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α -Linolenic Acid and Heart Disease

Duo Li^{a,b}, Nadia Attar-Bashi^b, and Andrew J. Sinclair^b

^aDepartment of Food Science, Hangzhou University of Commerce, Hangzhou, China 310035; and ^bDepartment of Food Science, Royal Melbourne Institute of Technology University, Melbourne, Australia 3000

Introduction

Dietary fat intake is known to play a critical role in influencing coronary heart disease (CHD) risk factors (1–3). Dietary saturated fatty acid (SFA) intake, especially myristic and palmitic acids, increases plasma cholesterol and low-density lipoprotein cholesterol (LDL-C) and is associated with CHD mortality (2). Polyunsaturated fatty acid (PUFA) has generally been associated with lowered plasma cholesterol and LDL-C and CHD risk (4). Recent research suggests that the diet-CHD relationship is more complex than previously recognized. The role of n-3 PUFA in the diet and tissues is of increasing interest in the medical research literature. α -Linolenic acid (ALA, 18:3n-3) is an essential fatty acid that can be used to synthesize longer chain n-3 PUFA such as eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) by desaturation and elongation steps. In the past three decades, a substantial number of studies have examined the effect of n-3 PUFA from marine sources on CHD mortality and risk factors (5). However, the relationship between ALA from plant sources and CHD mortality and risk factors is relatively less studied. In this chapter, we consider the effect of ALA on CHD mortality and risk factors, based on publications from the most recent international literature.

One issue to consider in any report on ALA is whether ALA itself has a biological role, or whether ALA is merely important as a substrate for the production of EPA, DPA, and DHA. In plants, ALA clearly plays an important role because lipoxygenase products of ALA include jasmonic acid and related phytohormones produced via the oxylipin cascade (6). The evidence in mammals is less clear. In humans, feeding high ALA diets (up to 15 g/d) in 4-wk studies led to significant increases in ALA, EPA, and DPA in plasma triacylglycerols (TAG) and phospholipids, and very little if any detectable increase in DHA in plasma, platelets, and white and red blood cells (7–9). Deuterium-labeling studies in humans with ALA showed that the percentage conversion of ALA to EPA and other long-chain n-3 PUFA was between 11–19% of the dose, and that the conversion rate was reduced by 40–54% when the diet was rich in linoleic acid (10). In a recent study, physiological compartmental analysis of ALA metabolism in adult humans was carried out. Subjects received a 1-g oral dose of an isotope tracer of ALA and only about 0.2% of the plasma ALA was destined for syn-

thesis of EPA; approximately 63% of the plasma EPA was accessible for production of DPA, and 37% of DPA was available for synthesis of DHA (11). The very limited conversion of ALA to EPA indicates that the biosynthesis of long-chain n-3 PUFA from ALA is limited in healthy individuals.

α -Linolenic Acid Protection Against Coronary Heart Disease

There is some evidence from recent prospective, cross-sectional and intervention studies that the dietary intake of ALA is protective against CHD.

Prospective Studies

Two prospective cohort investigations from the USA Health Professionals study and Nurses' Health study examined the effect of the intake of dietary fat and n-3 PUFA from plants on CHD in humans. In the first study, 43,757 healthy male professionals aged 40–75 yr, free of diagnosed cardiovascular disease (CVD) or diabetes were followed up for 6 yr from 1986 (12). Each subject completed a food-frequency questionnaire at the beginning of the study. The subjects returned food-frequency questionnaires in each 2-yr follow-up cycle. During the follow-up, 505 nonfatal myocardial infarctions and 229 deaths were documented. After adjustment for nondietary risk factors and total fat intake, intake of ALA was significantly negatively correlated with risk of myocardial infarction (relative risk 0.41 for a 1% increase in energy from ALA, $P < 0.01$). In the second study, the dietary intake of ALA was calculated from a food-frequency questionnaire completed in 1984 by 76,283 nurses aged 38–63 yr, free from previously diagnosed CVD and cancer. There were 597 cases of nonfatal myocardial infarction and 232 cases of fatal ischemic heart disease documented during 10 yr of follow-up. After the adjustment of confounding factors, such as age, standard coronary risk factors, and dietary intake of linoleic acid (LA) and other nutrients, the results showed that women who had a higher intake of ALA (≥ 5 –6 times/wk) were significantly associated with reduced risk of fatal ischemic heart disease compared with women who consumed ALA less than once per month in this study population ($P < 0.001$) (13). The major foods contributing to ALA in this study were mayonnaise or other creamy salad dressings and oil and vinegar salad dressings.

Cross-sectional Studies

The Family Heart Study, a cross-sectional study by the USA National Heart, Lung, and Blood Institute, found that higher intakes of either ALA or LA were inversely related to the prevalence of coronary artery disease (CAD) (14). Dietary intakes of 4584 volunteers were assessed with a semi-quantitative food-frequency questionnaire. After adjustment for confounding factors such as age, LA, and anthropometric, lifestyle, and metabolic factors, the prevalence odds ratios of CAD from the lowest to the highest quintile of ALA intake were 1.0, 0.77, 0.61, 0.58, and 0.60 for the men ($P = 0.012$) and 1.0, 0.57, 0.52, 0.30, and 0.42 for the women ($P = 0.014$). LA was also inversely related to the prevalence odds ratios of CAD in the multivariate model (0.60

and 0.61 in the second and third quintiles, respectively) after adjustment for ALA. It was noted that the combined effects of LA and ALA were stronger than the effect of either of the fatty acids individually.

Dietary Intervention Studies

The Lyon diet-heart study concluded that ALA prevented secondary CHD (15). In this study, the diet chosen was associated with a low mortality rate from CHD and all causes in the Seven-Countries Study (16). The Cretan diet had a high intake of ALA and was rich in antioxidants because it was rich in fruits and vegetables. Crete had a lower mortality rate from CHD compared with similar cohorts in other countries. Cretan participants had threefold higher serum concentrations of ALA compared with a similar cohort from the Netherlands (17). In the Lyon study, 605 patients who had suffered a first myocardial infarction were randomly divided into two groups, experimental ($n = 302$) and control ($n = 303$). Patients in the experimental group received a Mediterranean style diet (rapeseed oil and rapeseed oil based margarine). This diet was rich in ALA (ALA/LA ratio of 1:4), and total fat provided 30.5% of energy with S/M/P ratio of 0.9:1.4:1. The control group consumed a habitual diet which was poor in ALA (ALA/LA was 1:20) and in which total fat contributed 32.7% of energy with S/M/P ratio of 1.2:1:1. After 27 mon of follow-up, there were 16 cardiac deaths in the control and 3 in the experimental groups, 17 nonfatal myocardial infarctions in the control and 5 in the experimental groups, and the relative risk ratio for cardiac deaths and nonfatal myocardial infarction in the ALA-rich group was 0.27 ($P = 0.001$).

This study was continued after the original conclusion because there was a high adherence of the experimental group to the program over the total 46 mon of mean follow-up for each patient (18). It was found that the three composite outcomes [(i) cardiac death and nonfatal myocardial infarction; (ii) the preceding plus major secondary endpoints including unstable angina, stroke, heart failure, pulmonary or peripheral embolism; or (iii) the preceding plus minor events requiring hospital admission] were significantly reduced in the Mediterranean diet group compared with the Western diet group. The traditional risk factors such as high blood cholesterol and raised blood pressure were significantly and independently associated with recurrence of events. Plasma ALA, measured at 2 mon after randomization, was the only fatty acid that was significantly negatively associated with myocardial infarction plus cardiac death after adjustment for age, sex, smoking, total cholesterol, blood pressure, leukocyte count, and aspirin use.

Another study investigated the secondary prevention of CHD by ALA as mustard oil. In this study, 360 patients less than 1 d after acute myocardial infarction were randomized to one of three dietary groups: fish oil capsules (EPA, 1.08 g/d, and DHA, 0.72 g/d); mustard seed oil, 20 g/d (ALA, 2.9 g/d); and a control group (aluminum hydroxide, 100 mg/d) (19). After 1 yr, this study showed that there was a significant reduction in cardiac events in the fish oil and mustard oil groups compared with the control group (24.5% and 28.2%, respectively, vs. 34.7%; $P < 0.01$). Nonfatal infarctions were also significantly lower in the fish oil and mustard oil groups compared

with the placebo group (13.0% and 15.0% vs. 25.4%, $P < 0.05$). Total cardiac deaths were significantly reduced in the fish oil group but not in the mustard oil group compared with the placebo group. The fish oil and mustard oil groups also showed significant reductions in total cardiac arrhythmias, left ventricular enlargement, and angina pectoris compared with the placebo group.

The Multiple Risk Factor Intervention Trial (MRFIT) was a study of 12,866 men randomly assigned to either a usual care or special intervention group. The latter received advice and programs regarding reduction in smoking, blood pressure, and blood cholesterol. In this report, multivariate regression analysis was used to determine the effect of dietary PUFA intakes on 10-yr mortality rates in 6,250 usual care men (20). Dietary PUFA intake was calculated from four dietary recall interviews at baseline and at 1-, 2-, and 3-yr follow-up. Dietary intake of ALA was significantly negatively associated with CHD mortality rates ($P < 0.04$), total CVD ($P < 0.03$), and all-cause mortality ($P < 0.02$). There were also significant inverse associations for long chain n-3 PUFA on CHD mortality ($P < 0.02$), CVD mortality ($P < 0.006$), and all-cause mortality ($P < 0.02$).

The mechanisms whereby ALA could prevent CHD include reduction of blood pressure and levels of plasma/serum TAG and lipoprotein lipids, and support of antithrombotic and fibrinolytic activities, and of antiarrhythmia, anti-inflammatory, and anti-immunity actions.

Blood Pressure

The n-3 PUFA from fish have been found to reduce both systolic and diastolic blood pressure (BP), and this has been evaluated in a meta-analysis of 31 placebo-controlled trials in 1,356 subjects. The results indicated that systolic BP fell 3.4 mm Hg and diastolic BP fell 2.0 mm Hg following ingestion of 5.6 g/d of fish oil in hypertensive subjects (21).

Epidemiological, prospective, and cross-sectional studies found that vegetarians have a lower mortality from heart disease (22) and a lower diastolic blood pressure than omnivores in general populations (23,24). Berry and Hirsch (25) studied the relationship between adipose tissue fatty acids and blood pressure in 399 free-living male subjects (average age 37 yr). Stepwise regression analysis was performed to assess the separate contributions of age, body mass index, and adipose tissue fatty acids composition to the variance in BP. The analysis showed that adipose tissue LA was not associated with BP; however, they reported that an absolute 1% increase in adipose tissue ALA was associated with a decrease of 5 mm Hg in the systolic, diastolic, and composite mean arterial blood pressure. It was reported that ALA concentration had a disproportionate influence on the mean arterial blood pressure because it comprised 1/8 the amount of LA in adipose tissue.

The MARGARIN study was a prevention of CHD project. It investigated the association between dietary intake of ALA and LA, as assessed by a food-frequency questionnaire and levels of plasma cholesterol ester (CE), with CHD risk factors. The

study was a double blind, randomized placebo-controlled trial, which involved 266 subjects with hypercholesterolemia (6.0–8.0 mmol/L) and at least two other CHD risk factors (26). In multivariate analysis, CE ALA was inversely associated with diastolic blood pressure ($r = -0.13$; $P < 0.05$) and positively with serum TAG levels ($P < 0.01$), whereas the CE LA was inversely associated with serum TAG ($P < 0.01$). In the lowest quintile of CE ALA, mean dietary intake was 0.4% energy of ALA (1.2 g/d), 8.4% energy of LA, and an LA/ALA ratio of 21, and in the highest quintile 0.6% energy of ALA (1.7 g/d), 6.8% energy of LA, and an LA/ALA ratio of 12. In the highest quintile of CE ALA, the diastolic BP was 4 mm Hg lower and the serum TAG 0.3 mmol/L higher compared with the top quintile, suggesting that replacing LA with ALA might decrease diastolic blood pressure.

A recent animal study found that ALA deficiency in the perinatal period resulted in an increase in BP later in life in Sprague-Dawley rats (27). The authors suggested that although the study was carried out on rats, the implication of these findings is that an adequate ALA intake at an early age may help prevent increased BP in later life in humans.

Plasma and Lipoprotein Lipids

LA reduced plasma/serum total and LDL-C when substituted for carbohydrate in the diet, whereas saturated fatty acids were cholesterolemic and *cis* monounsaturated fatty acid was neutral in early studies (28,29). Recent dietary intervention studies found that like LA, ALA from plant sources is able to decrease plasma/serum total and LDL-C levels (30,31). McDonald *et al.* (30) reported that canola oil and sunflower oil had an equivalent hypercholesterolemic and antithrombotic effect in eight healthy young hypocholesterolemic men aged 19–32 yr. Approximately 75% of the fat in the diet was provided by a mixture of fats (tallow, lard, corn oil, butter, and vegetable shortening) during the 6 d pre- and postexperimental periods, and either canola oil or sunflower oil during the two 18-d experimental periods with 1.2, 7.9, and 0.8% of total fatty acid as ALA in the mixture of fats, canola oil, and sunflower oil, respectively. The canola oil and sunflower oil diets produced similar decreases in plasma total cholesterol of 20 and 15%, and LDL-C of 25 and 21%, respectively. It cannot be ruled out that the monounsaturated fatty acid and LA content of the canola diet contributed to the cholesterol-lowering effects seen.

Chan *et al.* (31) have compared the effect of dietary oleic acid (OA), LA, and ALA on plasma lipid metabolism in eight normolipidemic men aged 20–34 yr. A mixed-fat diet composed of conventional foods was fed for 6 d pre- and postexperimental periods. The subjects consumed four different experimental diets during four 18-d experimental periods. The four diets were identical in the proportions of nutrients: the diets provided 53% of energy from carbohydrate, 13% from protein, and 34% from fat. Diet 1 provided the 75% fat from a mixture of sunflower and olive oils; diet 2 from canola oil; diet 3 from soybean oil; and diet 4 from a mixture of sunflower, olive, and flaxseed oils. There were significant reductions in the mean concen-

trations of plasma total cholesterol (TC) (-18%), LDL-C (-22%), and VLDL-C (-41%) after the experimental diets than after the pre- and postexperimental mixed-fat diet ($P < 0.004$). Mean serum apolipoprotein B (-19%) and apolipoprotein A-I (-9%) concentrations were also significantly lower after the experimental diets ($P < 0.0007$). This study demonstrated that dietary ALA was as effective as OA and LA in lowering TC, LDL-C, VLDL-C, and serum apo B and apo A-I concentrations.

In a randomized, crossover feeding trial, 10 men with polygenic hypercholesterolemia were fed a Mediterranean-type cholesterol-lowering diet (control) and a diet of similar composition in which walnuts replaced approximately 35% of energy from monounsaturated fat for 6 wk for each diet (32). Compared with the control diet, the walnut diet reduced serum TC and LDL-C by 4.2% ($P = 0.176$) and 6% ($P = 0.087$), respectively. There was also a reduction in the apolipoprotein B level (6% reduction) in parallel with LDL-C reduction. Whole LDL was enriched with ALA and LA from walnuts. Also, LDL obtained during the walnut diet showed a 50% increase in association rates to the LDL receptor in human hepatoma HepG2 cells compared with LDL obtained during the control diet. There was a positive correlation between LDL uptake by the HepG2 cells and the ALA content of the TAG and cholesterol ester fraction of the LDL particles ($r^2 = 0.42$, $P < 0.05$). This data suggests that a walnut-enriched diet can increase the receptor-mediated LDL clearance, which might be responsible for the reduced LDL cholesterol levels on this diet.

Kelley *et al.* (33) studied the effect of a diet rich in flaxseed oil (6.3% energy from ALA) on blood lipids and coagulation status in 10 volunteers in a study lasting 126 d. This study was a comparison between a moderate fat diet rich in LA compared with a moderate fat diet rich in ALA. Subjects consumed a baseline diet (23.4% energy from fat, polyunsaturated/saturated (P/S) = 0.89, LA/ALA = 66) or the flax oil diet (28.8% energy from fat, P/S = 1.5, LA/ALA = 0.7) for 56 d and then changed to the other diet. The flax oil diet did not significantly alter serum TAG, TC, HDL-C, or LDL-C compared with the values for the baseline diet period. There was no difference between the diets on the bleeding time, prothrombin time, and partial prothrombin time in this study.

The n-3 PUFA, especially those from marine oils, have been shown to reduce serum TAG levels (5,34,35). In contrast, increased ALA intakes were associated with raised serum TAG compared with LA in a cross-sectional analysis (26) and with a significantly raised serum TAG compared with a high LA diet in a double blind, randomized study (36).

We have compared the effect of low, moderate, and high ALA/LA diets on plasma and lipoprotein lipids in vegetarian men (8). Three dietary periods in this study were (i) a low ALA (safflower oil and safflower oil-based margarine), (ii) a moderate ALA (canola oil and canola oil-based margarine), and (iii) a high ALA (linseed oil and linseed oil-based margarine) diet. Seventeen healthy male vegetarians (34 ± 8 yr of age) completed the study. All subjects consumed a low ALA diet for 14 d, and then were randomly divided into moderate ALA or high ALA diet groups for the following 28 d. Subjects were requested to refrain from consuming fish during the 42 d of the

intervention. There were no significant differences in plasma TAG, TC, LDL-C, or HDL-C concentrations between subjects on the diets rich in LA and low in ALA (31-g LA/d, 2-g ALA/d) and those on the diet containing almost equal amounts of LA and ALA (17-g LA/d, 15-g ALA/d), indicating that diets rich in either ALA or LA do not differ in their effect on plasma TAG levels. This confirms previous reports (37,38) suggesting that the plasma lipid-lowering effect of flaxseed (as opposed to flaxseed oil) is not due to its oil or ALA content but rather to its soluble fiber.

Vermunt *et al.* (39) reported that *trans* ALA may increase plasma ratios of LDL-C/HDL-C and TC/HDL-C. In this study, 88 healthy male volunteers from four European nations (France, Scotland, UK, and the Netherlands) consumed a *trans* ALA-free diet for 6 wk, followed by either high or low *trans* ALA for another 6 wk. Daily total *trans* ALA intake in the high *trans* group was 1.41 g. Experimental oils were delivered via margarines, cheeses, muffins, and biscuits. Compared with the low *trans* ALA diet, the high *trans* ALA diet significantly increased the plasma LDL-C, ratios of LDL-C/HDL-C by 8.1% (95% CI 1.4, 15.3), and the TC/HDL-C ratio by 5.1% (95% CI 0.4, 9.9). No effects were found on plasma concentrations of TC and HDL-C, TAG, apolipoprotein B and A-1, and lipoprotein(a).

Anti-Thrombotic and Fibrinolytic Activities

Arterial thrombosis is generally recognized to play a major role in the transition from stable to acute ischaemic heart and cerebral diseases, manifested by unstable angina, acute thrombotic infarction, and sudden death. Platelet aggregation is an early event in the development of thrombosis. It is initiated by thromboxane A₂ (TXA₂), a potent platelet aggregation agent and vascular contractor, produced from arachidonic acid (AA), a long chain n-6 PUFA in the platelet membrane (40,41). EPA is released from phospholipids of the platelet membrane and as a "false" substrate competes with AA for access to cyclooxygenase and produces an alternative form of thromboxane A₃ (TXA₃), which is relatively inactive in promoting platelet aggregation and vasoconstriction (42). This situation can lead to a reduced TXA₂ production and thus a lower thrombosis tendency (43,44). A diet with a high n-6/n-3 PUFA ratio can cause a high tissue n-6/n-3 PUFA ratio (i.e., increased AA/EPA ratio), which may promote production of TXA₂, leading to an increased thrombosis tendency (45,46). A number of other studies using diets rich in ALA have shown reductions in TXA₂ production (47-49).

Platelet EPA level doubled and collagen-induced platelet aggregation significantly declined ($P < 0.05$) after healthy male subjects consumed diets supplemented with 40-g of flaxseed oil ($n = 5$) for 23 d compared with a group which consumed an identical quantity of sunflower seed oil ($n = 6$) (50). Freese *et al.* (51) investigated the effect of dietary LA/ALA ratio on platelet aggregation in 20 male subjects using low-erucic acid rapeseed oil and high-oleic acid sunflower oil as the major fat source in a crossover design. LA/ALA ratio was 2.8 and 28 in the rapeseed oil and sunflower oil diets, respectively. Platelet aggregation induced by collagen was significantly ($P < 0.05$) reduced after using rapeseed oil compared with sunflower oil.

Chan *et al.* (52) investigated the effect of dietary ALA and its ratio to LA on platelet and plasma phospholipid fatty acids and prostanoid production in eight normolipidemic male subjects. The study consisted of two 42-d phases. Each was divided into a 6-d pre-experimental period, during which a mixed fat diet was fed, and two 18-d experimental periods, during which a mixture of sunflower and olive (low ALA, high LA/ALA ratio [LO-HI diet]), soybean (intermediate ALA, intermediate LA/ALA ratio), and canola oils (intermediate ALA, low LA/ALA ratio); and a mixture of sunflower, olive, and flax oil (high ALA, low LA/ALA ratio [HI-LO diet]) provided 77% of the fat in the diet. Each subject consumed each of the 4 experimental diets for 18 d per diet. The ALA content and the LA/ALA ratio of the four experimental diets were 0.8%, 27.4; 6.5%, 6.9; 6.6%, 3.0; and 13.4%, 2.7 respectively. There was a significant ($P < 0.05$) increase in platelet EPA level following the HI-LO diet compared with the LO-HI diet. Also, production of 6-keto-PGF₁ was significantly higher ($P < 0.05$) following the HI-LO diet than the LO-HI diet.

Evidence from dietary intervention studies has found that production of TXA₂ was decreased by LC n-3 PUFA in humans (53,54) and by plant ALA in humans (55,56). In one dietary intervention study, 15 healthy men (aged 37.7 ± 6.5 yr) were provided with foods enriched in ALA (cooking oil, margarine, salad dressing, and mayonnaise) and EPA and DHA (sausages and savory dip) and with foods naturally rich in n-3 fatty acids, such as flaxseed meal and fish. Subjects incorporated these foods into their diets at home for 4 wk. The average intake of EPA plus DHA was 1.8 g/d and of ALA was 9.0 g/d. EPA levels increased threefold in plasma, platelet, and mononuclear cell phospholipids. TXB₂, prostaglandin E₂, and interleukin 1 β synthesis decreased by 36, 26, and 20% ($P < 0.05$), respectively (56).

In a study of one healthy 64-yr-old male subject, in which a 7-wk intervention involved a diet rich in ALA from canola and flaxseed oils, it was found that the urinary excretion of 11-dehydrothromboxane B₂ declined by 34% from baseline level 7 wk after the n-6/n-3 ratio of dietary PUFA was reduced from 28 to 1. Return to the baseline diet brought about a rapid return of this metabolite to baseline levels. The excretion of 2,3-dinor-6-oxo-prostaglandin F₁- α was also reduced by approximately 32%; however, the levels remained low throughout the entire study. The dietary adjustment was brought about by substituting measured amounts of canola and flaxseed oils (3:1) for measured amounts of olive and corn oils (3:1) in an otherwise fat-free basal diet. This pilot study indicates that dietary ALA may be an effective modulator of thromboxane and prostacyclin biosynthesis (55).

The MARGARIN study from the Netherlands evaluated the effect of an increased intake of ALA on CVD risk factors after 2 yr. Subjects with multiple CVD risk factors (124 men, 158 women) participated in the double-blind intervention study (36), where they consumed margarine rich in either ALA ($n = 114$) or LA ($n = 110$). The average ALA intake was 6.3 g/d in the ALA group and 1.0 g/d in the LA groups. After 2 yr, the ALA group had a higher ratio of total to HDL cholesterol (+0.34; 95% CI: 0.12, 0.56), lower HDL cholesterol (-0.05 mmol/L; 95% CI: -0.10, 0), and higher serum TAG (+0.24 mmol/L; 95% CI: 0.02, 0.46), and after 1 yr, the ALA group had

lower plasma fibrinogen (-0.18 g/L; 95% CI: -0.31 , -0.04) than did the LA group adjusted for baseline values, gender, and lipid-lowering drugs (36).

Arterial Compliance

Arterial compliance or elasticity is an important index of circulatory function, which decreases with increasing CVD risk. Fish oil supplementation has been shown to improve arterial compliance in diabetic subjects (57), and to inhibit norepinephrine-mediated vasoconstriction, which also influences compliance (58).

A dietary intervention study found that ALA from flaxseed oil raised arterial compliance (59). Fifteen obese subjects (eight men, seven postmenopausal women) aged between 45–63 yr with markers of insulin resistance and with an absence of known metabolic disorders, consumed four diets for 4 wk each: two control diet periods, and two experimental periods (high ALA and low ALA) with flaxseed and sunola oils being the basic oils for the high and low ALA periods, respectively. In the two control periods, fat intake was 35% of energy; during the two experiment periods, fat intake fell to 26% of energy. Systemic arterial compliance was calculated from aortic flow velocity and aortic root driving pressure. Systemic arterial compliance during the first and last control periods was 0.42 ± 0.12 and 0.56 ± 0.21 units (mL/mm Hg). The arterial compliance rose significantly to 0.78 ± 0.28 ($P < 0.0001$) following the high ALA diet, and it was 0.62 ± 0.19 on the low ALA diet. The significant increase in arterial compliance ($P < 0.05$) with ALA reflected rapid functional improvement in the systemic arterial circulation despite the fact that insulin sensitivity and HDL-C decreased and LDL oxidizability increased with the high ALA diet.

Cardiac Arrhythmia

Results from animal models and cultured cells studies have indicated that marine n-3 PUFA (60–62), purified EPA (63), and plant n-3 PUFA (64,65) reduce cardiac arrhythmias. This effect may be at least partly responsible for the protective action of ALA on CHD mortality, because postinfarction patients assigned to a Mediterranean ALA-enriched diet had significantly reduced CHD mortality and morbidity compared with patients receiving no advised traditional diet controls (15). The protective effects in that study were attributed to ALA; however, there were no differences in primary recognized CVD risk factors such as blood pressure or plasma and lipoprotein lipids (15).

ALA from canola oil inhibits cardiac arrhythmias in rats when compared with oleic acid from olive oil and LA from soybean and sunflower oils (64). In that study, male Sprague-Dawley rats were randomly assigned to one of four experimental diet groups for 12 wk. The fat source in the diets was 12% olive (63% oleic acid), canola (55% oleic, 8% ALA), soybean (50% LA, 7% ALA), or sunflower seed oil (64% LA). Arrhythmias were induced by coronary artery occlusion and reperfusion. The rats fed the diet containing canola oil had a significantly lower incidence of ventricu-

lar fibrillation, mortality, and arrhythmia score during reperfusion than those fed the olive, soybean, and sunflower oil diets. The proportion of n-3 PUFA in myocardial phospholipids of canola oil group was significantly increased compared with the other diets.

In the dog, the antiarrhythmic effect of ALA was similar to that of EPA and DHA (62). Surgical myocardial infarction was produced by ligating the left anterior coronary artery and placing an inflatable cuff around the left circumflex artery. The dogs were trained to run on a treadmill and were screened for susceptibility to ventricular fibrillation when the cuff was inflated. In the control exercise, ischemia tests were conducted 1 wk before and 1 wk after infusion of free fatty acids of EPA, DHA, and ALA. ALA showed an antiarrhythmic activity similar to that of EPA and DHA. The mechanism of the antiarrhythmic action of n-3 PUFA is thought to be due to the n-3 PUFA stabilizing the electrical activity of cardiac myocytes by modulating the sodium and calcium currents in the myocytes, resulting in a prolonged relative refractory period (65).

Inflammation and Immunity

The n-3 PUFA have a regulatory influence on different processes of inflammatory and immune cell activation, and can provide positive effects on various states of immune diseases with a hyperinflammatory nature (66–68). Increased tissue levels of AA increase the eicosanoid family of inflammatory mediators such as prostaglandins, leukotrienes, and related metabolites, and through these regulate the activities of inflammatory cells, the production of cytokines, and the various balances within the immune system (69). The n-3 PUFA act as AA antagonists, decrease the levels of AA in cell membranes, and modulate the amount and types of eicosanoids (69,70). The n-3 PUFA might improve conditions of inflammation and immunity by eicosanoid-independent mechanisms. The n-3 PUFA can down-regulate the T-helper 1-type response, which is associated with chronic inflammatory disease. Components of both acquired and natural immunity, including the production of key inflammatory cytokines, can be influenced by n-3 PUFA. The n-3 PUFA decreased cytokine-induced adhesion molecule expression through mediated mechanisms, reducing inflammatory leucocyte-endothelium interactions and modifying lipid mediator synthesis, thus affecting the transendothelial migration of leucocytes and leucocyte trafficking in general. The n-3 fatty acids influence inflammatory cell activation processes from signal transduction to protein expression even involving effects at the genomic level.

Kelley *et al.* (71) studied the effect of a diet rich in flaxseed oil (6.3% energy from ALA) on immunocompetence in 10 volunteers in a study lasting 126 d. This study compared a moderate fat diet rich in LA with a moderate fat diet rich in ALA. Subjects consumed a baseline diet (23.4% energy from fat, P/S = 0.89, LA/ALA = 66) or the flax oil diet (28.8% energy from fat, P/S = 1.5, LA/ALA = 0.7) for 56 d and then changed to the other diet. On the flax oil diet it was found that the proliferation of

peripheral blood mononuclear cells was suppressed when they were cultured with phytohemagglutinin-P and concanavalin A; the flax oil diet also suppressed the delayed hypersensitivity response to seven recall antigens (71). There were no changes in the concentration of immunoglobulins in the serum or the number of helper cells, suppressor cells, and total T and B cells in the peripheral blood between the control and flax oil diet.

Summary

Results from prospective/epidemiological and dietary intervention studies indicate that ALA reduces cardiac mortality. The beneficial effects of ALA may operate via various mechanisms. ALA has been reported to prevent secondary CHD, reduce platelet aggregability, and decrease TXA₂ production. ALA has also been reported to have anti-inflammatory and anti-immunity activities through influence on eicosanoid-dependent and eicosanoid-independent mechanisms. Unlike LA, ALA from plant sources inconsistently decreases plasma/serum total and LDL cholesterol levels. Animal studies suggest that ALA can prevent cardiac arrhythmia and ventricular fibrillation, and it is as effective as EPA and DHA in the prevention of ventricular fibrillation. Whether the effects reported here are due to ALA itself or to longer chain n-3 PUFA formed from ALA is not known. Because most studies with ALA feeding show very little formation or accumulation of EPA in plasma lipids and almost no accumulation of DHA, this suggests that the reported benefits are from ALA itself.

References

1. Willett, W.C., Diet and Health: What Should We Eat? *Science* 264:532-537 (1994).
2. Kromhout, D., A. Menotti, B. Bloemberg, C. Aravanis, H. Blackburn, R. Buzina, A.S. Dontas, F. Fidanza, S. Giaipao, A. Jansen, M. Karvonen, M. Katan, A. Nissinen, S. Nedeljkovic, J. Pekkanen, M. Pekkarinen, S. Punsar, L. Rasanen, B. Simic, and H. Toshima, Dietary Saturated and *trans* Fatty Acids and Cholesterol and 25-Year Mortality from Coronary Heart Disease—The Seven Countries Study, *Prev. Med.* 24:308-315 (1995).
3. Key, T.J., M. Thorogood, P.N. Appleby, and M.L. Burr, Dietary Habits and Mortality in 11,000 Vegetarians and Health Conscious People: Results of a 17 Year Follow-Up, *Brit. Med. J.* 313:775-779 (1996).
4. Hegsted, D.M., M.A. Ausman, J.A. Jonson, and G.E. Dallal, Dietary Fat and Blood Lipids: An Evaluation of the Experimental Data, *Am. J. Clin. Nutr.* 57:875-883 (1993).
5. Bucher, H.C., P. Hengstler, C. Schindler, and G. Meier, n-3 Polyunsaturated Fatty Acids in Coronary Heart Disease: A Meta-Analysis of Randomized Controlled Trials, *Am. J. Med.* 112:298-304 (2002).
6. Koch, T., T. Krumm, V. Jung, J. Engelbert, and W. Boland, Differential Induction of Plant Volatile Biosynthesis in the Lima Bean by Early and Late Intermediates of the Octadecanoid-Signalling Pathway, *Plant Physiol.* 121:153-162 (1999).
7. Gerster, H., Can Adults Adequately Convert α -Linolenic Acid to Eicosapentaenoic Acid and Docosahexaenoic Acid? *Internat. J. Vit. Nutr. Res.* 68:159-173 (1998).

8. Li, D., A. Sinclair, A. Wilson, S. Nakkote, F. Kelly, L. Abedin, N. Mann, and A. Turner, Effect of Dietary Alpha-Linolenic Acid on Thrombotic Risk Factors in Vegetarian Men, *Am. J. Clin. Nutr.* 69:872–882 (1999).
9. Mantzioris, E., M.J. James, R.A. Gibson, and L.G. Cleland, Dietary Substitution with an α -Linolenic Acid-Rich Vegetable Oil Increases Eicosapentaenoic Acid Concentrations in Tissues, *Am. J. Clin. Nutr.* 59:1304–1309 (1994).
10. Emken, E.A., R.O. Adlof, and G.M. Gulley, Dietary Linoleic Acid Influences the Desaturation and Acylation of Deuterium-Labelled Linoleic and Alpha-Linolenic Acid in Young Adult Males, *Biochim. Biophys. Acta* 1213:277–288 (1994).
11. Pawlosky, R.J., J.R. Hibbeln, J.A. Novotny, and N. Salem, Jr., Physiological Compartmental Analysis of α -Linolenic Acid Metabolism in Adult Humans, *J. Lipid Res.* 42:1257–1265 (2001)
12. Ascherio, A., E.B. Rimm, E.L. Giovannucci, D. Spiegelman, M. Stampfer, and W.C. Willett, Dietary Fat and Risk of Coronary Heart Disease in Men: Cohort Follow-Up Study in the United States, *Brit. Med. J.* 313:84–90 (1996).
13. Hu, F.B., M.J. Stampfer, J.E. Manson, E.B. Rimm, A. Wolk, G.A. Colditz, C.H. Hennekens, and W.C. Willett, Dietary Intake of Alpha-Linolenic Acid and Risk of Fatal Ischemic Heart Disease Among Women, *Am. J. Clin. Nutr.* 69:890–897 (1999).
14. Djousse, L., J.S. Pankow, J.H. Eckfeldt, A.R. Folsom, P.N. Hopkins, M.A. Province, Y. Hong, and R.C. Ellison, Relation Between Dietary Linolenic Acid and Coronary Artery Disease in the National Heart, Lung, and Blood Institute Family Heart Study, *Am. J. Clin. Nutr.* 74:612–619 (2001).
15. de Lorgeril, M., S. Renaud, N. Mamelle, P. Salen, J.L. Martin, I. Monjaud, J. Guidollet, P. Touboul, and J. Delaye, Mediterranean Alpha-Linolenic Acid-Rich Diet in Secondary Prevention of Coronary Heart Disease, *Lancet* 343:1454–1459 (1994).
16. Keys, A., Wine, Garlic, and CHD in Seven Countries, *Lancet* 1:145–146 (1980).
17. Sandker, G.W., D. Kromhout, C. Aravanis, B.P. Bloemberg, R.P. Mensink, N. Karalias, and M.B. Katan, Serum Cholesteryl Ester Fatty Acids and Their Relation with Serum Lipids in Elderly Men in Crete and The Netherlands, *Eur. J. Clin. Nutr.* 47:201–208 (1993).
18. de Lorgeril, M., P. Salen, J.L. Martin, I. Monjaud, J. Delaye, and N. Mamelle, Mediterranean Diet, Traditional Risk Factors, and the Rate of Cardiovascular Complications after Myocardial Infarction: Final Report of the Lyon Diet Heart Study, *Circulation* 99:779–785 (1999).
19. Singh, R.B., M.A. Niaz, J.P. Sharma, R. Kumar, V. Rastogi, and M. Moshiri, Randomized, Double-Blind, Placebo-Controlled Trial of Fish Oil and Mustard Oil in Patients with Suspected Acute Myocardial Infarction: The Indian Experiment of Infarct Survival-4, *Cardiovasc. Drugs Ther.* 11:485–491 (1997).
20. Dolecek, T.A., Epidemiological Evidence of Relationships Between Dietary Polyunsaturated Fatty Acids and Mortality in the Multiple Risk Factor Intervention Trial, *Proc. Soc. Exp. Biol. Med.* 200:177–182 (1992).
21. Morris, M.C., F. Sack, and B. Rosner, Does Fish Oil Lower Blood Pressure? A Meta-Analysis of Controlled Trials, *Circulation* 88:523–533 (1993).
22. Key, T.J., G.E. Fraser, M. Thorogood, P.N. Appleby, V. Beral, G. Reeves, M.L. Burr, J. Chang-Claude, R. Frentzel-Beyme, J.W. Kuzma, J. Mann, and K. McPherson, Mortality in Vegetarians and Non-Vegetarians: A Collaborative Analysis of 8300 Deaths Among 76,000 Men and Women in Five Prospective Studies, *Public Health Nutr.* 1:33–41 (1998).

23. Rouse, I.L., L.J. Beilin, B.K. Armstrong, and R. Vandongen, Blood-Pressure-Lowering Effect of a Vegetarian Diet: Controlled Trial in Normotensive Subjects, *Lancet* 1:5–10 (1983).
24. Beilin, L.J., I.L. Rouse, B.K. Armstrong, B.M. Margetts, and R. Vandongen, Vegetarian Diet and Blood Pressure Levels: Incidental or Causal Association? *Am. J. Clin. Nutr.* 48:806–810 (1988).
25. Berry, E.M., and J. Hirsch, Does Dietary Linolenic Acid Influence Blood Pressure? *Am. J. Clin. Nutr.* 44:336–340 (1986).
26. Bemelmans, W.J., F.A. Muskiet, E.J. Feskens, J.H. de Vries, J. Broer, J.F. May, and B.M. Jong, Associations of Alpha-Linolenic Acid and Linoleic Acid with Risk Factors for Coronary Heart Disease, *Eur. J. Clin. Nutr.* 54:865–871 (2000).
27. Weisinger, H.S., J.A. Armitage, A.J. Sinclair, P.B. Vingrys, and R.S. Weisinger, Perinatal Omega-3 Fatty Acid Deficiency Affects Blood Pressure Later in Life, *Nature Med.* 7:258–259 (2001).
28. Keys, A., J.T. Anderson, and F. Grande, Serum Cholesterol Response to Changes in the Diet. IV. Particular Saturated Fatty Acids in Diet, *Metabolism* 14:776–787 (1965).
29. Hegsted, D.M., R.B. McGandy, M.L. Myers, and F.L. Stare, Quantitative Effects of Dietary Fat on Serum Cholesterol in Man, *Am. J. Clin. Nutr.* 17:281–295 (1965).
30. McDonald, B.E., J.M. Gerrard, V.M. Bruce, and E.J. Corner, Comparison of the Effect of Canola Oil and Sunflower Oil on Plasma Lipids and Lipoproteins and on *in vivo* Thromboxane A₂ and Prostacyclin Production in Healthy Young Men, *Am. J. Clin. Nutr.* 50:1382–1388 (1989).
31. Chan, J.K., V.M. Bruce, and B.E. McDonald, Dietary Alpha-Linolenic Acid is as Effective as Oleic Acid and Linoleic Acid in Lowering Blood Cholesterol in Normolipidemic Men, *Am. J. Clin. Nutr.* 53:1230–1234 (1991).
32. Munoz, S., M. Merlos, D. Zambon, C. Rodriguez, J. Sabate, E. Ros, and J.C. Laguna, Walnut-Enriched Diet Increases the Association of LDL from Hypercholesterolemic Men with Human He G2 Cells, *J. Lipid Res.* 42:2069–2076 (2001).
33. Kelley, D.S., G.J. Nelson, J.E. Love, L.B. Branch, P.C. Taylor, P.C. Schmidt, B.E. Mackey, and J.M. Iacono, Dietary Alpha-Linolenic Acid Alters Tissue Fatty Acid Composition, but Not Blood Lipids, Lipoproteins or Coagulation Status in Humans, *Lipids* 28:533–537 (1993).
34. Svaneborg, N., J.M. Moller, E.B. Schmidt, K. Varming, H.H. Lervang, and J. Dyerberg, The Acute Effects of a Single Very High Dose of n-3 Fatty Acids on Plasma Lipids and Lipoproteins in Healthy Subjects, *Lipids* 29:145–147 (1994).
35. Zampelas, A., A.S. Peel, B.J. Gould, J. Wright, and C.M. Williams, Polyunsaturated Fatty Acids of the n-6 and n-3 Series: Effects on Postprandial Lipid and Apolipoprotein Levels in Healthy Men, *Eur. J. Clin. Nutr.* 48:842–848 (1994).
36. Bemelmans, W.J., J. Broer, E.J. Feskens, A.J. Smit, F.A. Muskiet, J.D. Lefrandt, V.J. Bom, J.F. May, and B. Meyboom de-Jong, Effect of an Increased Intake of Alpha-Linolenic Acid and Group Nutritional Education on Cardiovascular Risk Factors: The Mediterranean Alpha-linolenic Enriched Groningen Dietary Intervention (MARGARIN) Study, *Am. J. Clin. Nutr.* 75:221–227 (2002).
37. Cunnane, S.C., S. Ganguli, C.R. Menard, A.C. Liede, M.J. Hamadeh, Z-Y. Chen, T.M.S. Wolever, and D.J.A. Jenkins, High α -Linolenic Acid Flaxseed (*Linum usitatissimum*): Some Nutritional Properties in Humans, *Br. J. Nutr.* 69: 443–453 (1993).
38. Cunnane, S.C., M.J. Hamadeh, A.C. Liede, L.U. Thompson, T.M.S., Wolever, and D.J.A. Jenkins, Nutritional Attributes of Traditional Flaxseed in Healthy Young Adults, *Am. J. Clin. Nutr.* 61:62–68 (1995).

39. Vermunt, S.H., B. Beaufrere, R.A. Riemersma, J.L. Sébédio, J.M. Chardigny, and R.P. Mensink, Dietary Trans Alpha-Linolenic Acid from Deodorised Rapeseed Oil and Plasma Lipids and Lipoproteins in Healthy Men: The TransLinE Study, *Br. J. Nutr.* 85:387–392 (2001).
40. Hamberg, M., J. Svensson, and B. Samuelsson, Thromboxanes: A New Group of Biologically Active Compounds Derived from Prostaglandin Endoperoxides, *Proc. Natl. Acad. Sci.* 72:2994–2998 (1975).
41. Moncada, S., and J.R. Vane, Arachidonic Acid Metabolites and the Interactions Between Platelets and Blood Vessel Walls, *N. Engl. J. Med.* 300:1142–1148 (1979).
42. Raz, A., M.S. Minkes, and P. Needleman, Endoperoxides and Thromboxanes: Structural Determinants for Platelet Aggregation and Vasoconstriction, *Biochim. Biophys. Acta* 488:305–311 (1977).
43. Leaf, A., and P.C. Weber, Cardiovascular Effects of n-3 Fatty Acids, *N. Engl. J. Med.* 318:549–557 (1988).
44. Dyerberg, J., Linolenate Derived Polyunsaturated Fatty Acids and Prevention of Atherosclerosis, *Nutr. Rev.* 44:125–134 (1986).
45. Kinsella, J.E., B. Lokesh, and R.A. Stone, Dietary n-3 Polyunsaturated Fatty Acids and Amelioration of Cardiovascular Disease: Possible Mechanisms, *Am. J. Clin. Nutr.* 52:1–28 (1990).
46. Okuyama, H., T. Kobayashi, and S. Watanabe, Dietary Fatty Acids—the n-6/n-3 Balance and Chronic Elderly Diseases: Excess Linoleic Acid and Relative n-3 Deficiency Syndrome Seen in Japan, *Prog. Lipid Res.* 35:409–457 (1996).
47. Abeywardena, M.Y., P.L. McLennan, and J.S. Charnock, Differential Effects of Dietary Fish Oil on Myocardial Prostaglandin I₂ and Thromboxane A₂ Production, *Am. Physiol.* 260:379–385 (1991).
48. Henry, M.M., J.N. Moore, E.B. Feldman, J.K. Fischer, and B. Russell, Effect of Dietary Alpha Linolenic Acid on Equine Monocyte Procoagulant Activity and Eicosanoid Synthesis, *Circ. Shock* 32:173–188 (1990).
49. Ikeda, I., H. Yoshida, M. Tomooka, A. Yosef, K. Imaizumi, H. Tsuji, and A. Seto, Effects of Long-Term Feeding of Marine Oils with Different Positional Distribution of Eicosapentaenoic and Docosahexaenoic Acids on Lipid Metabolism, Eicosanoids Production, and Platelet Aggregation in Hypercholesterolemic Rats, *Lipids* 33:897–904 (1998).
50. Allman, M.A., M.M. Pena, and D. Peng, Supplementation with Flaxseed Oil Versus Sunflower Seed Oil in Healthy Young Men Consuming a Low Fat Diet: Effects on Platelet Composition and Function, *Eur. J. Clin. Nutr.* 49:169–178 (1995).
51. Freese, R., M. Mutanen, L.M. Valsta, and I. Salminen, Comparison of the Effects of Two Diets Rich in Monounsaturated Fatty Acids Differing in Their Linoleic/ α -Linolenic Acid Ratio on Platelet Aggregation, *Thromb. Haemost.* 71:73–77 (1994).
52. Chan, J.K., B.E. McDonald, J.M. Gerrard, V.M. Bruce, B.J. Weaver, and B.J. Holub, Effect of Dietary α -Linolenic Acid and Its Ratio to Linoleic Acid on Platelet and Plasma Fatty Acids and Thrombogenesis, *Lipids* 28:811–817 (1993).
53. Ferretti, A., G.J. Nelson, P.C. Schmidt, G. Bartolini, D.S. Kelley, and V.P. Flanagan, Dietary Docosahexaenoic Acid Reduces the Thromboxane/Prostacyclin Synthetic Ratio in Humans, *J. Nutr. Biochem.* 9:88–92 (1998).
54. Schacky, C.V., W. Siess, S. Fischer, and P.C. Weber, A Comparative Study of Eicosapentaenoic Acid Metabolism by Human Platelets *in vivo* and *in vitro*, *J. Lipid Res.* 26:457–464 (1985).

55. Ferretti, A., and V.P. Flanagan, Antithromboxane Activity of Dietary Alpha-Linolenic Acid: A Pilot Study, *Prostaglandins Leukot. Essent. Fatty Acids* 54:451-455 (1996).
56. Mantzioris, E., L.G. Cleland, R.A. Gibson, M.A. Neumann, M. Demasi, and M.J. James, Biochemical Effects of a Diet Containing Foods Enriched with n-3 Fatty Acids, *Am. J. Clin. Nutr.* 72:42-48 (2000).
57. McVeigh, G.E., G.M. Brennan, J.N. Cohn, S.M. Finkelstein, R.J. Hayes, and G.D. Johnston, Fish Oil Improves Arterial Compliance in Non-Insulin-Dependent Diabetes Mellitus, *Arterioscler. Thromb.* 14:1425-1429 (1994).
58. Chin, J.F.P., A.P. Gust, P.J. Nestel, and A.M. Dart, Marine Oils Dose-Dependently Inhibit Vasoconstriction of Forearm Resistance Vessels in Humans, *Hypertension* 21:22-28 (1993).
59. Nestel, P.J., S.E. Pomeroy, T. Sasahara, T. Yamashita, Y.L. Liang, A.M. Dart, G.L. Jennings, M. Abbey, and J.D. Cameron, Arterial Compliance in Obese Subjects Is Improved with Dietary Plant n-3 Fatty Acid from Flaxseed Oil Despite Increased LDL Oxidizability, *Arterioscler. Thromb. Vasc. Biol.* 17:1163-1670 (1997).
60. McLennan, P.L., M.Y. Abeywardena, and J.S. Charnock, Dietary Fish Oil Prevents Ventricular Fibrillation Following Coronary Artery Occlusion and Reperfusion, *Am. Heart J.* 116:709-717 (1988).
61. McLennan, P.L., T.M. Bridle, M.Y. Abeywardena, and J.S. Charnock, Comparative Efficacy of n-3 and n-6 Polyunsaturated Fatty Acids in Modulating Ventricular Fibrillation Threshold in Marmoset Monkeys, *Am. J. Clin. Nutr.* 58:666-669 (1993).
62. Billman, G.E., H. Hallaq, and A. Leaf, Prevention of Ischemia-Induced Ventricular Fibrillation by Omega 3 Fatty Acids, *Proc. Natl. Acad. Sci. USA* 91:4427-4430 (1994).
63. Hallaq, H., A. Sellmayer, T.W. Smith, and A. Leaf, Protective Effect of Eicosapentaenoic Acid on Ouabain Toxicity in Neonatal Rat Cardiac Myocytes, *Proc. Natl. Acad. Sci. USA* 87:7834-7838 (1990).
64. McLennan, P.L., and J.A. Dallimore, Dietary Canola Oil Modifies Myocardial Fatty Acids and Inhibits Cardiac Arrhythmias in Rats, *J. Nutr.* 125:1003-1009 (1995).
65. Kang, J.X., and A. Leaf, Prevention of Fatal Cardiac Arrhythmias by Polyunsaturated Fatty Acids, *Am. J. Clin. Nutr.* 71:202S-207S (2000).
66. Jeffery, N.M., P. Sanderson, E.J. Sherrington, E.A. Newsholme, and P.C. Calder, The Ratio of n-6 to n-3 Polyunsaturated Fatty Acids in the Rat Diet Alters Serum Lipid Levels and Lymphocyte Functions, *Lipids* 31:737-745 (1996).
67. Inui, K., Y. Fukuta, A. Ikeda, H. Kameda, Y. Kokuba, and M. Sato, The Effect of Alpha-Linolenic Acid-Rich Emulsion on Fatty Acid Metabolism and Leukotriene Generation of the Colon in a Rat Model with Inflammatory Bowel Disease, *Ann. Nutr. Metab.* 40:175-182 (1996).
68. Benquet, C., K. Krzystyniak, R. Savard, F. Guertin, D. Oth, and M. Fournier, Modulation of Exercise-Induced Immunosuppression by Dietary Polyunsaturated Fatty Acids in Mice, *J. Toxicol. Environ. Health* 43:225-237 (1994).
69. Calder, P.C., P. Yaqoob, F. Thies, F.A. Wallace, and E.A. Miles, Fatty Acids and Lymphocyte Functions, *Br. J. Nutr.* 87:S31-48 (2002).
70. Grimm, H., K. Mayer, P. Mayser, and E. Eigenbrodt, Regulatory Potential of n-3 Fatty Acids in Immunological and Inflammatory Processes, *Br. J. Nutr.* 87:S59-67 (2002).
71. Kelley, D.S., L.B. Branch, J.E. Love, P.C. Taylor, Y.M. Rivera, and J.M. Iacono, Dietary Alpha-Linolenic Acid and Immunocompetence in Humans, *Am. J. Clin. Nutr.* 53:40-46 (1991).