

A review of omega-3 ethyl esters for cardiovascular prevention and treatment of increased blood triglyceride levels

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Abstract: The two marine omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), prevalent in fish and fish oils, have been investigated as a strategy towards prophylaxis of atherosclerosis. While the results with fish and fish oils have been not as clear cut, the data generated with the purified ethyl ester forms of these two fatty acids are consistent. Although slight differences in biological activity exist between EPA and DHA, both exert a number of positive actions against atherosclerosis and its complications. EPA and DHA as ethyl esters inhibit platelet aggregability, and reduce serum triglycerides, while leaving other serum lipids essentially unaltered. Glucose metabolism has been studied extensively, and no adverse effects were seen. Pro-atherogenic cytokines are reduced, as are markers of endothelial activation. Endothelial function is improved, vascular occlusion is reduced, and the course of coronary atherosclerosis is mitigated. Heart rate is reduced, and heart rate variability is increased by EPA and DHA. An antiarrhythmic effect can be demonstrated on the supraventricular and the ventricular level. More importantly, two large studies showed reductions in clinical endpoints like sudden cardiac death or major adverse cardiac events. As a consequence, relevant cardiac societies recommend using 1 g/day of EPA and DHA for cardiovascular prevention, after a myocardial infarction and for prevention of sudden cardiac death.

Keywords: sudden cardiac death, major adverse cardiac events, cardiovascular prevention, eicosapentaenoic acid, docosahexaenoic acid

Introduction

Epidemiological data demonstrate an inverse relation between consumption of fish and cardiovascular mortality and morbidity (von Schacky 2003). The active ingredients in fish were presumed to be eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, both omega-3 fatty acids found in fish (von Schacky 1987). A vast number of studies have been performed, investigating effects and mechanisms of these omega-3 fatty acids, especially with respect to cardiovascular prevention (von Schacky 2003). The vast majority of these studies were performed with fish oils containing various concentrations of EPA and DHA. Inherently, it was impossible to differentiate between the effects of the other fatty acids present in the fish oils used and EPA and DHA, let alone EPA versus DHA. In the early eighties, Mochida pharmaceutical company in Japan developed a large scale method to purify EPA and DHA in the chemical form of an ethyl ester. Subsequently, other companies, like Pronova (previously known as Norsk Hydro), were also able to purify EPA and DHA for use in humans. Since then, reports have appeared and keep appearing, investigating isolated and combined EPA and DHA in the form of ethyl esters (von Schacky 2003). This review focuses on these reports generated with EPA and/or DHA ethyl esters in the cardiovascular area of interest. Since differences exist in the metabolism of fatty

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acids between experimental animals and humans, this review will largely focus on studies reporting on data generated in human studies with a few relevant animal investigations. EPA and DHA have been studied jointly, as an 85% ethyl ester concentrate of Norwegian provenience (unless otherwise specified in this review: Omacor by Pronova). In other studies, EPA or DHA were studied singly or separately as ethyl esters (usually products by Mochida, Japan).

Method

Medline searches were conducted in March 2006 with the terms: purified eicosapentaenoic acid, eicosapentaenoic acid ethyl ester, eicosapentaenoic acid ethylester, ethyl eicosapentaenoate, ethyl eicosapentaenoic, purified docosahexaenoate, docosahexaenoic acid ethyl ester, docosahexaenoic acid ethylester, ethyl docosahexaenoate, ethyl docosahexaenoic, purified docosahexaenoic acid, purified eicosapentaenoate, concentrated eicosapentaenoic, concentrated docosahexaenoic, concentrated omega-3, concentrated n-3, Omacor, and all publications with cardiovascular topics were scrutinized. A Cochrane review pertinent to the field was used as a source for publications (Hooper et al 2004). Moreover, the author's personal database, generated during more than twenty years in the field was used. Not all reports, however, clearly state in the abstract, what chemical form of EPA and/or DHA was used, which might have led to omission of a small number of studies. Due to substantial disagreement over the correct methodology, reports on *ex vivo* oxidizability or *in vitro* work will not be reviewed.

Animal models

Largely, rats have been studied in animal investigations using omega-3 fatty acids: In a metabolic study, EPA appeared to inhibit intestinal cholesterol absorption and hepatic cholesterol synthesis (Mizuguchi et al 1993). Preliminary findings indicate that clearance of lipoproteins from serum might be increased (Mizuguchi et al 1993). In a model for non-insulin dependent diabetes mellitus, EPA prevented insulin resistance (Mori et al 1997). Since an increase in GLUT4 mRNA in skeletal muscle was found in parallel, this was interpreted as an effect mediated by a change in the fatty acid composition of the skeletal muscle membrane (Mori et al 1997). In stroke-prone hypertensive rats, EPA alleviated the age-dependent decrease in cerebral blood flow and improved glucose utilization (Katayama et al 1997). In salt-sensitive rats, EPA had no effect on development of hypertension and obesity, and insulin

resistance was found to be less pronounced (Mori, Murakawa, et al 1999). In male WBN/Kob rats, a model of spontaneous diabetes, EPA dose-dependently prevented development of diabetes and prevented insulin resistance (Nobukata et al 2000a). Also, platelet response to adenosine diphosphate and collagen was attenuated, and parameters of blood coagulation were shifted towards a less procoagulatory state (Nobukata et al 2000a). Moreover, endothelial cell migration increased and smooth muscle cell proliferation decreased (Nobukata et al 2000b). As compared with safflower oil feeding, EPA-fed diabetes-prone rats were leaner, had lower blood lipid levels, and a blunted insulin response to glucose testing (Minami et al 2002). Another study in the same rat model found platelet response to ADP and collagen reduced, and a number of coagulation and fibrinolysis parameters altered towards a less procoagulatory state (Mori, Nobukata, et al 2003). Also, migration activity of vascular endothelial cells was increased, and binding of vascular endothelial cells to vascular endothelial cell growth factor decreased (Mori, Nobukata, et al 2003). When EPA and DHA were compared, both reduced platelet response to collagen and subsequent formation of thromboxane A₂ to a similar degree (Nieuwenhuys and Hornstra 1998).

One study using rabbits has been published, in which hypercholesterolemic rabbits were treated either with a statin, EPA, or both. Carotid intima-media thickness was followed after sheathing with a cuff. EPA, but not pravastatin, tended to prevent intimal thickening ($p=0.058$). Combination treatment prevented intimal thickening to a significant degree (Yano et al 1997).

In one study, dogs were subjected to experimental myocardial infarction after 8 week supplementing with EPA. Unsupplemented dogs served as controls. EPA reduced infarct size and preserved contractile function, and the authors concluded that this effect was brought about by inhibition of neutrophil infiltration into and subsequently less damage to the infarcted myocardium (Otsuji et al 1993).

Taken together, animal studies performed in Japan largely in various rat models, were mainly aimed at testing EPA as a possible treatment in type 2 diabetes, and showed promising results.

Resorption in humans

When ingested by humans in the chemical form of phospholipids or triglycerides, peak levels of EPA and DHA in plasma are higher than when ingested as ethyl esters (Beckermann et al 1990; Visioli et al 2003). There is some debate as to whether this reflects better or faster absorption

of EPA and DHA as phospholipids or as triglycerides or whether it relates to a slower but equally effective absorption of EPA and DHA as ethyl esters (Rupp et al 2004). It has even been argued that a slower absorption leads to steadier plasma levels, and better antiarrhythmic effects (Rupp et al 2004). When given in comparable amounts as ethyl ester or as triglyceride, slight differences in fatty acid incorporation, but no differences in effects on lipids, parameters of hemostasis, or platelet function were observed (Hansen et al 1993). In the author's opinion, it is not clear whether there exists a meaningful difference in resorption or in the biological effects between ethyl ester or triglyceride forms of EPA and DHA.

Studies in healthy volunteers

In a 4 week study in healthy volunteers, EPA decreased platelet aggregability and whole blood viscosity (Terano et al 1983). In our own 6 day randomized crossover study using 6 g/day of either EPA or DHA, both fatty acids were rapidly absorbed and selectively incorporated into specific platelet phospholipid fractions (von Schacky and Weber 1985). Platelet response to collagen was reduced by both EPA and DHA, whereas platelet response to ADP was only reduced by DHA (von Schacky and Weber 1985). A 4 week study using 0.9 g/day EPA found levels of beta-thromboglobulin and pressor response to angiotensin II reduced (Yoshimura et al 1987). A kinetic study using 3g/day of either EPA or DHA found incorporation in plasma and cells of EPA to plateau at 6 weeks, whereas it took DHA 18 weeks to reach its peak (Marangoni et al 1993). Our own 6 week randomized, observer-blind study in 14 volunteers demonstrated mRNA levels of platelet-derived growth factor-A and -B, as measured *ex vivo* after supplementation of the volunteers diet with 7 g/day of EPA-DHA, to be reduced (Kaminski et al 1993). Our subsequent study demonstrated this effect to be due solely to EPA-DHA, and not to other unsaturated fatty acids. Moreover, reductions in monocyte chemoattractant protein-1 were observed (Baumann et al 1999). In a 7 week double-blind study in a total of 234 healthy men, 3.8 g/day EPA or 3.6 g/day DHA decreased triglycerides by 21% and 26%, respectively, high-density lipoprotein (HDL) increased by 0.06 mmol/l only in the DHA group (Grimsgaard et al 1997). In a 4 week study in 33 healthy persons, EPA, but not DHA, decreased mean platelet volume, and raised platelet count (Park and Harris 2002). DHA was studied in postmenopausal women receiving (n=18) or not receiving (n=14) hormone replacement therapy in a 4 week randomized crossover trial

(Stark and Holub 2004). DHA lowered triglycerides and heart rate, and increased HDL in all women. Retroconversion of DHA to EPA was less in the women using hormone replacement therapy (Stark and Holub 2004).

In the studies mentioned in the preceding paragraph, consistent data on fatty acid metabolism were reported: After ingestion of purified EPA, blood levels of EPA increased. After ingestion of purified DHA, blood levels of DHA increased. No study using purified EPA found increases in DHA, strongly arguing against EPA being metabolized to DHA in large amounts. However, all studies with DHA supplements found increases in EPA, strongly implying retroconversion of DHA to EPA. After supplementation with EPA, but not with DHA, levels in docosapentaenoic acid increased. Identical data have also been reported from patients (eg, Mori, Watts, et al 2000).

EPA and DHA lower human triglyceride levels EPA-DHA

As mentioned above, from studies in healthy volunteers, it had already become clear that EPA and DHA lower blood triglycerides. EPA and DHA combined in fish oil do so in a dose-dependent manner (von Schacky 1987). These findings have been amplified in patients with hypertriglyceridemia. In twenty-two patients with coronary artery disease, 3.4 g/day of EPA-DHA reduced triglycerides by 17% (Eritsland et al 1989). A 50% ethyl ester concentrate of EPA-DHA ("Himega") was compared with a triglyceride-based fish oil in 13 hypertriglyceridemic persons in a twelve week randomized crossover study (Simons et al 1990). Both preparations lowered triglycerides by 50% in doses of 2 g/day or 4 g/day (Simons et al 1990). 3.4 g/day EPA-DHA, but not alpha-linolenic acid or linoleic acid, lowered triglycerides in a randomized 6 week study in hypercholesterolemic men (Kestin et al 1990). Four g/day of EPA-DHA were compared with corn oil in a 5-6 month randomized study in 20 hypertriglyceridemic patients undergoing coronary bypass grafting in mid-study (Nilsen et al 1991). At end of study, plasma triglycerides were reduced by 39% by EPA-DHA, a number of other parameters related to bleeding were unchanged (Nilsen et al 1991). A 10 week randomized study compared 5.1 g/day EPA-DHA with 6 g/day corn oil in 156 persons (Bonna et al 1992). Triglycerides were reduced by 21% in the EPA-DHA group, with calculations pointing towards EPA being more effective (Bonna et al 1992). In a randomized, double blind 6 week study in 43 hypertensive persons 3.4 g/day of

EPA–DHA reduced triglycerides by 21%, whereas the corn oil control did not (Lungershausen et al 1994). Similar findings were reported from a similar study (McKeone et al 1997). The same dose of the same preparations for 12 week was tested in 57 persons in the same manner. Triglycerides were reduced by 28% by EPA–DHA (Grundt et al 1995). In 40 patients with severe hypertriglyceridemia (500–2000 mg/dL), 3.4 g/day EPA–DHA given for four months, reduced triglycerides by 45%, as compared with the patients randomized to placebo (Harris et al 1997). In a 6 month randomized study in 935 persons with hypertriglyceridemia and other cardiovascular risk factors, 1.7 g/day EPA–DHA reduced hypertriglyceridemia (Sirtori et al 1998). In a randomized study in 28 hypertriglyceridemic persons comparing 3.4 g/day EPA–DHA with gemfibrozil, both reduced triglycerides (Stalenhoef et al 2000). In 14 patients with familial combined hyperlipidemia, 3.4 g/day EPA–DHA lowered triglycerides by 44% in a randomized crossover study (Eritsland et al 1996). In a randomized study in 14 patients with familial combined hyperlipidemia, 3.4 g/day EPA–DHA reduced triglycerides by 44%, whereas placebo did not (Calabresi et al 2004).

Purified EPA and/versus DHA

In an open study, 2.7 g/day of EPA reduced triglycerides in primary hypercholesterolemia (Nozaki et al 1992). In 141 postmenopausal mildly hypertriglyceridemic Japanese women randomized to hormone replacement therapy \pm 1.8g EPA/day, EPA decreased triglycerides by 27% (Kurabayashi et al 2000). In a randomized comparative trial with 3g/day of either EPA or DHA in 49 normolipidemic persons, EPA decreased triglycerides significantly, whereas DHA did not (Rambjor et al 1996). In mildly dyslipidemic men, 4 g daily of EPA or DHA for 6 weeks reduced triglycerides by 18% and 20%, respectively (Mori, Burke, et al 2000). Similar findings were reported in a randomized controlled 6 week study comparing 4 g/day EPA with 4 g/day DHA in diabetic patients with hypertension (Woodman et al 2002).

EPA–DHA \pm statins

In a randomized study comparing 3 g/day of EPA–DHA (“Himega”) with 40 mg/day of pravastatin in 32 patients for 6 weeks, EPA–DHA, but not pravastatin, reduced triglycerides by 30%, whereas pravastatin lowered low-density lipoprotein (LDL) (Contacos et al 1993). Combination therapy combined the effects (Contacos et al 1993). In a 5 week randomized study in 41 persons with combined hyperlipidemia, addition of 3.4 g/day EPA–DHA

further reduced triglycerides, when added to treatment with 20 mg simvastatin (Nordoy et al 1998). This effect was observed as being more pronounced in the post-prandial state (Nordoy et al 2000). In a similar 8 week study in 14 patients with familial combined hyperlipidemia, triglycerides were lowered by 27%, as compared with placebo (Calabresi et al 2000). In a randomized one-year study in 59 simvastatin-treated coronary heart disease patients, 3.4 g/day EPA–DHA lowered triglycerides by 20%–30%, whereas they remained stable in the corn oil-treated controls (Durrington et al 2001). In a 6 week, randomized, placebo-controlled, 2 x 2 factorial study in 52 men with visceral obesity, both 3.4 g/day EPA–DHA and 40 mg atorvastatin reduced triglycerides by some 25%, with the effect being additive in the men treated with both (Chan et al 2002).

The triglyceride lowering effect is also reported in some of the studies with clinical endpoints, using the ethyl esters of EPA and DHA, which are reported in more detail below. In the SHOT trial, triglycerides were lowered from 175 ± 99 mg/dL to 142 ± 89 mg/dL (Eritsland et al 1996). In the GISSI Prevenzione trial, triglycerides were lowered by 3.4% by 0.85 g/day EPA–DHA (GISSI 1999). In a smaller, shorter Norwegian study, triglycerides were lowered by 18.56% by 1.7 g/day EPA–DHA (Nilsen et al 2001). A slight effect was also seen in Japan EPA Lipid Intervention Study (JELIS), conducted with EPA, a trial yet to be published.

Taken together, it has been proven beyond reasonable doubt that EPA–DHA lower triglycerides in humans. Reductions are greater when initial levels are higher. Reductions are greater when triglyceride levels are measured in the postprandial state than when measured in the fasting state (eg, Nordoy et al 2000). The effect appears dose-dependent, and does not wane over time. Combination therapy with a statin appeared safe in these mechanistic studies. Adding EPA–DHA to a statin adds to the triglyceride-lowering effect of the statin. It is unclear at present whether EPA lowers triglycerides more effectively than DHA.

Effects on other serum lipids

The effects of fish oil on serum lipids are reviewed in (Harris 1996). A recent review details the effects of purified EPA or DHA on serum lipids (Mori and Woodman 2006).

EPA–DHA

Total cholesterol has been found to be decreased or unaltered (Eritsland et al 1989; Grundt et al 1995; Harris et al 1997;

Nordoy et al 1998; Calabresi et al 2000; Durrington et al 2001). Rather consistently, LDL has been seen to be increased (Simons et al 1990; Calabresi et al 2000; Stalenhoef et al 2000) with a few exceptions (Chan et al 2002). This may be due to the fact that the more buoyant, fast-floating LDL-subclasses increase, while the denser, slow-floating LDL-subclasses decrease (Calabresi et al 2000; Stalenhoef et al 2000). The more buoyant LDL subclasses are thought to be less atherogenic. HDL was increased in some studies up to 15%, whereas in others it remained unaltered (eg, Nordoy et al 1998; Durrington et al 2001; Calabresi et al 2004). In one study, the increase was due to an increase in HDL-2 after supplementation with EPA–DHA (Calabresi et al 2004). Apolipoprotein A usually increased when studied.

EPA and/versus DHA

In open studies, 1.8 g or 2.7 g EPA per day reduced cholesterol (Nozaki et al 1992; Tsuruta et al 1996). In trials comparing similar amounts of EPA to DHA, EPA, but not DHA decreased total cholesterol slightly (Rambjor et al 1996; Grimsgaard et al 1997). In more recent comparative studies, no effects of either EPA or DHA were seen on total cholesterol, HDL, or LDL levels (Mori, Burke, et al 2000; Woodman et al 2002). DHA, but not EPA, increased LDL particle size (Mori, Burke, et al 2000; Woodman et al 2003b). After either EPA or DHA no clear-cut effects on HDL were demonstrated (Nozaki et al 1992; Rambjor et al 1996; Mori, Burke, et al 2000; Woodman et al 2002).

Taken together, there appears to be some diversity as to the effects of EPA–DHA on cholesterol, LDL, and HDL. This may be due to the differences in the populations or patients studied. However, since results obtained with EPA–DHA versus results obtained with either EPA or DHA diverge, there may be even other sources of heterogeneity. They remain to be clarified.

Studies in patients using surrogate endpoints

Glucose metabolism

In an eight week study using 900 mg/day or 1800 mg/day EPA in 24 patients with non-insulin dependent diabetes mellitus, glycemic control was not altered (Westerveld et al 1993). Microalbuminuria was reduced by 900 mg/day EPA after three months, the effect being sustained after 12 months, but left glycemic control unaltered in non-insulin dependent diabetes patients with nephropathy (Shimizu et al 1995b). This effect was also seen in a similar study, using

1800 mg/day EPA in 115 patients for 4 weeks (Shimizu et al 1995a). In 64 hyperlipidemic coronary artery patients, glycemic control was unaltered after one year of 3.4 g/day EPA–DHA (Durrington et al 2001). In a 6 week double blind study, comparing 4 g each of EPA with DHA and with olive oil in 59 hypertensive diabetics, both EPA and DHA increased fasting glucose levels (by 1.40 ± 0.29 mmol and 0.98 ± 0.29 mmol respectively), but neither had an effect on glycated hemoglobin, fasting insulin or C-peptide (Woodman et al 2002). In a one-year study, partly double-blind in 935 persons with hypertriglyceridemia and other cardiovascular risk factors, 1.7 g/day EPA–DHA had no effect on parameters of glucose metabolism (Sirtori et al 1998). Taken together, neither EPA–DHA nor EPA nor DHA has a meaningful effect on glucose metabolism (Farmer et al 2006).

Blood rheology

Erythrocyte deformability was not altered by 900 mg/day or 1800 mg/day EPA for eight weeks (Westerveld et al 1993).

Inflammatory markers/cytokines

In a 6 week study in 59 hypertensive type 2 diabetics, 4 g/day of EPA or DHA reduced oxidant stress, as assessed as urinary F_2 isoprostane excretion, but C-reactive protein, interleukin-6 or tumor necrosis factor- α were unaltered, relative to the olive oil treated controls (Mori, Woodman, et al 2003). A similar picture emerged, when P-selectin and interleukin 6 were studied after 0.85 g/day EPA–DHA (Lee et al 2006). After a myocardial infarction 300 patients were given either EPA–DHA or corn oil in a randomized one-year study (Grundt et al 2003). Homocysteine was found to be reduced in the EPA–DHA-treated patients, but E-selectin and soluble intercellular adhesion molecule-1 were not altered (Grundt et al 2003). In a 3 year randomized study comparing dietary counseling with no counseling, both ± 2.4 g/day EPA–DHA in 563 elderly men with long-standing hyperlipidemia, dietary counseling and EPA–DHA both reduced soluble intercellular adhesion molecule-1 and soluble thrombomodulin (Hjerkinn et al 2005). Thus, markers of inflammation like C-reactive protein remained largely unaltered in patients with cardiovascular disorders, while markers of endothelial activation appear to be reduced.

Hemostatic parameters

Ivy bleeding time was prolonged, but other hemostaseologic parameters were not significantly altered, after four weeks of 3.4 g/day EPA–DHA in 40 patients after myocardial

infarction (Smith et al 1989). Platelet activating factor-induced platelet aggregation was reduced by 900 mg/day EPA for eight weeks in patients with diabetes mellitus type 2, the effect being more pronounced after 1800 mg/day of EPA (Westerveld et al 1993). Platelet aggregation as induced by collagen or ADP was not found to be altered (Westerveld et al 1993). Bleeding time increased in one study in coronary artery patients (Eritsland et al 1989). Fibrinolytic activity, as assessed by PAI activity levels, was found to be increased by 1800 mg/day of EPA in patients with coronary artery disease (Tsuruta et al 1996). After 3.4 g/day EPA–DHA, a similar picture emerged (Nordoy et al 2000). In 59 hypertensive type 2 diabetics, 4 g/day of EPA and 4 g/day DHA were compared with olive oil in a 6 week randomized, double blind study (Woodman et al 2003a). DHA, but not EPA reduced platelet response to collagen and associated thromboxane A₂ formation (Woodman et al 2003a). Other parameters like fibrinolytic activity, or vascular function were not found to differ after the three interventions (Woodman et al 2003a). A three month randomized study comparing 0.85 g/EPA–DHA with usual care in 77 post myocardial infarction patients saw no differences in levels of fibrinogen or D-Dimer. von Willebrand factor, however increased (Lee et al 2006). Thus, by and large, the available evidence indicates that EPA–DHA reduce platelet aggregability, and have a slight anticoagulatory effect, both evidenced in a prolonged bleeding time. DHA appears more effective in terms of inhibition of platelet aggregability than EPA. However, in patients on aspirin, as most cardiovascular patients are, the effects are probably negligible.

Hemodynamic parameters

In hypertensive patients, fish oils reduced blood pressure dose-dependently, with meaningful effects being achieved only at high doses (von Schacky 2003). Animal studies indicated that the blood pressure lowering ingredients are EPA and DHA (cf, Mori, Bao, et al 1999). In humans, only a limited number of studies have been performed with EPA and DHA ethylesters: 3.4 g/day EPA–DHA were compared with 9.2 g/day alpha-linolenic acid and to 14.3 g/day linoleic acid in a 6 week randomized study in 33 normotensive men (Kestin et al 1990). EPA–DHA, but no other intervention, lowered blood pressure (Kestin et al 1990). A double blind randomized cross-over trial in treated hypertensive patients with 3.4 g/day EPA–DHA found systolic/diastolic blood pressure to be reduced by 3.1/1.8 mm Hg after 6 weeks, whereas corn oil had no effect (Lungershausen et al 1994). In a randomized 12 week study using 3.4 g/day EPA–DHA

in 57 hyperlipidemic patients, blood pressure was reduced, whereas it remained stable in the control patients using corn oil (Grundt et al 1995). In 32 mildly hypertensive, but otherwise healthy patients, 3.4 g/day EPA–DHA for 4 months lowered systolic/diastolic blood pressure, as assessed by ambulatory 24 h recording by 6/5 mm Hg, whereas olive oil had no effect in the controls (Prisco et al 1998). In a 7 week double-blind randomized study in 224 normotensive healthy men, 3.4 g/day EPA and DHA were compared. DHA lowered resting heart rate by 2.2 beats per minute (bpm), whereas EPA lowered it by 1.9 bpm. Heart rate was unaltered in the control group (Grimsgaard et al 1998). In a subsample of 52 patients, diastolic filling of the heart improved, as assessed by echocardiography (Grimsgaard et al 1998). Interestingly, in a randomized 6 week study comparing 4 g/day of EPA to DHA and to olive oil in 59 overweight mildly hyperlipidemic men, only DHA reduced blood pressure (Mori, Bao, et al 1999). The ambulatory blood pressure recordings of the persons having ingested DHA were 5.8/3.3 (systolic/diastolic) mm Hg lower than in the olive oil group, whereas EPA had no effect (Mori, Bao, et al 1999). Moreover heart rate was reduced by DHA (daytime -3.7 ± 1.2 bpm, nighttime 2.9 ± 1.2 bpm), whereas EPA did not have an effect (Mori, Bao, et al 1999). In a one-year randomized, double-blind study in 45 hypertensive heart transplant recipients, 3.4 g/day EPA–DHA held blood pressure constant, whereas it increased by $8 \pm 3/3 \pm 2$ mm Hg in the placebo-treated control group (Holm et al 2001). Moreover, renal function was preserved in the group treated with EPA–DHA (Holm et al 2001). Thus, there clearly is a blood pressure lowering effect of EPA–DHA. Interestingly, blood pressure is lowered by DHA, but not by EPA. The same holds true for heart rate. The finding that diastolic filling is improved within 7 weeks of EPA–DHA adds to the rationale to perform studies in congestive heart failure, where diastolic filling plays a major role.

Endothelial function

A small number of studies found endothelial function to be improved after fish oil ingestion by humans (von Schacky 2003). Endothelial function can be assessed by ultrasound tracking of the vasomotory activity of the brachial artery after occlusion, a method called flow mediated dilatation (FMD). The method calls for a positive control with sublingual glyceryl trinitrate to achieve maximal dilatation. This method was used in the three investigations with ethyl esters of EPA and DHA. Using 3.4 g/day of EPA–DHA in 30 hypercholesterolemic persons for four months,

endothelial function was improved, whereas it remained stable in the controls, randomized to corn oil in this double-blind study (Goodfellow et al 2000). Maximal vasodilatory response was unaltered (Goodfellow et al 2000). Another way of assessing endothelial function is a plethysmographic approach, which calls for intraarterial infusion of vasoactive substances like acetylcholine, norepinephrine, and sometimes sodium nitroprusside in various dosages. A randomized, double-blind design was also used to compare 4 g/day EPA with 4 g/day DHA and with 4 g/day olive oil (control) in 59 overweight, mildly hyperlipidemic men (Mori, Watts, et al 2000). In this study, DHA, but not EPA improved the response to acetylcholine (Mori, Watts, et al 2000). DHA, however, improved the response to sodium nitroprusside and attenuated the constrictory response to norepinephrine (Mori, Watts, et al 2000). Olive oil had no effect (Mori, Watts, et al 2000). It was concluded that endothelium-independent mechanisms are responsible for the blood pressure lowering effect of DHA (Mori, Watts, et al 2000). In a randomized crossover trial for 6 weeks, using FMD as a method to assess endothelial function, 20 children with familial (combined) hyperlipidemia received 1.2 g/day DHA (Engler et al 2004). Endothelial function improved, but levels of total cholesterol, LDL, and HDL increased, as compared with the National Cholesterol Education Panel (NCEP)-II diet during the control period (Engler et al 2004). Thus, whether tested with ultrasound or plethysmographically, the available evidence indicates that EPA–DHA improve endothelial function in patients with cardiovascular disorders. When compared, DHA, but not EPA, was found to be effective.

Studies in patients using intermediate endpoints

Vascular occlusion

Neither as an ethyl ester nor as a triglyceride do EPA–DHA affect the rate of restenosis after percutaneous coronary angioplasty, as systematically reviewed in a meta-analysis (Balk et al 2006). Whether EPA and/or DHA as an ethyl ester have an effect on carotid intima-media thickness, another popular intermediate endpoint, has not been studied to the authors' knowledge. A study in 39 patients with coronary artery disease found a reduction in anginal attacks, glyceryl trinitrate consumption, and an increase in exercised time brought about by 10 g/day of a fish oil concentrate for 12 weeks, whereas the parameters remained basically stable in the olive oil-treated controls (Salachas et al 1994). Interestingly, however, a profound positive effect of 3.4 g/

day EPA–DHA on the patency of newly constructed hemodialysis polytetrafluorethylene grafts was observed in a randomized one-year study in 24 patients (Schmitz et al 2002). Patency was 75.6% in the 12 patients randomized to EPA–DHA, but only 14.9% in the corn oil control group (Schmitz et al 2002). Interestingly, venous outflow resistance and systemic blood pressure were decreased (Schmitz et al 2002).

More importantly, however, a randomized trial in 610 patients undergoing coronary bypass surgery was performed to assess the effect of 3.4 g/day of EPA–DHA on the one-year patency of the anastomoses (Shunt Occlusion Trial [SHOT]; Eritsland et al 1996). EPA and DHA increased in serum phospholipids. In 95% of patients, angiography was performed at one year. Considering distal vein graft anastomoses, 27% were occluded in the intervention group, while 33% were occluded in the control group ($p=0.034$). 43% of patients in the intervention group had a distal anastomosis occluded, whereas the corresponding number in the control group was 51% ($p=0.05$). The occlusion rate of the considerably smaller number internal mammary artery grafts was low, and remained unaffected by the treatments studied (Eritsland et al 1996). Vein graft patency correlated with the amounts of EPA–DHA present in serum phospholipid fatty acids (Eritsland et al 1996).

Thus, as demonstrated in animal models (von Schacky 2003), EPA–DHA prevent occlusion of arterial grafts, be it venous or polytetrafluorethylene. While EPA–DHA appear not to have an effect on pain-free walking distance in patients with peripheral arterial disease (Sommerfeldt and Hiatt 2004), they might be a promising approach to improve graft patency in these patients after surgery.

Arrhythmia

Omega-3 fatty acids exert antiarrhythmic effects in humans. These effects are assessed in various ways:

- By assessing heart rate variability, which can be done with a 24 h electrocardiogram. It has been demonstrated repeatedly that high heart rate variability is associated with a low likelihood of sudden cardiac death. A number of studies had already demonstrated, that fish oils increase heart rate variability (Schmidt et al 2005). In a crossover trial, 10 patients with coronary artery disease were treated with either 3 g or 6 g per day EPA–DHA (Villa et al 2002). The variance of the RR intervals from electrocardiogram recordings increased after both doses (not significantly so in the small group). The ratio of the low to high frequency, an index of interaction between

sympathetic and vagal modulation, decreased significantly after both doses, indicating reduced sympathetic tone (Villa et al 2002). More recently, a larger study was performed in 58 elderly nursing home residents (Holguin et al 2005). The effects of 2 g/day EPA–DHA were compared with 2 g/day of soy oil on heart rate variability. The average time- and frequency-domain parameters of heart rate variability increased significantly during the six month intervention period after EPA–DHA and after soy bean oil, with EPA–DHA being more effective (Holguin et al 2005). Further studies are under way (Pater et al 2003).

- By studying patients with implanted cardioverters/defibrillators. These devices not only deliver electric shocks in order to restore a regular rhythm, but first try to resolve the arrhythmia by electrically stimulating the heart. Since the devices have recording capabilities, read-outs can be used to assess rhythm disturbances. This approach has been used in Fish oil Against Arrhythmia Trial (FAAT), a randomized, double-blind 12 month study in 402 patients, using 2.6 g/day EPA–DHA (Leaf et al 2005). This intervention increased the levels of EPA and DHA in the red blood cells in the actively treated group from $3.5 \pm 0.1\%$ to $7.6 \pm 0.3\%$. There was a trend toward a longer time to first action of the device because of ventricular tachycardia or fibrillation ($p=0.057$), the primary endpoint (Leaf et al 2005). When “probable events” were included in the analysis (secondary endpoint), the time to first event was significantly longer in the actively treated group (relative risk [RR] 0.69, 95% confidence interval [CI] 0.49–0.97, $p=0.033$). The picture became clearer, when only patients compliant for 11 months were analyzed. Here, the RR was 0.62 (95% CI 0.39–0.97, $p=0.034$). The results were not as clear-cut as expected because of a high non-compliance rate in both groups: 36.5% in the EPA–DHA group versus 34.2% in the control group, which left the analyzable number of participants below the initially estimated study size. Adverse events were evenly distributed between the EPA–DHA group and the control group (Leaf et al 2005). Another randomized study using 1.8 g/day EPA–DHA in a similar population of 200 patients saw an increase of EPA and DHA from 4.7% to 8.3% in the patients’ red blood cells (Raitt et al 2005). In the intervention group, the time to first episode (primary endpoint) was slightly shorter, the difference being far from significant ($p=0.19$). It has been argued that the initial levels of EPA and DHA in this study were

high enough to obscure any treatment effect (Leaf et al 2005), an argument consistent with the concept of the omega-3 index (Harris and von Schacky 2004). However, it has also been argued that the ventricular tachycardias and fibrillations seen in these patients do not have the same etiology as sudden death in persons with acute myocardial infarction.

- In the early postoperative phase, new onset of atrial fibrillation is the most common complication, eg, observed in roughly 33% of patients undergoing coronary bypass surgery (Calo et al 2005). In a trial in 160 patients, randomized to receiving either 1.7 g/day EPA–DHA or usual care for at least 5 days before until discharge (around day 7), new development of atrial fibrillation was the primary endpoint (Calo et al 2005). This occurred in 33.3% of the patients in the control group, but only in 15.2% of the intervention group ($p=0.013$). The hospital stay (secondary endpoint) was shorter in the intervention group (7.3 ± 2.1 days) than in the control group (8.3 ± 2.6 days) ($p=0.017$).

All studies using intermediate endpoints mentioned were not of a size to observe differences in clinical events. However, the omega-3 fatty acid preparations were usually well tolerated, and serious events like death or major adverse cardiac events tended to occur less frequently in the treated groups (no formal meta-analysis performed).

Studies in patients with clinical endpoints

A 1.5 year randomized, double-blind, study was performed in 300 patients, recruited between the fourth and the eighth day after a myocardial infarction (Nilsen et al 2001). 150 patients received 3.4 g/day EPA–DHA, 150 patients received identical corn oil capsules. Although the intervention caused the expected effects on triglycerides and HDL, a significant difference in clinical endpoints was not observed (Nilsen et al 2001). A primary endpoint was not defined for this study, and therefore study size was not estimated. From a statistical point of view, the study appears underpowered to assess an effect of the intervention on clinical endpoints.

More importantly, a reduction of total mortality, mostly driven by a reduction in sudden cardiac death, by ingestion of 0.85 g/day EPA–DHA was demonstrated by the GISSI-Prevenzione study (GISSI 1999; Marchioli et al 2002). GISSI-Prevenzione was a randomized, open-label 3.5 year study performed in Italy in 11 323 persons having survived a myocardial infarction for a median of 16 days (GISSI 1999; Marchioli et al 2002). A factorial design was used, since

vitamin E was also to be tested, but showed no effect. The primary endpoint, a combination of death, non-fatal myocardial infarction, and stroke was reduced by 10% or 15% ($p=0.048$ or $p=0.008$ respectively), depending on the analysis (two-way or four-way). The secondary endpoints of total mortality (20%) cardiovascular mortality (30%), and sudden death (45%) were all highly significantly reduced by the intervention (with confidence intervals less than 1), but non-fatal myocardial infarctions were not (GISSI 1999; Marchioli et al 2002). The intervention was safe, and only gastrointestinal disturbances were reported as side-effects in some 5% of participants. Time course analyses of the occurrence of the clinical endpoints demonstrated diverging curves, all favoring the intervention (Marchioli et al 2002). Importantly, treatment effects could be discerned early, eg, in the case of total mortality after 90 days ($p=0.037$, CI 0.59–0.97), or in the case of sudden cardiac death after 120 days ($p=0.048$, CI 0.22–0.99). Since the results were largely driven by the reduction in sudden cardiac death, they were interpreted as an antiarrhythmic effect of EPA–DHA, in keeping with the studies mentioned above, and a previous trial showing a similar result (Burr et al 1989). Less progression and more regression of coronary lesions combined with fewer unstable plaques, resulting in smaller myocardial infarction would be a complementary explanation (von Schacky 2003).

A randomized study to assess the effect of 1.8 g/day EPA in 18 645 hypercholesterolemic persons was conducted in Japan. The study design has been published (Yokoyama and Origasea, 2003). Major adverse cardiac events were reduced (presented as a late breaking clinical trial at the American Heart Association meeting in November 2005). A final publication, however, is pending.

In contrast to fish, the natural source of EPA and DHA, ethyl ester preparations of EPA +/and DHA contain no mercury. Mercury has been found to be associated with the risk of myocardial infarction (Guallar et al 2002). Depending on the amount of mercury present in the fish consumed versus the amount of EPA and DHA present, mercury might counteract the positive effect of EPA and DHA. This could have contributed, among other factors, to the negative effect of fish intake in a study conducted in men with angina (Burr et al 2003). Therefore, the positive effects of EPA +/and DHA can be discerned more clearly, when studied as ethyl esters. This is also reflected by the results of a Cochrane analysis (Hooper et al 2004). When the trials using fish are

Table 1 Effects of purified eicosapentaenoic and docosahexaenoic acid, as observed in human studies, only significant differences were considered for inclusion. Arrows reflect semi-quantitatively the findings from the literature

	EPA	DHA
Triglycerides	↓↓	↓↓
Cholesterol	↔	↔
LDL	↑	↑
HDL	↔?	↑?
platelet aggregability	(↓)	↓
mean platelet volume	↓	↔
blood pressure	↔	↓
heart rate	↓	↓↓
endothelial function	↔	↑
glucose metabolism	↔	↔

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Note: Information sourced from von Schacky and Weber 1985; Nozaki et al 1992; Rambjor et al 1996; Grimsgaard et al 1997, 1998; Mori, Bao, et al 1999; Mori, Burke, et al 2000; Mori, Watts, et al 2000; Woodman et al 2000, 2002, 2003a, 2003b; Park and Harris 2002; Mori and Woodman 2006.

removed from the analyses, a clear picture emerges in favor of EPA +/and DHA.

Conclusion

While differences exist in the biological action between EPA and DHA, there is no reason to think that one of the two fatty acids is biologically inactive. Quite to the contrary; as outlined above, both favorably act on lipid parameters, and other parameters pertinent to atherosclerosis, while exerting no negative effects on glucose metabolism (Table 1). A large body of evidence demonstrates that many factors pertinent to the development of atherosclerosis and its complications are positively influenced by EPA and DHA as ethyl esters. As reviewed here, these factors comprise, but are not limited to triglycerides, platelet aggregation, hemostatic parameters, blood pressure, and endothelial function. Moreover, EPA and DHA have antiarrhythmic properties. In large scale clinical studies, this translates into survival benefits for patients after a myocardial infarction. Ongoing studies will further clarify this picture. However, as of now, important cardiac societies, like the American Heart Association or the European Society for Cardiology recommend EPA and DHA for prevention of sudden cardiac death, for the treatment after a myocardial infarction and for cardiovascular prevention (Kris-Etherton et al 2002; De Backer et al 2003; Priori et al 2003; van der Werf et al 2003). A clear cut recommendation to use ethyl esters of EPA and DHA, however, has not been given.

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