

Update on alpha-linolenic acid

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Consumption of omega 3 fatty acids is known to have health benefits. For many years, the importance of the only member of the omega 3 family considered to be essential, alpha-linolenic acid (ALA), has been overlooked. Current research indicates that ALA, along with its longer chain metabolites, may play an important role in many physiological functions. Potential benefits of ALA include cardioprotective effects, modulation of the inflammatory response, and a positive impact on both central nervous system function and behavior. Recommended levels for ALA intake have been set, yet the possible advantages of its consumption are just being revealed.

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INTRODUCTION

It is now recognized that in humans α -linolenic acid (ALA 18:3 ω) an omega-3 (n-3) fatty acid, is an essential fatty acid (EFA) that cannot be synthesized by the body and therefore must be supplied by dietary sources. However, this recognition has been long in gestation. In 1976, Cuthbertson¹ reviewed the requirements for infant formula and claimed that alpha-linolenic acid was not essential and only linoleic acid was required to replace breast milk. His claim was challenged by Crawford et al.² who argued that the evidence available at that time not only favored the essentiality of alpha-linolenic acid but also the independent need for arachidonic acid and docosahexaenoic acid for infants. The position of the essentiality of ALA was confirmed by the 1978 World Health Organization/Food and Agriculture Organization Expert Consultation on the Role of Dietary Fats and Oils in Human Nutrition.³ For many years there was little interest in ALA and issues were raised concerning the danger of consuming highly unsaturated fatty acids that were susceptible to peroxidation. Holman⁴ argued that omega-3 fatty acids, although susceptible to peroxidation, were in practice, themselves protective. His

conclusions have recently been confirmed by the discovery of neuroprotectins, powerful antioxidants derived from docosahexaenoic acid.⁵

ALA is abundant in certain plant foods including walnuts, rapeseed (canola), several legumes, flaxseed, and green leafy vegetables.⁶ ALA is the precursor of three important longer-chain n-3 fatty acids, eicosapentaenoic acid (EPA 20:5 ω 3), docosapentaenoic acid (DPA ω 3 22:5 ω 3), and docosahexaenoic acid (DHA 22:6 ω 3), which have vital roles in brain development and function, cardiovascular health, and inflammatory response.⁷⁻¹⁰ Omega-3 fatty acids are incorporated into the membrane lipid bilayer in virtually all body cells and affect membrane composition, eicosanoid biosynthesis, cell signaling cascades, and gene expression.¹¹

EPA, DPA ω 3, and DHA are found in large quantities in fish oils. Most attention has been given to EPA and DHA with DPA ω 3 being ignored. However, DPA ω 3 is the main ALA metabolite in the cell membranes of most large land mammals with the exception of *Homo sapiens*.^{12,13} EPA and DHA derived from fish oil have been investigated widely, but there have been significantly fewer studies on ALA from plants. In a recent evidence-based systematic review of the health effects of omega-3 fatty

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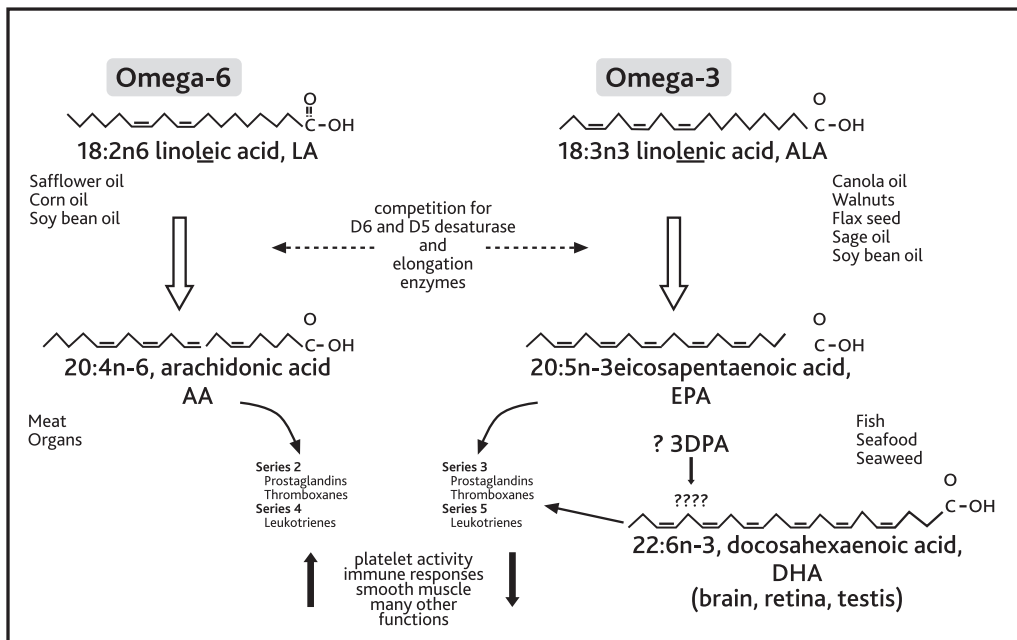


Figure 1 Metabolism and dietary sources of omega-6 and omega-3 fatty acids.

acids,¹⁴ 166 studies were included that examined the impact on cardiovascular risk factors and clinical outcomes. Only 18 of the studies looked at the effects of ALA. Furthermore, for approximately one-third of the clinical conditions examined, no studies at all evaluated ALA. This is unfortunate as ALA may have independent, therapeutic properties similar to those of other n-3 fatty acids. It may also be of particular importance for neural development.¹⁵ Land-based sources of ALA are varied and plentiful while the seafood stocks are under threat. Thus, it seemed worthwhile to review the literature on the value of ALA in the human food chain.

ABSORPTION AND METABOLISM OF ALA

As a rule, dietary fats are absorbed very efficiently from the digestive tract, and ALA is no exception. Burdge¹⁶ recently reported that absorption levels of ALA are 96% or more. There are several possible metabolic fates for ALA that enters the bloodstream. The body can store the fatty acids in adipose tissue, use them for acetyl-CoA or energy production through β -oxidation, synthesize other non-essential saturated or monounsaturated fatty acids (MUFA), or convert them to longer-chain n-3 polyunsaturated fatty acids (PUFA) in the liver. The activity of the desaturation/elongation pathway is of unique importance as it is responsible for the synthesis of EPA and DHA. What is presently understood concerning conversion of ALA to EPA, DPA ω 3, and DHA is that the first step in the pathway, addition of a fourth double bond by Δ 6 desaturase, is

considered to be the rate-limiting step. This is followed by elongation (addition of two carbon atoms) and an additional desaturation by the enzyme Δ 5 desaturase, with the product being 20:5n-3 or EPA (Figure 1). Several possibilities have been suggested for the precise pathway for production of DHA (22:6n-3) from EPA in humans. It was assumed that the conversion of DPA ω 3 to DHA would be carried out by a Δ 4 desaturase but, thus far, little or no Δ 4 desaturase has been found. Sprecher¹⁷ has provided evidence for elongation of 22:5 ω 3 to 24:5 ω 3, which is then desaturated by the rate limiting Δ 6 desaturase to yield a 24:6 ω 3. Two carbons are then cleaved in the peroxisomes to yield DHA that is then exported to the reticulo-endothelial system. Thus, the insertion of the last double bond in DHA production in human metabolism may be rather indirect and somewhat inefficient. There are some doubts as to the validity of the Sprecher shunt, but it provides a possible explanation for the small proportions of DHA produced and the build up of DPA ω 3, despite ample ALA available in foods and in body tissues.

Alpha-linolenic acid is partially converted to EPA in humans (8–20%), while conversion rates of ALA to DHA are estimated at 0.5–9%.^{18,19} The sex difference in metabolism is well known. Studies in women of reproductive age showed a substantially greater (2.5-fold) rate of conversion of ALA to EPA than that measured in healthy men. Thus, the ability to produce long-chain metabolites is gender dependent. It appears that women have a lower partitioning of ALA to β -oxidation, leaving more of it available for conversion to EPA.^{16,20} Other possible explanations include a direct effect of estrogen on conversion

rates.^{16,21} Gender differences have also been observed in the conversion rates of ALA to DHA. In males it is estimated that only 0.5–4% of ALA is converted to DHA while in females the rates are thought to be as high as 9%.^{18,19} It is hypothesized that demands for DHA by the fetus during pregnancy may stimulate female physiology to more readily synthesize this fatty acid.

n-6:n-3 Ratio

Dietary patterns have changed over time and in many developed nations consumption of n-6 fatty acids has risen dramatically. According to Simopoulos,²² in the past, the ratio of n-6 to n-3 essential fatty acids was 1 : 1. However, in the modern Western diet the ratio is approximately 15 : 1. The n-6 and n-3 fatty acids are metabolized by the same set of enzymes to their respective long chain metabolites. The competition for these enzymes between the omega 6 and omega 3 as well as between EFAs and other fatty acids has been known since 1966.²³ It has been shown that when ALA intake in the diet is increased, an increased proportion of both ALA and EPA is found consistently in both plasma and cell lipids.^{7,20,23} In addition, it is thought that a higher relative intake of n-6 fatty acids increases production of arachidonic acid (20:4n-6), which in turn is used to produce pro-thrombotic and pro-inflammatory n-6 metabolites.^{24,25} Metabolites of n-3 origin are anti-inflammatory and anti-arrhythmic.^{8,19,24,25} A high n-6 : n-3 ratio is thought to promote the pathogenesis of many diseases, including cardiovascular disease, cancer, osteoporosis, and inflammatory and autoimmune diseases.^{26–28}

CARDIOPROTECTIVE EFFECTS OF ALA

In systematic reviews of the literature, numerous well-designed studies have been carried out to support the protective role of long-chain n-3 polyunsaturated fatty acids derived from fish in coronary heart disease (CHD).^{10,14} However, the data concerning the role of ALA is less definitive. On one hand, Balk et al.¹⁴ described the evidence for a cardioprotective role of ALA as “inconclusive” and Wang et al.¹⁰ stated there is no high-quality evidence to support the role of ALA in preventing myocardial infarction and cardiac death. In contrast, a meta-analysis that looked at five prospective cohort studies reported that high ALA intake was associated with reduced risk of fatal heart disease (combined relative risk 0.79, 95% CI 0.60–1.04).²⁹ There are several studies that strongly indicate that ALA may indeed be important in maintaining heart health. Because ALA is a precursor of the longer chained n-3 fatty acids, it contributes to the body pools of EPA and DHA that have been associated with improved vascular tone, heart rate, serum lipid

levels, platelet function, inflammatory responses, arrhythmia, growth rates of atherosclerotic plaques, and blood pressure.^{26,27,30} Most of these beneficial effects have also been associated specifically with ALA consumption.

Interest in the role of ALA in the prevention of heart disease arose following the publication of the Lyon Heart Study carried out in France.³¹ This study provided the first evidence that ALA consumption may play an important role in reduction of heart disease. In this secondary prevention trial, which included 608 patients with known CHD, participants in the intervention group were encouraged to follow the Mediterranean diet and were supplied with canola margarine rich in ALA. At 27-month follow-up, a 73% reduction in cardiovascular events was observed along with a 70% decrease in overall mortality. ALA intake in the intervention group was three times higher than that in the control subjects. Although it appears that ALA may have contributed to the improvement in cardiac health, there were numerous independent variables in the study, which makes it impossible to isolate the effects of ALA per se.

Mozaffarian²⁶ reviewed the findings from observational studies that evaluated the relationship between ALA intake and risk of CHD or mortality. In most studies, particularly those in the American population, an inverse association between ALA consumption and incidence of CHD was found. For example, the Cardiovascular Health Study,³² which included a prospective cohort of close to 6000 older men and women, found that each one standard deviation increase in plasma phospholipid ALA levels (a biomarker of ALA intake) was associated with a 52% decrease in risk for fatal CHD. In The Nurses Health Study,^{33,34} over 75,000 women were followed using dietary intake records to assess ALA consumption. ALA intake was associated with a significantly lower risk of sudden cardiac death. These results help support the hypothesis that ALA may have antiarrhythmic properties. Data from another large cohort, the National Heart, Lung, and Blood Institute Family Heart Study,³⁵ included measurement of calcified atherosclerotic plaque in the coronary arteries (CAC) of 2004 participants aged 32 to 93 years. When the lowest to highest quintiles of ALA consumption were correlated to CAC, it was found that consumption of ALA was associated with a lower prevalence of CAC in a dose-response fashion.

It is interesting to note that in the Health Professionals Study²⁵ (a prospective cohort of 45,722 men), the strongest evidence linking ALA intake and CHD risk was observed in participants that consumed very little seafood. In men who consumed <100 mg of long-chain n-3 (EPA + DHA), each 1 g/day ALA intake was associated with a 58% lower risk of nonfatal heart attack and a 47% lower risk of CHD. These data strongly support the direct role of ALA consumption in decreasing CHD risk

and indicate that intake of plant sources of n-3 fatty acids may be of particular importance in sectors of the populations that do not eat fatty fish.

Despite the highly convincing results reported in these studies, it must be noted that when biomarkers of intake are not measured, ALA consumption is determined entirely by self-report. The wide use of food frequency questionnaires to estimate dietary intake is problematic as they provide only semiquantitative data and are characterized by large random error.³⁶ The inaccuracies of assessing dietary exposures are well-known, and cannot be ignored when evaluating the reliability of nutritional studies.

Experimental studies have also been carried out to assess the specific effects of diets enriched with ALA on blood clotting. Freese et al.³⁷ showed that in healthy men, supplementing the diet with canola oil for 6 weeks (2.3% energy from ALA) reduced *in vitro* platelet aggregation. An additional study determined platelet composition and function in individuals that consumed either 40 g/d flaxseed oil (a rich source of ALA) or equal amounts of sunflower seed oil.³⁸ Consumption of flaxseed doubled EPA levels in platelets and significantly decreased aggregation response. These results are thought to be beneficial in preventing thrombosis connected to CHD.

It is difficult to determine the exact impact of ALA on heart health because very few studies have focused exclusively on ALA. However, available data consistently support a beneficial effect. Why, then, is there a seeming lack of consensus between the individual studies described here and the results of large systematic evidence-based reviews? This is most likely due to the fact that there are only a small number of well-designed, high-quality studies investigating the role of ALA in cardiovascular disease. Large-scale randomized trials with ALA have not been carried out and observational studies alone cannot decisively establish causal relationships. Recommendations from a workshop sponsored by the National Heart, Lung, and Blood Institute and Office of Dietary Supplements on omega-3 fatty acids and cardiac arrhythmogenesis³⁹ specified the need for more epidemiological data on ALA intake and sudden cardiac death and cardiac arrest, including large-scale, double-blind randomized trials with well-defined end points. Although the data available today is not conclusive, the continual appearance of new studies and professional opinions of scientists from around the world^{19,26,30,35,40-42} support the ever-growing body of research that ALA has cardioprotective effects in its own right.

THE ROLE OF ALA IN INFLAMMATION

Inflammation is a central pathological component in CHD disease. Other diseases such as rheumatoid arthritis,

psoriasis, Crohn's disease, chronic obstructive pulmonary disease, and irritable bowel syndrome (IBD) are also characterized by high levels of inflammation.²⁸ It is thought that n-3 fatty acids are able to reduce disease-promoting inflammatory responses.⁴³ Eicosanoids, a class of bioactive molecules, act as inflammatory mediators and play a role in platelet aggregation. They are produced from both the n-3 and n-6 fatty acid families and include leukotrienes, prostaglandins and thromboxanes.⁸ Eicosanoids derived from n-3 fatty acids are considered to be antithrombotic, anti-inflammatory, and vasodilating.^{19,42,44} These physiological effects are attributed, for the most part, to the longer chained n-3 fatty acids (EPA and DHA), but some research has looked specifically at the effects of ALA.

In order to assess the role of ALA on systemic inflammation, men suffering from dyslipidemia were provided a high-ALA diet (canola oil 15 ml/day) or a safflower oil control diet.⁴⁵ After 3 months, in individuals consuming the canola oil diet, systemic inflammation was significantly reduced, as measured by C-reactive protein, serum amyloid A, and interleukin-6. Additional evidence that ALA consumption affects biomarkers of inflammation and endothelial activation was observed in a cross-sectional study of 727 women in the Nurses Health Study.⁴⁶ An inverse relationship was observed between ALA intake and plasma concentrations of C-reactive protein, IL-6, E-selectin, soluble intercellular cell adhesion molecule 1, and soluble vascular cell adhesion molecule 1. This provides support for the role of ALA as an anti-inflammatory agent that also lowers endothelial activation. Ferrucci et al.⁴⁷ also reported that higher levels of ALA in the blood were associated with lower levels of inflammatory biomarkers. His study included 1123 individuals, aged 20–98 years. Fatty acids in fasting plasma were analyzed in relation to numerous inflammatory markers. Decreased levels of C-reactive protein and IL-1ra levels were associated with higher plasma ALA levels.

Zhao et al.⁴⁸ have shown that dietary ALA elicits anti-inflammatory effects by inhibiting IL-6, IL-1beta, and TNF-alpha production in peripheral blood mononuclear cells cultured from hypercholesterolemic individuals exposed to a diet high in ALA (6.5% of energy). Thus, consumption of ALA may provide protection against heart disease and other inflammatory diseases by reduction of inflammatory cytokines.

OTHER POTENTIAL BENEFITS OF ALA CONSUMPTION ON THE CENTRAL NERVOUS SYSTEM, BEHAVIOR, AND IN AUTOIMMUNE DISEASES

New studies continually appear suggesting that ALA may be of physiological importance in a wide range of disorders and diseases.

Attention deficit hyperactivity disorder: A recent study by Joshi et al.⁴⁹ reported a significant improvement in the symptoms of ADHD in children that had received flax oil and Vitamin C supplements.

Neuroprotection: Lauritzen et al.⁵⁰ reported that ALA prevented neuronal death in an animal model of transient global ischemia; it also protected animals treated with kainate against seizures and hippocampal lesions. An additional study reported neuroprotective effects in rat models of spinal cord ischemia leading to paraplegia.⁵¹ ALA treatment preserved neurological function and animals sustained only mild-to-moderate injury. This suggests that ALA can induce protection against ischemia in spinal injury, preventing necrosis and apoptosis of motor neurons.

Autoimmune diseases: Work in animal models by Reiffen et al.⁵² suggests that the addition of flaxseed oil (70% ALA) to the diet may attenuate the severity of systemic lupus erythematosus (SLE or lupus). SLE mice fed flaxseed exhibited lower titers of antibodies to DNA and to cardiolipin and less severe kidney damage than mice fed other diets, including fish oil.

POSSIBLE ADVERSE EFFECTS OF ALA CONSUMPTION

In the many publications dealing with omega-3 fatty acid consumption, very few adverse effects have been reported aside from mild gastrointestinal symptoms.^{10,14,19} However, concern has arisen from one meta-analysis that reported an increased incidence of prostate cancer risk in men with high intake or high blood levels of ALA (combined relative risk 1.70; 95% CI 1.12–2.58).²⁹ The data presented was heterogeneous and the authors themselves stated it is uncertain if the results present a genuine effect. The results have yet to be confirmed. More recent reviews did not confirm or refute this possible deleterious effect of ALA.¹⁴

RECOMMENDATIONS FOR ALA INTAKE

The present dietary reference intakes state that to achieve nutritional adequacy, ALA should provide 0.6–1.2% of energy with up to 10% provided by longer chained fatty acids.⁵³ In contrast to ALA, which is essential, EPA and DHA have no minimum requirements, and in large doses, longer chain n-3 fatty acids present a health risk. Recommendations by the European Commission for fatty acid composition in infant formulas require that ALA be included at levels of at least 50–100 mg/100 kcal. Here too, there is no set minimum for EPA or DHA, but maximum safe levels have been established.⁵⁴ The fact that several major scientific and medical associations have published nutritional guidelines including recom-

mendations specifically for ALA emphasizes its perceived importance in health promotion and disease prevention.

CONCLUSION

Based on the studies and research presented here, it can be concluded that increasing ALA in the daily diet is a safe, viable option for meeting dietary requirements and maintaining the suggested n-6 : n-3 ratio. Foods naturally rich in ALA should be included in the diet and manufacturers can help the public meet dietary recommendations by increasing ALA content in processed foods. Results of many of the studies described here indicate that ALA is not only essential, it also has therapeutic properties. Nevertheless, it is clear that additional well-designed research is needed in order to establish unequivocal support for health claims.

Although there is some interconversion of the omega 3 fatty acids, each fatty acid has its own place in biology. It is important to remember that of the omega-3 fatty acids, ALA is the parent molecule, and greater attention should be paid to its independent physiological function.

REFERENCES

1. Cuthbertson WF. Essential fatty acids in infancy. *Am J Clin Nutr.* 1976;29:559–568.
2. Crawford MA, Casperd NM, Sinclair AJ. The long chain metabolites of linoleic and linolenic acids in liver and brain in herbivores and carnivores. *Comp Biochem Physiol.* 1976;54B:395–401.
3. World Health Organization/Food and Agriculture Organization. *International Expert Consultation on the Role of Dietary Fats and Oils in Human Nutrition. Nutrition Report no 3.* Rome, FAO:1978
4. Holman RT. Nutritional and functional requirements for essential fatty acids. *Prog Clin Biol Res.* 1986;222:211–228.
5. Lukiw WJ, Cui JG, Marcheselli VL, et al. A role for docosa-hexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J Clin Invest.* 2005;115:2774–2783.
6. Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr.* 2006;83(Suppl):S1526–S1535.
7. Holman RT. The slow discovery of the importance of 3 essential fatty acids in human health *J Nutr.* 1998; 128(Suppl):S427–S433.
8. Das UN. Essential fatty acids – a review. *Curr Pharm Biotechnol.* 2006;7:467–482.
9. Leaf A. Omega-3 fatty acids and prevention of arrhythmias. *Curr Opin Lipidol.* 2007;18:31–34.
10. Wang C, Chung M, Balk E, et al. n-3 Fatty acids from fish or fish-oil supplements, but not -linolenic acid, benefit cardiovascular disease outcomes in primary and secondary-prevention studies: a systematic review. *Am J Clin Nutr.* 2006;83:5–17.

11. Shahidi F, Miraliakbari H. Omega-3 fatty acids in health and disease: part 2—health effects of omega-3 fatty acids in autoimmune diseases, mental health, and gene expression. *J Med Food*. 2005;8:133–148.
12. Crawford MA, Gale MM, Woodford MH. Linoleic acid and linolenic acid elongation products in muscle tissue of *Syncerus caffer* and other ruminant species. *Biochem J*. 1969;115:25–27.
13. Crawford MA, Casperd NM, Sinclair AJ. The long chain metabolites of linoleic and linolenic acids in liver and brain in herbivores and carnivores. *Comp Biochem Physiol*. 1978;54B:395–401.
14. Balk EM, Horsley TA, Newberry SJ, et al. A collaborative effort to apply the evidence-based review process to the field of nutrition: challenges, benefits, and lessons learned. *Am J Clin Nutr*. 2007;85:1448–1456.
15. Sinclair AJ, Crawford MA. The incorporation of linolenic and docosahexaenoic acid into liver and brain lipids of developing rats. *FEBS Lett*. 1972;26:127–129.
16. Burdge GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot Essent Fatty Acids*. 2006;75:161–168.
17. Sprecher H. The roles of anabolic and catabolic reactions in the synthesis and recycling of polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids*. 2002;67:79–83.
18. Burdge GC. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care*. 2004;7:137–144.
19. DeFilippis AP, Sperling LS. Understanding omega-3s. *Am Heart J*. 2006;151:564–570.
20. Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev*. 2005;45:581–589.
21. Giltay EJ, Gooren LJ, Toorians AW, Katan MB, Zock PL. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr*. 2004;80:1167–1174.
22. Simopoulos AP. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed Pharmacother*. 2006;60:502–507.
23. Brenner RR, Peluffo RO. Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic and linolenic acids. *J Biol Chem*. 1966;241:5213–5219.
24. Galli C, Agradi E, Petroni A, Tremoli E. Differential effects of dietary fatty acids on the accumulation of arachidonic acid and its metabolic conversion through the cyclooxygenase and lipoxygenase in platelets and vascular tissue. *Lipids*. 1981;16:165–172.
25. Mozaffarian D, Ascherio A, Hu FB, et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation*. 2005;111:157–164.
26. Mozaffarian D. Does alpha-linolenic acid intake reduce the risk of coronary heart disease? A review of the evidence. *Altern Ther Health Med*. 2005;11:24–30.
27. Mori TA. Omega-3 fatty acids and hypertension in humans. *Clin Exp Pharmacol Physiol*. 2006;33:842–846.
28. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr*. 2002;21:495–505.
29. Brouwer IA, Katan MB, Zock PL. Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *J Nutr*. 2004;134:919–922.
30. Robinson JG, Stone NJ. Antiatherosclerotic and antithrombotic effects of omega-3 fatty acids. *Am J Cardiol*. 2006;98(4A):39i–49i.
31. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet*. 1994;343:1454–1459.
32. Lemaitre RN, King IB, Mozaffarian D, et al. 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;77:319–325.
33. Hu FB, Stampfer MJ, Manson JE, et al. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *Am J Clin Nutr*. 1999;70:1001–1008.
34. Albert CM, Oh K, Whang W, et al. Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. *Circulation*. 2005;112:3232–3238.
35. Djousse L, Arnett DK, Carr JJ, et al. Dietary linolenic acid is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the National Heart, Lung, and Blood Institute Family Heart Study. *Circulation*. 2005;7:111:2921–2926.
36. Rutishauser IH. Dietary intake measurements. *Public Health Nutr*. 2005;8:1100–1107.
37. Freese R, Mutanen M, Valsta LM, Salminen I. Comparison of the effects of two diets rich in monounsaturated fatty acids differing in their linoleic/alpha-linolenic acid ratio on platelet aggregation. *Thromb Haemost*. 1994;71:73–77.
38. Allman MA, Pena MM, Pang D. Supplementation with flaxseed oil versus sunflowerseed oil in healthy young men consuming a low fat diet: effects on platelet composition and function. *Eur J Clin Nutr*. 1995;49:169–178.
39. London B, Albert C, Anderson ME, et al. Omega-3 fatty acids and cardiac arrhythmias: prior studies and recommendations for future research: a report from the National Heart, Lung, and Blood Institute and Office of Dietary Supplements Omega-3 Fatty Acids and their Role in Cardiac Arrhythmogenesis Workshop. *Circulation*. 2007;116:e320–335.
40. Crawford M, Galli C, Visioli F, et al. Role of plant-derived omega-3 fatty acids in human nutrition. *Ann Nutr Metab*. 2000;44:263–265.
41. Nannicini F, Sofi F, Avanzi G, Abbate R, Gensini GF. Alpha-linolenic acid and cardiovascular diseases – omega-3 fatty acids beyond eicosapentaenoic acid and docosahexaenoic acid. *Minerva Cardioangiol*. 2006;54:431–442.
42. Simopoulos AP. Essential fatty acids in health and chronic disease. *Am J Clin Nutr*. 1999;70(Suppl):S560–S569.
43. Calder PC. Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids*. 2006;75:197–202.
44. Gil A. Polyunsaturated fatty acids and inflammatory diseases. *Biomed Pharmacother*. 2002;56:388–396.
45. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A. Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. *Atherosclerosis*. 2003;167:237–242.
46. Lopez-Garcia E, Schulze MB, Manson JE, et al. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr*. 2004;134:1806–1811.
47. Ferrucci L, Cherubini A, Bandinelli S, et al. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab*. 2006;91:439–446.
48. Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM. Dietary alpha-linolenic acid inhibits proin-

- flammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. *Am J Clin Nutr.* 2007;85:385–391.
49. Joshi K, Lad S, Kale M, et al. Supplementation with flax oil and vitamin C improves the outcome of Attention Deficit Hyperactivity Disorder (ADHD). *Prostaglandins Leukot Essent Fatty Acids.* 2006;74:17–21.
 50. Lauritzen I, Blondeau N, Heurteaux C, et al. Polyunsaturated fatty acids are potent neuroprotectors. *EMBO J.* 2000;19:1784–1793.
 51. Lang-Lazdunski L, Blondeau N, Jarretou G, et al. Linolenic acid prevents neuronal cell death and paraplegia after transient spinal cord ischemia in rats. *J Vasc Surg.* 2003;38:564–575.
 52. Reifen R, Blank M, Afek A, et al. Dietary polyunsaturated fatty acids decrease anti-dsDNA and anti-cardiolipin antibodies production in idiotype induced mouse model of systemic lupus erythematosus. *Lupus.* 1998;7:192–197.
 53. Institute of Medicine. *Dietary Reference Intakes: Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Washington, DC: National Academies Press, 2002.
 54. European Commission. Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae. SCF/CS/NUT/IF/65 Final 18 May 2003. Available at: http://ec.europa.eu/food/fs/sc/scf/out199_en.pdf. Accessed on 28 April 2008.