Dietary modulations in the prevention of coronary artery disease: a special emphasis on vitamins and fish oil

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Coronary artery disease (CAD) is the most common cause of death in most countries. The etiology of CAD is complex, and many risk factors have been described. Traditional risk factors for CAD can only explain about half of the clinical events; therefore, interest in nutritional factors, such as vitamins and fish oil, has been maintained. Curr Opin Cardiol 2002, 17:559–567 © 2002 Lippincott Williams & Wilkins, Inc.

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Supported by grants from the Swedish Medical Research Council and funds from the Department of Veterans Affairs.

Current Opinion in Cardiology 2002, 17:559–567

Abbreviations

cNOS	constitutive NO synthase
CAD	coronary artery disease
HDL	high-density lipoprotein
LDL	low-density lipoprotein
NO	nitric oxide
PUFA	polyunsaturated fatty acids
SOD	superoxide dismutase

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Coronary artery disease (CAD) is the most common cause of death in most countries. The etiology of CAD is complex, and many risk factors have been described. In one review, 246 potential risk factors were mentioned [1]. Among them, 46 were related to diet. Traditional risk factors for CAD can only explain about half of the clinical events; therefore, interest in nutritional factors, such as vitamins and fish oil, has been maintained.

Vitamins and coronary artery disease

Substantial evidence suggests that low folic acid intake can increase the risk of CAD. In many studies, high blood homocysteine levels have been associated with higher risks of CAD [2]. Homocysteine is a sulfhydryl-containing amino acid derived from the demethylation of dietary methionine. Homocysteine can be transformed back to methionine; in this process, folic acid and vitamin B12 are required. Alternatively, homocysteine can be converted to cysteine; in this process, vitamin B6 is required.

A meta-analysis of 35 studies showed a correlation between total homocysteine in plasma in 4000 patients with CAD and in 22,000 control subjects [3]. Total homocysteine was 26% higher in the patient group. In another study [4], the correlation between total homocysteine in plasma and mortality in 600 patients with CAD determined by coronary angiography was studied and showed a strong positive correlation. Increased plasma levels of homocysteine can be normalized by supplementation of 0.2–0.4 mg of folic acid daily. Addition of vitamins B6 and B12 results in an even better effect. However, if normalization of plasma homocysteine results in a decrease in morbidity and mortality in patients with CAD is not yet known. Higher folic acid intake has been found to be associated with lower risk of CAD [5]. The average daily American intake of folic acid with food is about 0.2 mg, and the food-fortification program in the United States has added about 0.1 mg per day [6]. Thus, most people seem to consume less than 0.4 mg daily unless they use supplements containing folic acid. Alcohol intake interferes with folate absorption.

Intake of vitamin B6 below the recommended daily allowance (RDA) of 2 mg is associated with an increased risk of CAD, but this association may depend on folic acid intake [5]. Persons who decrease their consumption of red meat without increasing the consumption of legumes may have low vitamin B6 levels [6]. Elderly persons with

DOI: 10.1097/01.HCO.0000024187.40973.5B

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low gastric acidity have low blood levels of vitamin B12, which is associated with higher homocysteine levels [7] and may increase the risk of vascular disease [8].

A recent study in monkeys [9] showed that supplementation with B vitamins (folic acid, vitamin B6, and vitamin B12) prevented hyperhomocystinemia, but this was not sufficient to prevent development of endothelial dysfunction or formation of atherosclerotic lesions, implying that interventions to lower plasma homocysteine may have limited clinical benefit.

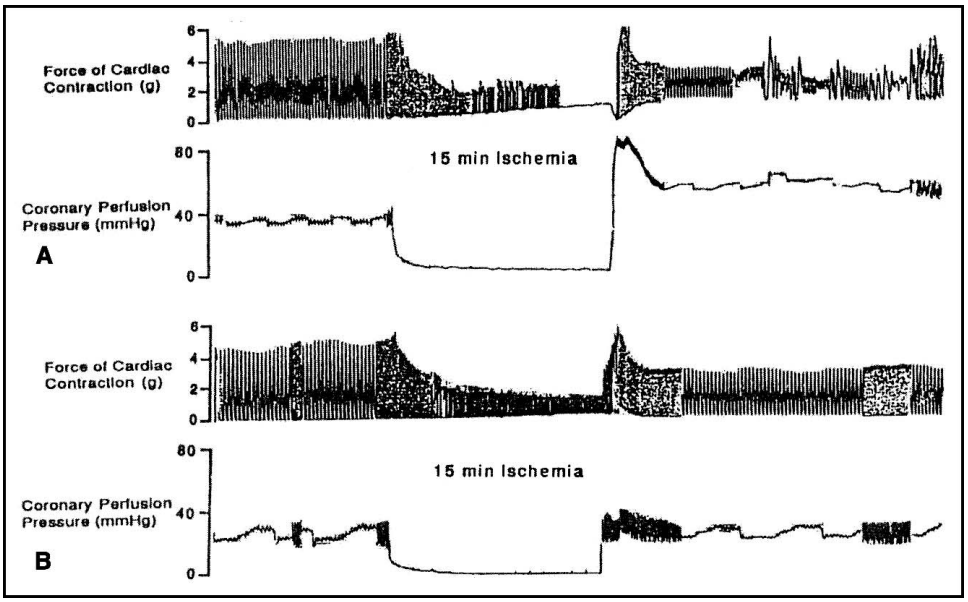
The most interesting vitamin and antioxidant in prevention of CAD may be vitamin E. Epidemiologic studies have shown an inverse relation between the consumption of vitamin E and morbidity and mortality in persons with CAD [10]. Vitamin E is the name of a group of tocopherols and tocotrienols. Four types of each, named α-, β-, δ-, and γ-tocopherol and -tocotrienol, are found in nature. Until recently, all commercial vitamin E supplements contained only α-tocopherol. The reason for this is that since the discovery of vitamin E in 1922, α-tocopherol was regarded the most potent form of vitamin E. This was based on studies showing α-tocopherol to be the most powerful form of vitamin E relative to antifertility effect in rats. In this model, α-tocopherol was found to be 10 times more potent than γ-tocopherol, and since then, α-tocopherol's biologic activity has been regarded to be 10 times higher than that of γ-tocopherol.

Vitamin E protects the polyunsaturated fatty acids in the cell membranes from oxidation by free radicals. It blocks the oxidative modification of low-density lipoprotein (LDL) cholesterol. Generation of free radicals in the endothelium is an important early step in the development of CAD. This may be a common mechanism underlying many of the risk factors for CAD. When the antioxidant capacity has been reduced, LDL particles trapped in the vascular wall become oxidized. This results in breakdown of nitric oxide (NO) and activation of the redox-sensitive transcription factor NF-B, which leads to generation of adhesion molecules for monocytes on the endothelial surface and movement of monocytes and macrophages into the vascular intima. The reaction between NO and the free oxygen radical O_2^- leads to the formation of the toxic substance peroxynitrite.

As a consequence, the amount of antioxidants and scavengers, such as vitamin E, in the vascular wall may be of major importance, and intake of tocopherols with food or as supplements could play an important role in the prevention of CAD. This has resulted in the extensive use of vitamin E and other antioxidants during the past decade. In Sweden, a survey was performed on all scientific studies published in this field from 1989 to1996. The conclusion was that antioxidants, such as vitamin E, taken with food, can prevent CAD, but data from existing clinical studies of supplements show no evidence that intake of antioxidants as supplements have a preventive effect. However, it is the authors' opinion that use of the wrong type of tocopherols may be the basis for failure of these studies to show a beneficial effect of vitamin E [11].

Our interest in the tocopherols originated many years ago during studies of the stability of fish oils.

Figure 1. Effect of supplementation of stable fish oil (Eskimo-3) in rats on cardiac dynamics in hearts exposed to 15 minutes of ischemia and 10 minutes of reperfusion



Ischemia–reperfusion is associated with a marked decrease in force of cardiac contraction, an increase in coronary perfusion pressure, and appearance of ventricular arrhythmias. These changes were less marked in the hearts from fish oil-fed rats **(B)** than in hearts from control rats **(A)**.

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We noticed that α-tocopherol sometimes could be a weak antioxidant and could even be a pro-oxidant. When we combined different tocopherols, the antioxidant effect sometimes became much stronger. We then performed studies in human erythrocytes, in which we induced lipid peroxidation by addition of hydrogen peroxide. We introduced different mixtures of α-, β-, δ-, and γ-tocopherol into the lipid layers of the cell membrane before exposure to hydrogen peroxide. We observed that a mixture of γ-, δ-, and α-tocopherols in 5:2:1 ratio to have a much better antioxidant effect than α-tocopherol alone. This mixture is similar to that found in nature. The concentration of tocopherols was also important, and high concentrations had no antioxidant effect. Interestingly, the total uptake of tocopherols in the cell membrane was much higher after incubation of cells with the mixed tocopherol preparation compared with the same concentration of α-tocopherol [12] (Fig. 1). Lipid peroxidation induced a decrease of polyunsaturated fatty acids in the cell membrane, and this decrease was inhibited by the mixed tocopherol preparation.

We have examined the effects of α-tocopherol alone versus γ-tocopherol rich preparation of mixed tocopherols in several experimental and clinical investigations. We found that the mixed tocopherol preparation had much more favorable effects than α-tocopherol alone on constitutive NO synthase (cNOS) and superoxide dismutase (SOD) activity and protein expression in rats and humans [11,13]. The mixed tocopherol preparation was more efficient than α-tocopherol alone in decreasing platelet aggregation and inhibiting thrombus formation [11]. Also, the mixed tocopherol preparation was more efficient in decreasing arterial superoxide anion generation and lipid peroxidation [11]. LDL oxidation decreased more and endogenous SOD activity in plasma and arterial tissue increased more after mixed tocopherol preparation, as did MnSOD and Cu/Zn SOD protein expression in arterial tissues. Only the mixed preparation increased cNOS protein expression [14].

We incubated human platelets with α-, δ-, and γ-tocopherol or a combination of the three (1:2:5 ratio). All three forms of tocopherols had similar effects on human platelet aggregation. The three forms together appeared to attenuate platelet aggregation, at least in part, via a decrease in free radical generation and an increase in platelet cNOS activity. Interestingly, the combination of tocopherols had a synergistic platelet inhibitory effect [15].

We have further investigated the mechanism of the effect of γ-tocopherol in experiments where we studied the role of this agent in oxidized LDL-induced NF-κB activation and apoptosis in human coronary artery endothelial cells. Treatment of the cells with γ-tocopherol attenuated the activation of NF-κB and also reduced oxidized LDL-induced apoptosis [16]. The attenuation of oxidized LDL-induced activation of NF-κB and apoptosis shown in our studies provides a unique mechanism for the beneficial effect of the γ-tocopherol–rich mixed preparation in persons with CAD.

In other experiments [17], we compared the effect of the mixed tocopherol preparation with that of α-tocopherol alone on SOD activity and iNOS expression in cultured myocytes exposed to hypoxia and reoxygenation (H-R). H-R resulted in myocyte injury (determined by LDH release), a decrease in SOD activity, and upregulation of iNOS expression and activity. The mixed tocopherol preparation was much superior to α-tocopherol alone in terms of myocyte protection from the adverse effects of H-R.

When we compared the effect of the mixed tocopherol preparation with α-tocopherol after 8-weeks supplementation in humans, the mixed tocopherol preparation, but not α-tocopherol alone, inhibited ADP-induced platelet aggregation. Also, the release of NO from and cNOS activation in platelets were much higher after the mixed tocopherol preparation [13]. The mixed tocopherol preparation increased SOD protein levels in the platelets, suggesting that these tocopherols upregulate intrinsic SOD expression at a protein level, a process which may also be important in the effect of the mixed tocopherol preparation on platelet aggregation. We also observed an increase in cNOS activation in the leukocytes that was pronounced after supplementation with mixed tocopherols (*vs* α-tocopherol alone). NO is a key determinant in the development of atherosclerosis, and inhibition of NO synthesis increases leukocyte–endothelial interaction [18].

The results of these investigations suggest that the mixed tocopherol preparation has stronger antioxidant effect than α-tocopherol alone and that α-tocopherol alone may not be an ideal antioxidant. Lack of efficacy of commercial tocopherol preparations in clinical trials may reflect absence of γ- and δ-tocopherols in these preparations. Results from recent clinical studies have confirmed our hypothesis that α-tocopherol alone has limited clinical benefit.

In observational and randomized trials, α-tocopherol alone has been used, and the results have been negative. In some observational studies in subjects without known CAD, supplementation with vitamin E has been associated with a 20 to 40% decrease in the risk of CAD [19]. In one study, intake of vitamin in food but not as supplements, was associated with a decreased risk of CAD [20]. Several large, controlled randomized studies showed no effect on cardiovascular events of 300 to 400 mg of α-tocopherol taken alone over 4 to 5 years [20–23].

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Supplementation of diet with α-tocopherol can decrease γ-tocopherol levels in the blood and tissues as shown by our group and others. This may be undesirable because γ-tocopherol has properties that are not shared by α-tocopherol. Further, it is the γ-tocopherol fraction, not the α-tocopherol, that is expressed in low amounts in the plasma in patients with CAD [24]. One study showed that the strongest explanation for the difference between mortality in CAD between Sweden and Lithuania is the plasma level of γ-tocopherol [25]. γ-Tocopherol is superior to α-tocopherol in removal of peroxynitrite [26]. Also, other electrophile toxic substances can form stable complexes with γ-tocopherol. For this reaction, a free nucleophil 5 position is necessary, which is found in γ-tocopherol but not in α-tocopherol. The amount of nitrotyrosine is a measure of the amount of toxic peroxynitrite. Several studies have shown high levels of nitrotyrosine in atherosclerotic vessels and myocardial infarcts.

When tocopherols repair oxidized chain-breaking antioxidants, the tocopherols themselves are turned into inactive radicals and have to be reactivated. Vitamin C can reactivate tocopherols. However, little evidence supports any benefit of vitamin C beyond the range of the typical diet in the United States or the current RDA of 90 mg for men and 75 mg for women (35 mg higher for smokers) [6]. Minimal effects may be expected from supplements because tissues become saturated at about these levels of intake [6]. Plasma ascorbic acid concentration was found to be inversely related to mortality from CAD [27]. It is possible, however, that the association is with another nutrient or nutrients contained in foods containing ascorbic acid, such as fruit and vegetables.

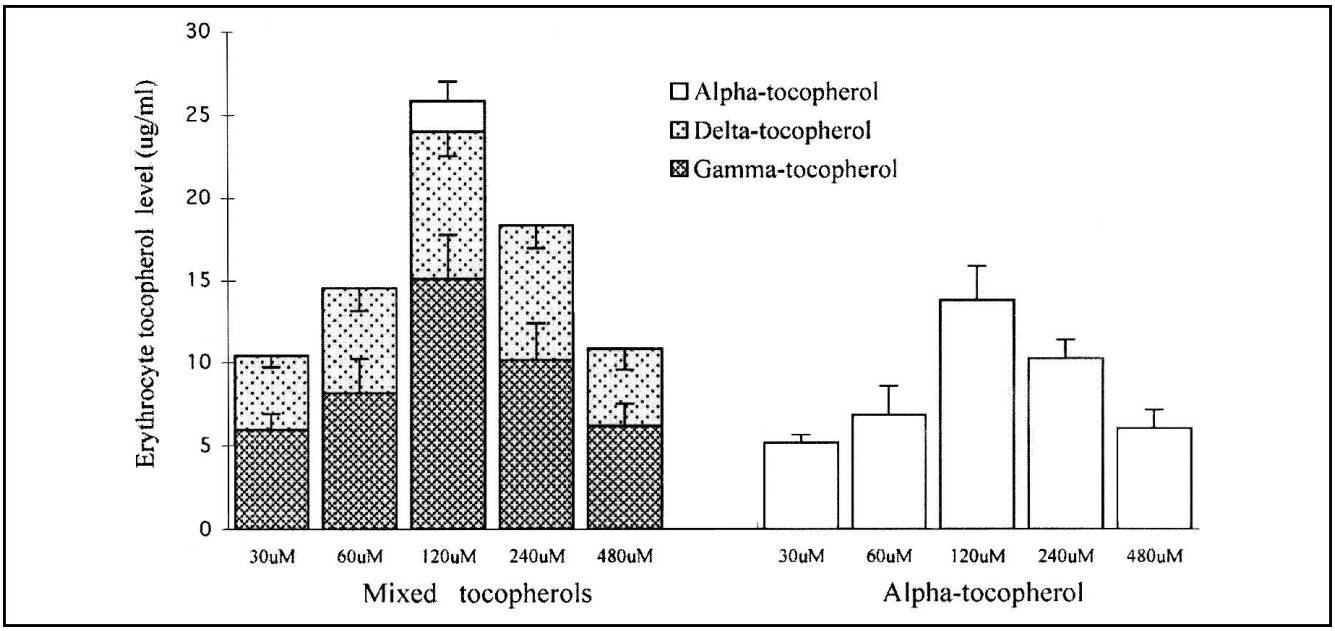
There are no data in support of any effect of supplementation above RDA of either vitamin A or vitamin D, 5000 IU and 400 IU, respectively, in prevention of CAD. On the contrary, intake of vitamin A in the range of twice the RDA may even be harmful and has been associated with an increased risk of osteoporosis and hip fracture [28]. Cod liver oil is an important source of vitamin A. People in Sweden and Norway, who have the highest frequency of osteoporosis and hip fracture worldwide, have had a high consumption of cod liver oil.

Thus, there is little evidence that intake of separate vitamins in high concentrations would be beneficial to those with CAD. However, as will be discussed, under certain circumstances, such as with intake of marine n-3 polyunsaturated fatty acids (PUFA), an additional supply of a mixture of antioxidants as found in fish is needed.

Fish oil and coronary artery disease

We became interested in fish oils containing n-3 fatty acids more than 20 years ago when two Danish physicians, Bang and Dyerberg, reported that the frequency of CAD among Greenland Eskimos was just one tenth of that among Scandinavians. This finding was surprising because the fat content of the Eskimo diet is high. The explanation was that the Eskimo diet contains a high amount of n-3 PUFA from fatty fish and fish-eating

Figure 2. Uptake of different tocopherols in human erythrocytes after incubation with a mixed tocopherol preparation (Cardi-E) containing α-, δ-, γ-tocopherol or α-tocopherol alone



The uptake of tocopherol was much higher after incubation with the mixed tocopherol preparation.

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mammals. We noticed the intake of fish containing n-3 PUFA had decreased by about 80% in Scandinavia during the past 80 years. A century ago, it was not uncommon for fish to be eaten 7 days a week, and salmon was often served 5 days a week in Sweden.

We observed in our studies that victims of sudden cardiac death had much lower levels of n-3 PUFA in their hearts than people dying from other causes [29]. From there, it was not a long step to try to prevent sudden death and CAD by increasing the amount of n-3 PUFA in the diet by supplementation with fish oil. A problem we faced at that time was that fish oil easily became rancid because of loss of antioxidants during purification of the fish oil from environmental poisons, such as mercury and pesticides. The original antioxidants in the fish had to be restored. After several years of research, this problem was solved, and stable fish oil was available for our studies. It was soon found that the stable fish oil did not only have a better taste but also had more beneficial effects.

We were able to show that supplementation with this fish oil in an experimental model reduced incidence and severity of anoxia-induced cardiac arrhythmias (Fig. 2) [30]. This seems to be the result of a direct effect of the fish oil on the heart. Arrhythmias can be induced by thromboxane and prevented by prostacyclin. The increased prostacyclin-to-thromboxane ratio after intake of fish oil [31] may thus be important in this effect. There is also a strong positive correlation between tissue levels of marine n-3 PUFAs and heart rate variability, which may contribute to these results [32]. Interestingly, in this study, patients with a high wine intake also had high heart rate variability, but further analysis showed that these patients also had a high fish intake. When corrected for this confounding variable, there was no correlation between wine consumption and heart rate variability.

Recently, several large clinical studies have confirmed the effect of fish oil on CAD. Fifteen large studies enrolling more than 60,000 subjects have shown a decreased mortality in CAD and in total mortality of about 30% after intake of fish oil, fatty fish, or n-3 PUFA [33]. In a randomized controlled trial on the effect of intake of fatty fish or natural fish oil, 2033 men who had recovered from myocardial infarction were studied for 2 years [34]. The fish–fish oil group showed a 29% decrease in 2-year all-cause mortality. In another study of more than 11,000 patients surviving a recent myocardial infarction [22], intake of 1 g daily of n-3 PUFA for 3.5 years resulted in a 20% decrease in total deaths, a 30% decrease in cardiovascular deaths, and a 45% decrease in sudden deaths.

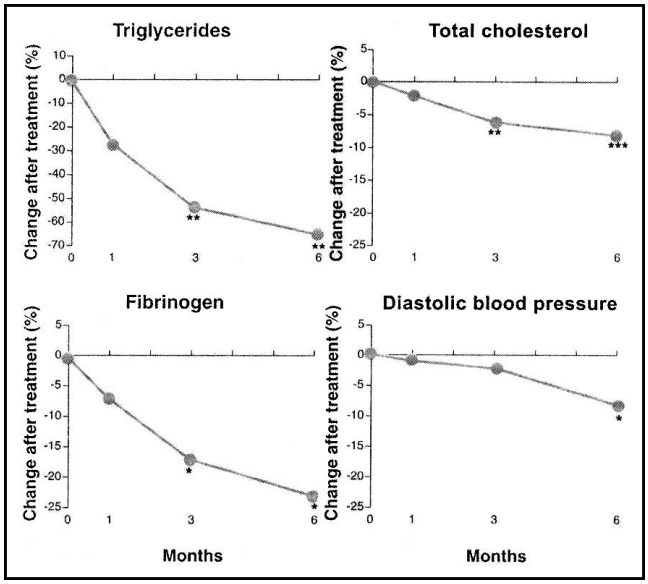
Interestingly, these patients already had conventional treatment, such as aspirin, ß-blockers, and angiotensin-converting enzyme inhibitors. Thus, there is no doubt that fish oil has beneficial effects in the outcome of patients with CAD. A major part of the effect seems to be the result of reduced incidence and severity of cardiac arrhythmias.

Recently, it was reported that n-3 marine PUFA in tissues were strongly associated with a decreased risk of sudden death among men without evidence of previous CAD [35]. Interestingly, there was no correlation between the n-3 PUFA α-linolenic acid and sudden death. We also found no increase in the blood levels of the marine n-3 PUFA, EPA, and DHA in humans supplemented with linseed oil, which may not be a good source of n-3 PUFA in humans.

Fish oil also has important effects on blood lipids. Hypertriglyceridemia has been identified as an independent risk factor for CAD and probably is an important factor in the development of atherosclerosis. Intake of fish oil usually results in a marked decrease in triglycerides. Different preparations have different effects. For example, intake of 15 mL of natural stable fish oil daily containing about 5 g of n-3 PUFA for 6 months resulted in a 64% decrease in triglycerides in one study [36] (Fig. 3), and intake of six capsules of highly concentrated, unstable fish oil daily, also containing about 5 g of n-3 PUFA for 6 months, resulted in only a 27% decrease in triglycerides [37].

High levels of plasma triglycerides are an especially important risk factor for CAD among women, and women

Figure 3. Effect of 6 months of dietary supplementation with stable fish oil on triglycerides, total cholesterol, fibrinogen in plasma, and diastolic blood pressure



Maximal effect was seen after 6 months.

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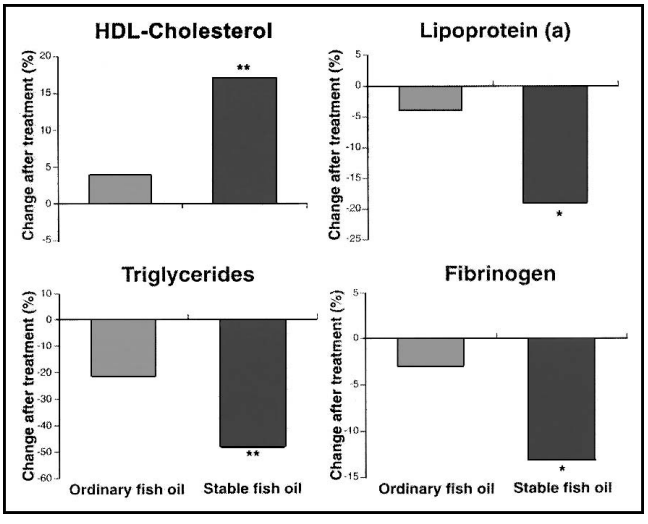
on estrogen replacement often have increased plasma triglyceride levels. Women taking oral contraceptives often use stable fish oil to decrease the risk of venous thromboembolism. Stable fish oil was found to have an antithrombotic effect [38], which may be caused by decreased platelet aggregation as a result of increase in NO and SOD [38].

When two natural fish oils, one stable and one unstable, were compared, superior effects were observed with the stable oil. Intake of the stable fish oil resulted in a more potent decrease in triglyceride levels [39] (Fig. 4). Stable fish oil was also more potent than unstable fish oil in decreasing LDL cholesterol [40], total cholesterol, and lipoprotein (a), and in increasing high-density lipoprotein (HDL) cholesterol [41] (Fig. 4). In addition to its effect on the plasma concentration of various cholesterol, fish oil was also shown to make the LDL cholesterol particle larger and less dense and thus less prone to induce atherosclerosis. Lipoprotein (a) is believed to be one of the most important risk factors for CAD. It is a lipoprotein with structural similarities to LDL cholesterol and plasminogen, an important protein in the fibrinolytic system. When lipoprotein (a) replaces plasminogen in the thrombus, the risk of developing myocardial infarction is increased. Few compounds other than fish oil have been shown to lower plasma lipoprotein (a). Nicotinic acid (vitamin B3) is such a substance, but it has certain side effects in contrast to fish oil, which has no serious side effects.

Figure 4. Effect of 3 to 4 weeks of dietary supplementation with ordinary and stable fish oil on high-density lipoprotein cholesterol, lipoprotein (a), triglycerides, and fibrinogen in plasma



The two fish oils contained the same amount of fatty acids, the only difference being that the stable fish oil (Eskimo-3) contained a mixture of different natural antioxidants (Pufanox) to restore the antioxidant activity, whereas the ordinary fish oil contained an ordinary amount of vitamin E only. The stable fish oil had more potent effects than the ordinary fish oil.

A major mechanism of the effect of fish oil in atherosclerotic CAD may be its influence on the inflammatory process. Fish oil intake increases the fluidity of the cell membranes because of the structure of the fatty acids. Whereas saturated fatty acids have a straight structure, n-3 PUFAs, like EPA and DHA, are markedly curved because of their double bonds. Saturated straight fatty acids are closely packed together in the cell membrane, which becomes stiff, whereas curved fatty acids, such as EPA and DHA, cannot be packed so closely. They need more space and make the cell membrane more plastic and less stiff. This is important for enzyme function and function of receptors. Some membrane-bound receptors have been shown to be particularly sensitive to their fatty acid environments. Among these, adrenergic and insulin receptors are particularly sensitive to the fatty acid environment.

Intake of fish oil results in replacement of arachidonic acid by the n-3 PUFA in the phospholipid bilayer of the cell membranes. This in turn results in less production of arachidonic acid-derived eicosanoids, 2-series prostaglandins, and 4-series leukotrienes and an increased production of EPA-derived eicosanoids, 3-series prostaglandins, and 5-series leukotrienes. These latter compounds are often less biologically potent than the arachidonic acid-derived analog. This results in significant inhibitory effects on processes underlying inflammation, such as platelet aggregation, vasoconstriction, neutrophil function, and immunity. Fish oil alters the function of several mediators involved in the communication between cells (eicosanoids, cytokines, NO) and alters expression of leukocyte adhesion molecules. The production of cytokines and NO is partly regulated by eicosanoids. However, many of the effects of fish oil seem to be exerted in an eicosanoid-independent manner, *eg,* by expression of key genes.

Oxidizing free radicals provoke degradation of the double bonds of PUFA in cellular membranes, resulting in the formation of short-chain fatty aldehydes, such as malondialdehyde (MDA), which can be used as an index of oxidative damage. Erythrocyte MDA increases the rigidity and decreases deformability of erythrocytes. PUFA are highly susceptible to autooxidation because of their relatively weak C-H methylene bonds, which readily undergo free radical-mediated hydrogen abstraction. Fish oils containing highly unsaturated n-3 PUFA in pure form are readily susceptible to autoxidation during aerobic conditions. Studies on human urine and plasma have shown that levels of lipid peroxidation products are increased after intake of some fish oils. Studies have also shown a decrease in tissue vitamin E content and an increase in *in vitro* susceptibility to lipid peroxidation in tissues after intake of some fish oils. The *in vitro* stability of different commercially available fish oil preparations varies markedly [42,43] (Table 1). This

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Table 1. Stability of different fish oils

	Stability, days	Vitamin E, IU/g		Stability, days	Vitamin E, IU/g
Fish oil 1*	1	6.8	Fish oil 8	14	1.0
Fish oil 2*	3	20.0	Fish oil 9	14	1.5
Fish oil 3*	4	4.4	Fish oil 10	14	8.5
Fish oil 4*	4	5.0	Fish oil 11	14	3.7
Fish oil 5	6	4.4	Fish oil 12	16	0.3
Fish oil 6	10	1.4	Fish oil 13	21	1.5
Fish oil 7	13	1.0	Natural stable fish oil	200	4.5

Stability, time to rancidity (peroxide value 20) after exposure of the oil to air at room temperature.
*Chemically modified fish oils. Other fish oils are natural.

table shows that the stability of 14 different fish oil preparations varies between 1 and 200 days. Chemically modified, highly concentrated fish oils are usually markedly more unstable than natural fish oils. As seen, most fish oil products available today are unstable, *ie*, they become rancid when exposed to air, and after intake, they can induce consumption of antioxidants such as vitamin E. This will lead to formation of free radicals, which can cause cellular injury. There is no direct association, however, between the content of vitamin E in the fish oils and their stability (Table 1). Even fish oil preparations containing large amounts of vitamin E can be unstable. The problem with the instability of fish oils has not become widely known until recently. Oily fish, crude fish oil preparations, and even some commercially available fish oils contain toxic contaminants, such as pesticides and mercury. These have to be removed, and during this process the antioxidants in the fish oils are lost and have to be restored to keep the stability of the fish oil. The effects observed after intake of an unstable fish oil are the net effects of the positive actions of the n-3 fatty acids and the negative results of the formation of free radicals.

Recently, it has been shown that intake of highly concentrated n-3 PUFA preparations can have adverse effects [44]. Thus, intake of such preparations for 6 months almost doubled the frequency of angina pectoris and significantly increased markers of inflammation and atherosclerotic activity, such as soluble E-selectin and soluble vascular cell adhesion molecule-1 (VCAM-1). Also, the use of nitrates was significantly higher in the group treated with highly concentrated n-3 PUFA preparations compared with control subjects. The adverse effects were ascribed to the increased lipid peroxidation with increased thiobarbituric acid-reactive substances seen in these patients. It was also pointed out that highly concentrated n-3 PUFA preparations induce oxidation far more easily than natural fish oil. NF-κB, which is an important transcription factor in chronic inflammatory diseases because it acts on different genes that encode for proinflammatory processes, for example, E-selection, and VCAM-1 genes are activated by oxidants. Intake of highly concentrated n-3 PUFA preparations results in increased amounts of oxidants, which can potentially induce increased levels of inflammatory markers via the NF-κB pathway [44]. Hau *et al.* showed that highly concentrated, unstable fish oil preparations increase the susceptibility of LDL to oxidation [45]. Others have found similar effects [46].

Fish oil in the natural, stable form also has marked effect not only on triglycerides but also on cholesterol, fibrinogen, and blood pressure [36,39,41] (Fig. 3). Interestingly, stable fish oil also seems to have more pronounced effects on the inflammatory process than unstable fish oil. It is therefore not surprising that intake of stable fish oil has better effects than unstable fish oil on joint stiffness [40] and on the decrease in plasma fibrinogen [39].

The *in vitro* stability of fish oils has been shown to be inversely correlated to *in vivo* lipid peroxidation as measured by plasma levels of MDA and correlated to the ratio between the vasodilator prostacyclin and the vasoconstrictor thromboxane A2 [47]. Intake of several different fish oil products has been found to increase blood glucose, probably because of increased lipid peroxidation in pancreas with decreased production of insulin. After intake of stable fish oil, no such increase in blood glucose is seen [48].

It is now well established that fish oil has a role in the prevention and management of CAD. The same is true for drugs, such as statins and aspirin. The combination of fish oil and statins is especially interesting in combined hyperlipidemia, where fibrates have been suggested as the first choice treatment. However, in a recent meta-analysis, this treatment was found to increase total mortality by 17% [49]. We found that fish oil and statins in combination [50] show a synergistic effect on cholesterol and triglycerides. Patients with hyperlipidemia were given 10 mg of simvastatin (Zocor) daily or 10 mL of natural, stable fish oil (Eskimo-3) or a combination of the two. Simvastatin treatment resulted in a decrease of total

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cholesterol, LDL cholesterol, and the LDL-to-HDL ratio but no change in HDL cholesterol or triglycerides. The stable fish oil reduced triglycerides, total cholesterol, LDL cholesterol, and the LDL-to-HDL ratio and increased HDL cholesterol. The largest decrease in triglycerides, total cholesterol, LDL cholesterol, and LDL-to-HDL ratio was seen in the group given the combination of fish oil and simvastatin.

In another study, we found that fish oil counteracted the potential adverse effects of low-dose aspirin, *eg*, the decrease in prostacyclin and the increase in leukotrienes [51]. The protection of prostacyclin synthesis by fish oil may prevent gastrointestinal upset sometimes induced by aspirin.

Obesity is an important factor in the pathogenesis of CAD. Recent studies show that intake of fish oil containing n-3 PUFA induces weight loss in people with obesity. It has thus been shown that intake of fish oil instead of corn oil containing n-6 PUFA can induce weight loss. Consumption of diets rich in n-3 PUFA leads to less body fat deposition than consumption of fat rich in saturated, monosaturated, or n-6 PUFA [52]. n-3 PUFA oxidize more fat and produce more heat than n-6 PUFA [53]. n-3 PUFA induce expression of genes encoding proteins involved in two biochemical processes associated with enhanced heat production, peroxisomal fatty acid oxidation, and uncoupled oxidative phosphorylation, resulting in less efficient lipid deposition [53]. The long chain n-3 PUFA EPA and DHA are potent activators of the transcription factors PPARs (peroxisomal proliferator-activated receptors), and activation of PPAR is pivotal to induction of several genes, including those encoding peroxisomal fatty acid oxidation.

In conclusion, fish oil has many interesting effects, suggesting a major role of this oil in prevention and management of CAD, especially if the fish oil preparation is stable and does not induce lipid peroxidation. The effects of vitamins are far less convincing, but intake of vitamin E with food or as combinations of different tocopherols as found in nature may play a role in prevention of CAD. Probably subjects with low intake of fatty fish containing omega-3 fatty acids or low intake of γ-tocopherol with the food will benefit most. The efficacy of stable fish oil on cholesterol shows considerable interindividual variations. Future research should focus on identifying genetic loci responsible for this variability by use of molecular genetic information, such as apolipoprotein E genotypes and LDL receptor gene mutations.

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