted in Figure 10. DHA contents vary greatly across cultures, primarily reflecting marine food intakes, whereas EPA contents are lower and tend to be less variable. Breast milk DHA contents in American women are among the lowest in the world, generally reflecting the low intake of marine foods in this country.

Infant plasma and red blood cell DHA concentrations are determined by availability of this nutrient from formula or human milk and increase with the dose of DHA in human milk or formula at levels up to $\approx 0.7\%$ of milk formula fatty acids, after

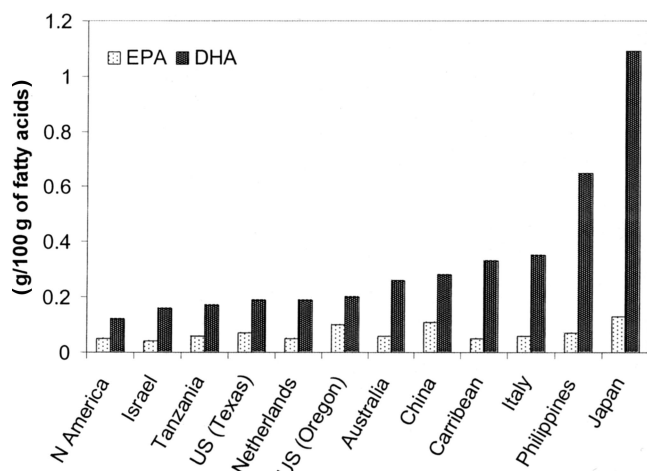


FIGURE 10. Recently published reports of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) contents in human milk in various countries (94–102).

which infant red blood cell DHA concentrations reach near saturation concentrations (103, 104). About 80% of all infant formula sold in the United States now contains DHA, and because infants require a balanced ratio of n–6 to n–3 fatty acid, these formulas also include ARA. Current US infant formulas contain between 0.1% and 0.35% of fat as DHA. Recent reports suggest that both dose and duration of DHA exposure determine the extent to which infants benefit from the dietary DHA (5, 105).

SUMMARY

Human plasma and tissues are responsive to dietary intake of the long-chain n–3 fatty acids, and levels increase in plasma and tissues in a dose-dependent manner. The most effective way to increase a particular n–3 fatty acid is to provide that specific dietary fatty acid, because interconversion of the n–3 fatty acids is limited in humans. ALA accumulates only to a minor extent, most likely as the result of increased oxidation at higher doses, and modestly raises EPA but not DHA. Plasma phospholipid EPA concentrations increase in a linear manner in response to dietary EPA, whereas dietary DHA causes a dose-dependent, saturable increase in plasma phospholipid DHA concentrations with doses up to ≈ 2 g/d. Both DHA and EPA similarly reduce ARA concentrations in plasma. Tissue contents of long-chain n–3 fatty acids increase in response to dietary DHA or EPA. Human milk content of DHA depends on maternal intake of this nutrient, and infant plasma DHA concentrations are responsive to the DHA amounts in their milk or formula feedings. The dose-response information provided herein should be useful in predicting efficient doses of n–3 fatty acids for supplementation studies and for developing recommendations for intakes of specific n–3 fatty acids.

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