Risk Stratification by the "EPA+DHA Level" and the "EPA/AA Ratio"

Focus on Anti-Inflammatory and Antiarrhythmogenic Effects of Long-Chain Omega-3 Fatty Acids

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Abstract

The identification of risks associated with sudden cardiac death requires further investigations. The question was addressed whether parameters can be established which not only describe an increased risk for an enhanced electrical instability of the heart but also of inflammatory events underlying plaque rupture. Emphasis is placed on dose-dependent effects of the long-chain omega-(ω-)3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Since free acids of EPA and DHA are required for most of their biological effects, it appears essential not only to build up stores in the body for release of these fatty acids, but also to provide a sustained uptake of EPA and DHA in the form of ethyl esters. In contrast to rapidly absorbed triacylglycerols from fish, ethyl esters are taken up more slowly within 24 h. For the administration of 1 g/day highly purified EPA+DHA ethyl esters (Omacor®) to healthy volunteers, it is shown that EPA is increased from 0.6% to 1.4% within 10 days, while DHA is increased from 2.9% to 4.3%. After withdrawal, EPA and DHA approach baseline values within 10 days. A gas chromatographic procedure was established which requires only 10 µl of whole blood for the identification of more than 35 fatty acids. Evidence is summarized strengthening the concept that a low "EPA+DHA level" presents a risk for sudden cardiac death and that the administration of 840 mg/day of EPA+DHA ethyl esters raises the "EPA+DHA level" to approximately 6% that is associated with a marked protection from sudden cardiac death. For reducing pro-inflammatory eicosanoids and cytokines, a higher "EPA+DHA level" is required which can be achieved with an intake of 2–4 g/day of 84% EPA+DHA ethyl esters. For assessing influences from pro-inflammatory eicosanoids and cytokines, the EPA/arachidonic acid ratio ("EPA/AA ratio") was identified as diagnostic parameter. To assess the dietary EPA+DHA intake, fatty acids were determined in fish dishes of the cafeteria of the Philipps University Hospital Marburg, Germany. The EPA+DHA content of the popular Alaska Pollock was 125 ± 70 mg/100 g. A once daily fish dish can thus not provide the 840 mg/day EPA+DHA administered in the GISSI Prevention Study in the form of ethyl ester which markedly reduced the risk of sudden cardiac death in postmyocardial infarction patients. Nonetheless, at least two preferably oily fish meals per week should be consumed as preventive measure by persons without coronary artery disease. With documented coronary heart disease, it was advised to consume approximately 1 g/day of EPA+DHA.

Key Words: Omega-3 fatty acids · EPA · DHA · Omacor® · Sudden cardiac death

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Risikostratifizierung mit dem „EPA+DHA-Spiegel" und dem „EPA/AA-Quotienten" unter Berücksichtigung antiinflammatorischer und antiarrhythmogener Wirkungen langkettiger Omega-3-Fettsäuren

Zusammenfassung

Die Identifizierung von Risikofaktoren für den plötzlichen Herztod bedarf weiterer Untersuchungen. Es wurde daher versucht, Parameter zu etablieren, die nicht nur eine erhöhte elektrische Instabilität des Herzens, sondern auch proinflammatorische Prozesse, die u.a. an einer Plaqueruptur beteiligt sind, beschreiben. Im Vordergrund stehen dosisabhängige Wirkungen von Eicosapentaensäure (EPA) und Docosahexaensäure (DHA). Da die freien Säuren von EPA und DHA für die meisten ihrer biologischen Wirkungen benötigt werden, ist es entscheidend, nicht nur Speicher dieser Fettsäuren im Körper für deren Freisetzung aufzubauen, sondern auch eine länger dauernde Aufnahme durch Ethylester zu gewährleisten. Im Gegensatz zu Triacylglycerolen aus Fisch werden Ethylester kontinuierlicher über 24

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h aufgenommen. Nach Verabreichung von 1 g/Tag hochgereinigter Omega-(ω-)3-Fettsäuren (Omacor®) wurde ein Anstieg bei EPA von 0,6% auf 1,4% und bei DHA von 2,9% auf 4,3% beobachtet. Nach dem Absetzen kehrten die EPA- und DHA-Spiegel innerhalb von 10 Tagen auf die Ausgangswerte zurück. Es wurde ein gaschromatographisches Verfahren etabliert, das es ermöglicht, in 10 µl Gesamtblut über 35 Fettsäuren zu bestimmen. Diese Daten unterstützen das Konzept, dass ein niedriger „EPA+DHA-Spiegel" ein Risiko für den plötzlichen Herztod ist und dass die Verabreichung von 840 mg/Tag EPA+DHA-Ethylestern zu einem 6%igen EPA+DHA-Spiegel im Blut führt, der mit einem verminderten Risiko für den plötzlichen Herztod assoziiert ist. Um proinflammatorische Eicosanoide und Zytokine zu reduzieren, wird ein höherer „EPA+DHA-Spiegel" benötigt, wie er durch Einnahme von 2–4 g/Tag 84%igen EPA+DHA-Ethylestern erreicht wird. Um Einflüsse von proinflammatorischen Eicosanoiden und Zytokinen zu verfolgen, wird der EPA/Arachidonsäure-Quotient („EPA/AA-Quotient") als diagnostischer Parameter beschrieben. Zur Beurteilung der EPA+DHA-Aufnahme in der Nahrung wurden Fischgerichte der Cafeteria des Klinikums der Philipps-Universität Marburg untersucht. Der EPA+DHA-Gehalt von häufig angebotenem Alaska-Seelachs betrug 125 ± 70 mg/100 g. Daraus folgt, dass dieser Fischverzehr nicht die bei der GISSI-Präventionsstudie verabreichte Menge von 840 mg/Tag EPA+DHA-Ethylester ersetzen kann, bei der es zu einer Abnahme des Risikos von plötzlichem Herztod bei Postinfarktpatienten kam. Es bleibt dennoch eine wichtige Empfehlung für Personen ohne koronare Herzerkrankung, möglichst fettreichen Kaltwasserfisch mindestens zweimal in der Woche zu verzehren. Bei bekannter koronarer Herzerkrankung wurde eine tägliche Aufnahme von ca. 1 g EPA+DHA empfohlen.

Schlüsselwörter: Omega-3-Fettsäuren · EPA · DHA · Omacor® · Plötzlicher Herztod

Introduction

Risk stratification for prevention and therapy of cardiac death remains a challenge particularly in aging populations, where the burden of disease and disability is increasing rapidly. Established parameters relate primarily to prevention or treatment of hypertension, lipid disorders and diabetes. In view of the remaining high mortality and morbidity from cardiovascular diseases, it appears that the current treatment is not adequate or additional risks exist which have not yet been identified. Risks which are at present not adequately assessed and treated involve inflammation in various heart diseases [59]. Of recent interest became the plaque rupture of atherosclerotic vessels arising from the presence of macrophages and activated T-lymphocytes. As exemplified by a prospective study on C-reactive protein, homocysteine, and plasma lipid levels, only C-reactive protein levels were significantly associated with the risk of sudden cardiac death [3]. In addition to inflammatory and ischemic events, the risk of sudden death and ventricular fibrillation is increased when pump function of the heart becomes critically impaired. In patients with idiopathic dilated cardiomyopathy, parameters reflecting the risk of ventricular arrhythmias have recently been described in a prospective study [24]. Reduced left ventricular ejection fraction and lack of β-blocker use were important arrhythmia risk predictors, whereas signal-averaged ECG, baroreflex sensitivity, heart rate variability, and T-wave alternans did not seem to be helpful for arrhythmia risk stratification [24]. Dilated hearts exhibit an increased wall stress favoring the opening of stretch-activated membrane channels [21]. The resulting electrical instability contributes to the increased risk of ventricular tachyarrhythmias. The question arises, therefore, whether risk parameters can be established which not only describe an increased risk for inflammatory events but also of an enhanced electrical instability. Of particular interest in this respect are factors related to the constituents of lipids. While the level of triacylglycerols has been recognized as cardiovascular risk [16], the actual composition of triacylglycerols with respect to its molecular components is still underestimated. Lipids are composed of various fatty acids whose nature can vary depending on a number of dietary and neuroendocrine influences. The present study provides evidence which strengthens the concept that the ω-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in addition to the ω-6 fatty acid arachidonic acid (AA) provide parameters for stratification of risks associated with inflammatory and arrhythmogenic events. The question is also addressed whether polyunsaturated fatty acids differ with respect to activation of transcription factors such as peroxisome proliferator-activated receptors (PPARs). It is examined whether free fatty acids are involved in these actions and which parameters determine a critical free fatty acid concentration required for desirable effects. Conclusions on EPA and DHA incorporation in

blood lipids are mainly derived from the administration of Omacor® which contains highly purified (84%) EPA and DHA ethyl esters [66, 75]. Incorporation data are available for the doses 4 and 8 g/day and patients with inflammatory IgA nephropathy [15]. For examining the incorporation of 1 g/day, we administered Omacor® to normal healthy volunteers. In the present study, it is assessed whether EPA and DHA are dose-dependently incorporated into blood and whether the EPA/AA ratio is affected. Based on the dose-dependent incorporation of EPA and DHA of Omacor®, the "EPA+DHA level" and the "EPA/AA ratio" are described as risk-identifying parameters.

Inflammation and Eicosanoids

In the past, inflammatory mediators have been seen primarily in the context of pro-inflammatory cytokines and eicosanoids such as leukotrienes. The parent fatty acid of pro-inflammatory leukotrienes is arachidonic acid (AA). The amount of prostanoids formed depends not only on the activation of AA-releasing enzymes such as phospholipase A_2 (PLA_2) but also on the membrane concentration of AA. As measured by the 6-keto-prostaglandin (PG)$F_{1\alpha}$, the prostacyclin production was closely correlated with the phospholipid AA concentration [58]. The membrane AA concentration of releasable AA is, therefore, an important determinant most probably not only of prostanoids but also of leukotrienes. The AA concentration is affected particularly by adrenergic influences. In animal experiments, chronically administered norepinephrine increased the proportion of AA in cardiac phosphatidylcholine which was associated with a reduced linoleic acid concentration [18]. We observed an increased AA concentration also in the aorta of rats after chronic swimming exercise which is associated with enhanced adrenergic influences [58]. This AA-raising effect of catecholamines appears to hold only for intact animals, because in cultured cardiomyocytes isoproterenol did not alter the fatty acid profile [25]. The pathogenetic relevance of AA is exemplified in patients with high risk for atherosclerosis on continuous ambulatory peritoneal dialysis [28]. The AA level in serum was significantly higher in these patients while EPA was lower. No differences were observed in the amount of dietary AA intake. It was suggested that the ratio of AA/EPA could be used as a marker of atherogenicity [28].

Increasing evidence supports the concept that AA effects (20:4) can be counteracted by EPA (20:5) by several mechanisms including a competition for cyclooxygenases (COX) and lipoxygenases. Eicosanoids derived from EPA exhibit less inflammatory and thrombogenic effects (Figure 1). Thromboxane (TX)A_3 derived from EPA is weakly proaggregatory when compared with the AA-derived TXA_2 [19]. Also the influence of pro-inflammatory PGE_2 and the leukotriene (LT)B_4, which is a chemoattractant and activator of neutrophils, is affected by the presence of EPA. Although PGE_3 has a similar action as PGE_2, it is produced with low efficiency. LTB_5 has little inflammatory activity when compared with LTB_4 [33]. There is also increasing evidence that in addition to competitive mechanisms between EPA and AA, platelet aggregation can be affected directly by DHA [37].

Of particular interest are findings on the highly inducible COX-2 which is involved in the overproduction of prostaglandins in inflammatory sites [1]. EPA greatly decreased the interleukin-(IL-)1β-induced COX-2 expression in endothelial cells [1]. Although it is well documented that ω-3 fatty acids suppress cytokine production such as tumor necrosis factor-(TNF-)α and IL-1β, the underlying mechanisms still remain unclear. It appears that interfering with the synthesis of TXA_2 and PGE_2 also affects cytokine production [33].

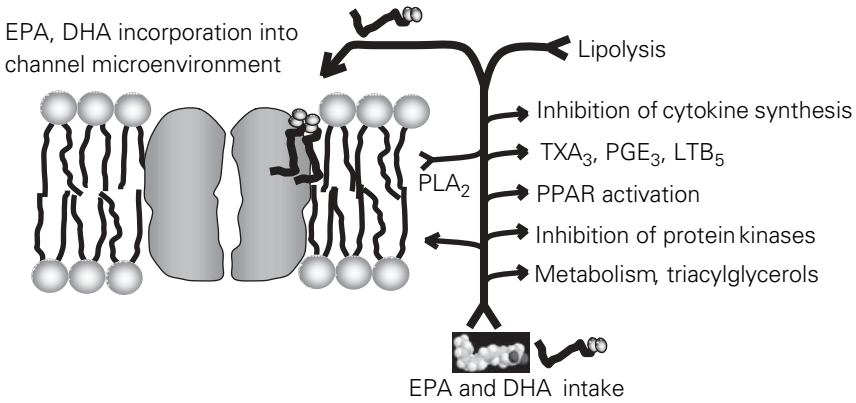


Figure 1. Schematic representation of the effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA attenuates some of the actions of arachidonic acid (AA) particularly via the synthesis of TXA_3, PGE_3 and LTB_5.

Abbildung 1. Vereinfachte Darstellung der Effekte von Eicosapentaensäure (EPA) und Docosahexaensäure (DHA). EPA vermindert einige der Wirkungen von Arachidonsäure (AA), vor allem über die Synthese von TXA_3, PGE_3 und LTB_5.

Inflammation and PPARs

Anti-inflammatory effects of EPA and DHA can arise not only from interactions with eicosanoid generation but also from activation of PPARs. Anti-inflammatory actions occur in addition to the well-established triacylglycerol lowering action of PPARα activators such as fenofibrate and the antidiabetic effect of PPARγ activators (thiazolidinediones). Physiological activators of the PPAR transcription factors are fatty acids, fatty acid derivatives, and eicosanoids [20]. Fibrates reduced the IL-1-induced IL-6 release in smooth muscle cells [12]. Both PPARα and PPARγ activators inhibited thrombin-induced endothelin-1 synthesis [13] and were involved in the initiation of apoptosis of TNF-α-activated macrophages [10]. PPARα activation also reduced the expression of adhesion molecules in endothelial cells [49]. PPARγ inhibited the endothelial expression of chemokines induced by the Th1-cytokine interferon (IFN)γ [34, 48]. PPAR activators have, therefore, also been seen as important regulators of gene expression in vascular cells in relation to atherogenesis [47].

Lack of anti-inflammatory actions could be of particular interest in hypertrophied pressure-overloaded hearts which exhibit a reduced PPARα expression [6]. The reduced PPARα has been linked primarily with a shift in fuel metabolism in favor of glucose utilization with various beneficial effects. Thus, a drug-induced increase in glucose utilization stimulates the promoter activity of the Ca^{2+} pump (SERCA2) gene of sarcoplasmic reticulum [79]. As a lead compound for increasing the SERCA2 expression we used the CPT-1 inhibitor etomoxir. The chemical structure of etomoxir consists of a long-chain fatty acid residue and it has, therefore, also a PPARα-activating action [62]. Since partial inhibition of fatty acid utilization results in cytoplasmic accumulation of free fatty acids and lipids, any CPT-1 inhibition is expected to induce PPARα activation by increasing the presence of physiological PPARα ligands. It remains to be shown, whether the etomoxir-induced increased SERCA2 activity during progression of heart failure [67] has also to be attributed to reduced pro-inflammatory mechanisms due to PPARα activation. Of particular interest in this respect, aging in rats was also associated with a reduced PPARα expression [30]. It remains an intriguing possibility that pro-inflammatory reactions are thus enhanced by pressure overload and aging.

The question arises, therefore, whether ω-3 fatty acids have greater effects on activation of PPARs compared with other fatty acids. Although PPARα binds oleic acid and EPA with nearly equal affinity, only EPA activated PPARα in hepatocytes [35]. For any PPARα activation, increased concentrations of the respective free fatty acids are required in the cytoplasm. In accordance, lipid-lowering fibrates which activate PPARα were originally synthesized as non-metabolizable branched fatty acids which accumulate. On similar lines, it can be argued that EPA is elevated, since it is incorporated to a lesser extent into triacylglycerols. The increased cytoplasmic EPA concentration is expected to result in PPARα activation [35]. Of interest is that also the PPARγ mRNA was increased by EPA [9]. For DHA, beneficial effects on the vascular wall through inhibition of oxidative stress generation and anti-inflammatory action linked to PPARα activation have been described [14]. In this respect, inhibitory effects of EPA and DHA on second messenger-regulated protein kinases [52] could also play a role.

Free Fatty Acids: Enhanced Release Due to Sympathetic Activation

EPA and DHA have to be present as free fatty acids before they can be converted into eicosanoids or can activate PPARs. Major sources for EPA and DHA release are adipose tissue and phospholipid membranes. It is of importance that polyunsaturated fatty acids such as EPA, DHA and AA are incorporated to a greater extent into the inner position of phospholipids [63]. Since a raised sympathetic nervous system activity leads to activation of PLA_2 which mobilizes fatty acids from the inner position of phospholipids, an overproportional increase of these polyunsaturated fatty acids occurs. This has been shown for pigs after coronary occlusion which were fed a diet enriched in EPA and DHA triacylglycerols [54]. Compared with pigs fed saturated fat, an overproportional increase of the EPA and DHA concentration was found in the raised myocardial free fatty acids (Figure 2).

Oral Intake of ω-3 Fatty Acids: Slowed Uptake of Ethyl Esters versus Triacylglycerols

Whether a required free EPA and DHA level is reached, depends not only on the fatty acid release from stores but also on the absorption of orally administered EPA and DHA. In fish, EPA and DHA occur as triacylglycerols which can be transesterified with ethanol in a large scale. From the ensuing mixture of saturated and highly unsaturated ethyl esters, nearly homogeneous EPA and

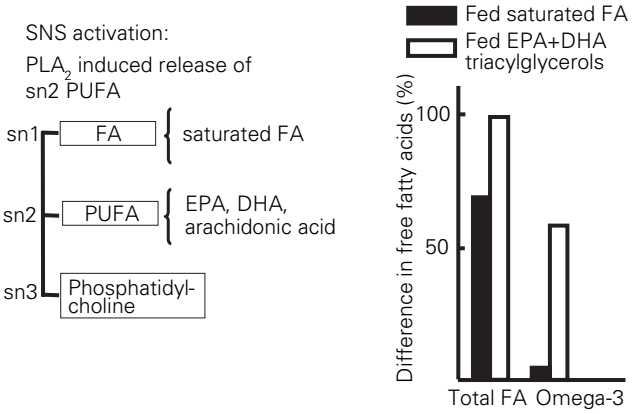


Figure 2. Enhanced release of EPA and DHA from the sn2 position of membrane phospholipids after activation of PLA_2 by a rise in sympathetic activity. After coronary occlusion, pigs fed EPA and DHA triacylglycerols [54] exhibited an overproportional increase of the EPA and DHA concentration in the raised myocardial free fatty acids (FA). Controls were pigs fed saturated fat. Data are adapted from [54]. PUFA: polyunsaturated fatty acids.

Abbildung 2. Verstärkte Freisetzung von EPA und DHA aus der sn2-Position von Membranphospholipiden nach Aktivierung der PLA_2 als Folge einer gesteigerten Sympathikusaktivität. Nach Koronarverschluss bei Schweinen, die EPA- und DHA-Triacylglycerole erhielten [54], waren die EPA- und die DHA-Konzentration in den erhöhten myokardialen freien Fettsäuren (FA) überproportional gesteigert. Kontrollschweine wurden mit gesättigtem Fett gefüttert. Die Daten werden aus [54] übernommen. PUFA: hochungesättigte Fettsäuren.

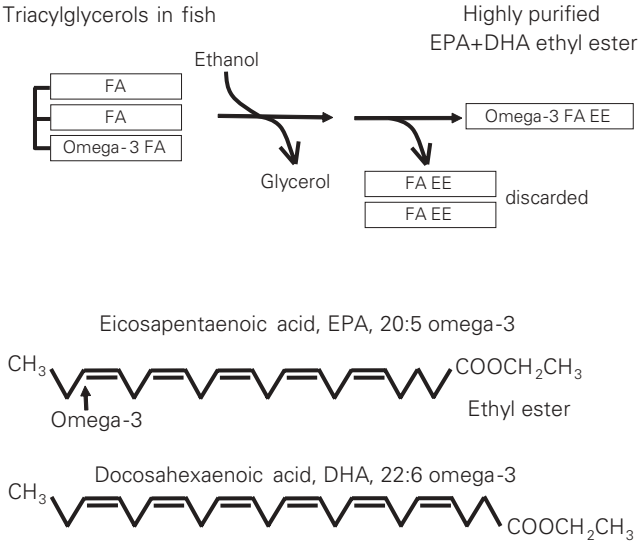
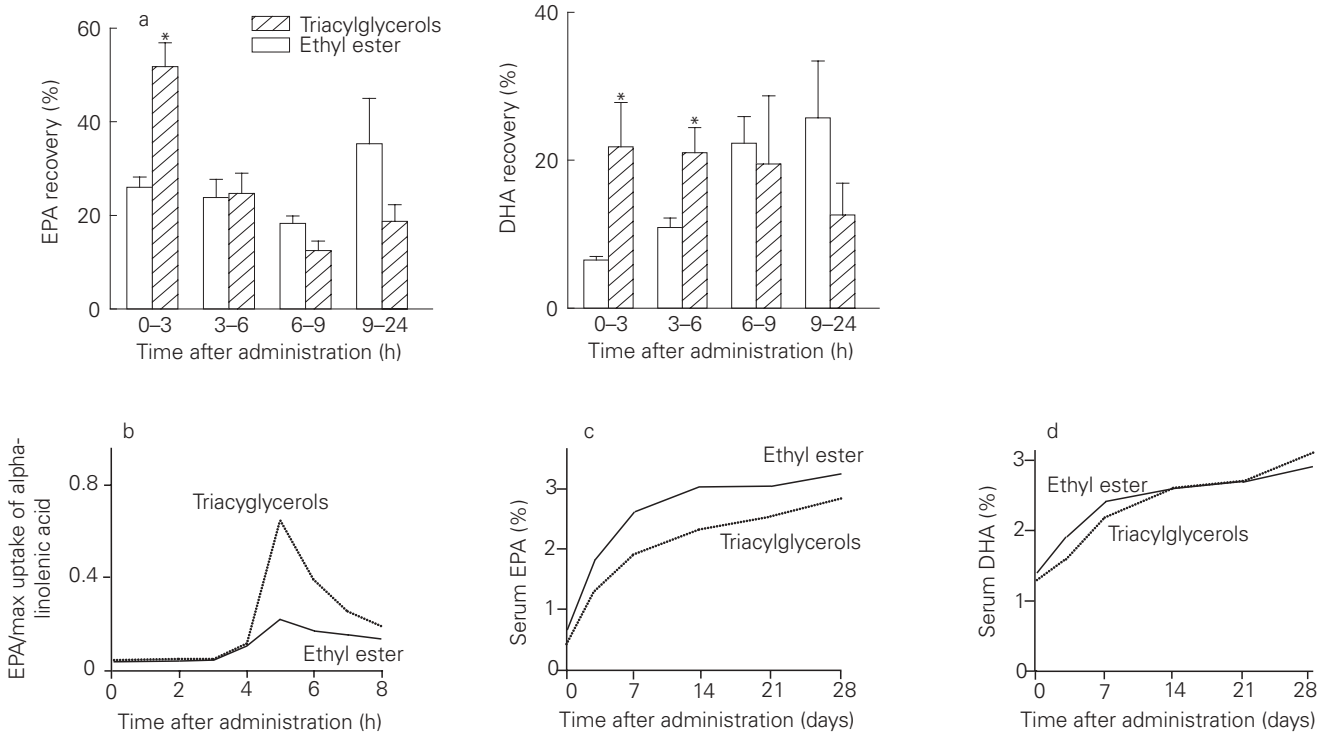


Figure 3. Transesterification of triacylglycerols from fish leading to a highly purified EPA and DHA ethyl ester preparation. EPA and DHA are ω-3 fatty acids since the first double bond is at the position 3 when counting from the methyl end.

Abbildung 3. Die Umesterung der Triacylglycerole von Fisch führt zu hochgereinigten EPA- und DHA-Ethylestern. EPA und DHA sind ω-3-Fettsäuren, da sich die erste Doppelbindung am dritten Kohlenstoffatom befindet, wenn vom Methylende gezählt wird.

DHA ethyl esters can be isolated (Figure 3). It is of particular interest that the duodenal uptake rates differ between triacylglycerols and ethyl esters. Triacylglycerols are rapidly degraded in the duodenum by pancreatic lipase and, in the case of polyunsaturated fatty acids, by carboxylester hydrolase. Compared to triacylglycerols, the ethyl esters of EPA and DHA are absorbed more slowly. This has been shown in rats when EPA and DHA were administered by gavage either as triacylglycerols or ethyl esters [31] (Figure 4a). Within 3 h after administration, the recovery in the lymph of the respective fatty acids was greater in the case of triacylglycerols [31]. After 15 h, the recovery from ethyl esters was approximately doubled compared with triacylglycerols. One of the consequences is that the plasma EPA and DHA level is maintained at a higher level in the second half of a 24-h period which could be of importance, since malignant ventricular arrhythmias are more abundant in the early morning hours [36]. Plasma EPA and DHA levels arising from fish consumption during the preceding day would thus be expected to be lower than in the case of an ethyl ester administration. The different absorption kinetics seen in the rat appear to hold also for humans. Thus, the recovery of EPA in the blood was lower within an 8-h period when compared with triacylglycerols [40] (Figure 4b), while there was no difference in the long-term incorporation of EPA and DHA involving time periods up to 28 days [44] (Figures 4c and 4d). Although the absorption of ethyl esters is increased by co-ingestion with a high-fat meal, the absorption of EPA ethyl ester was still lower [41]. It remains to be examined to what extent the 45% risk reduction of sudden death observed in the GISSI Prevention Study [23, 45, 46] has to be attributed to the administration of ethyl esters of EPA and DHA.

Since no data was available on the blood incorporation of EPA and DHA after administration of 1 g/day Omacor®, we conducted a study in eleven normal healthy volunteers. For establishing risk stratification parameters based on the uptake of EPA and DHA, we used analytical conditions for minimizing the required blood volume. After administration of 1 g/day Omacor®, levels of fatty acids were determined in 20 µl of whole blood using gas chromatography. Whole blood had previously also been used in the Physicians' Health Study [2]. The EPA concentration increased from 0.6% to 1.4% within 10 days leading to a plateau value (Figure 5). DHA values increased from 2.9% to 4.3%. After



Figures 4a to 4d. a) Recovery of EPA and DHA in the lymph of rats administered EPA and DHA either as triacylglycerol or ethyl ester by gavage. *p < 0.05 versus ethyl esters. The total recovery after 24 h did not differ significantly between triacylglycerols and ethyl esters. Data are adapted from Ikeda et al. [31]. b) Short-term plasma incorporation of EPA after administration of 1 g EPA to humans either as ethyl ester or triacylglycerol. Data are adapted from Lawson & Hughes [40]. Long-term serum incorporation of c) EPA and d) DHA. Triacylglycerol administration to humans corresponded to 1.26 g EPA and 0.66 g DHA/day, while the 84% ethyl ester administration corresponded to 1.06 g EPA and 0.64 g DHA/day. Data are adapted from Luley et al. [44].

Abbildungen 4a bis 4d. a) Wiederfindung von EPA und DHA in der Lymphe von Ratten, denen EPA und DHA entweder als Triacylglycerole oder Ethylester mit der Schlundsonde verabreicht wurde. *p < 0,05 gegen Ethylester. Die Gesamtwiederfindung nach 24 h unterschied sich nicht zwischen Triacylglycerolen und Ethylestern. Die Daten wurden von Ikeda et al. [31] übernommen. b) Kurzzeit-Plasmaspiegel von EPA nach Verabreichung von 1 g EPA beim Menschen entweder als Ethylester oder Triacylglycerol. Daten wurden von Lawson & Hughes [40] übernommen. Langzeit-Serumspiegel von c) EPA und d) DHA. Bei Triacylglycerolen wurden beim Menschen 1,26 g EPA und 0,66 g DHA/Tag und bei den 84%igen Ethylestern 1,06 g EPA und 0,64 g DHA/Tag verabreicht. Die Daten werden von Luley et al. [44] übernommen.

Omacor® discontinuation, the values approached the prestudy level within 10 days, whereby the decline in DHA appeared to be less pronounced. The data show that within the present time scale of EPA and DHA ethyl ester administration, no EPA and DHA stores were formed which could maintain the blood EPA and DHA level.

In view of the competitive action of EPA and AA particularly on pro-inflammatory processes, the AA concentration and the EPA/AA ratio were calculated. During the intake of 1 g/day Omacor®, the AA was not significantly reduced (not shown). The ratio of EPA/AA increased, however, from 0.05 to 0.14 demonstrating a higher probability of EPA release compared with AA. In the study of Donadio et al. involving a 4 g/day Omacor® administration, this ratio rose from 0.09 to 0.47 after 6 weeks while the EPA+DHA level was 10.3% [15]. When the daily dose was 8 g/day, the ratio increased from 0.11 to 0.64 (EPA+DHA = 12.3%) demonstrating that the doubling of the EPA and DHA intake had only a moderate further effect on the EPA/AA ratio. The marked increase in the EPA/AA ratio with 4 g/day Omacor® is expected to be associated with a reduced production of pro-inflammatory eicosanoids and cytokines. Also, an enhanced activation of PPARs is expected.

Clinical Endpoints of Reduced Pro-Inflammatory Processes

Evidence in favor of reduced pro-inflammatory actions leading to clinically relevant endpoints was provided by Donadio et al. [15] in a prospective randomized study in

patients with severe IgA nephropathy receiving 4 or 8 g/day Omacor® for 2 years. As might be inferred from the not markedly different EPA/AA ratios, both doses were similar in slowing the rate of renal function loss as deduced from the smaller rise in serum creatinine [15]. As regards underlying protective mechanisms, it has been shown that in mesangial cell cultures, EPA and DHA were effective in suppressing the cell proliferation which is a hallmark of IgA nephropathy [78]. In this respect, it would be of interest to examine whether beneficial effects of 4 g/day Omacor® can be seen also in heart diseases associated with marked inflammation arising from virus infection or autoimmune disorders. With respect to pro-inflammatory processes associated with plaque rupture, there is evidence that the number of macrophages was reduced after administration of 1.4 g EPA and DHA in the form of triacylglycerols [73]. Fewer plaques had thin fibrous caps, signs of inflammation, and the number of macrophages in plaques was reduced.

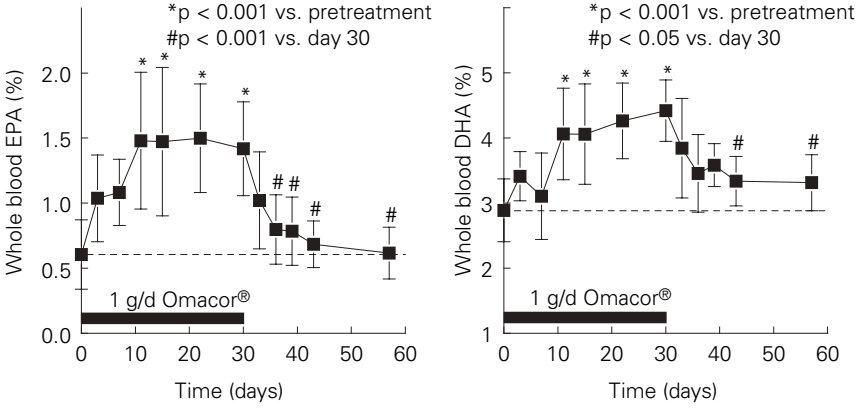


Figure 5. Blood levels of EPA and DHA after 1 g/day Omacor® administration in eleven normal healthy volunteers. Fatty acids were extracted from 10 µl whole blood essentially as described previously [58] which was followed by transesterification with methanolic KOH. A Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector and a Supelco SP-2560 (100 m × 0.25 mm × 0.2 µm) column (Sigma-Aldrich) was used. Integration was performed with "PeakSimple" from SRI Instruments (Torrance, CA, USA). Omacor® was purchased. Statistical analysis was performed by repeated measures analysis of variance and the Tukey-Kramer multiple comparisons test using the "GraphPad InStat" (San Diego, CA, USA) package. The data are based on eleven persons during the administration of Omacor® and nine persons after withdrawal.

Abbildung 5. Blutspiegel von EPA und DHA nach 1 g/Tag Omacor®-Verabreichung bei elf normalen gesunden Freiwilligen. Die Fettsäuren wurden in 10 µl Vollblut wie früher beschrieben extrahiert [58], die Umesterung erfolgte mit Methanol-KOH. Die Auftrennung wurde in einem Hewlett-Packard-5890-Gaschromatographen auf einer Supelco-SP-2560-(100 m × 0,25 mm × 0,2 µm)Säule (Sigma-Aldrich) durchgeführt. Die Detektion erfolgte mit einem Flammenionisationdetektor und die Integration mit dem „PeakSimple"-Programm von SRI Instruments (Torrance, CA, USA). Omacor® wurde käuflich erworben. Die statistische Analyse erfolgte durch eine "repeated measures"-Varianzanalyse und einen multiplen Tukey-Kramer-Vergleich unter Verwendung des „GraphPad-InStat"-Programms (San Diego, CA, USA). Die Daten basieren auf elf Personen während der Verabreichung von Omacor® und neun Personen nach dem Absetzen.

Antiarrhythmogenic Effects of EPA and DHA

The present study shows that 1 g/day Omacor® raises the whole blood EPA+DHA level from 3.5% to 5.7%. The question arises whether this increase has beneficial effects which could be inferred from previous epidemiologic studies in populations with a variable EPA and DHA blood content and whether a link can be provided with the data of the GISSI Prevention Study [23, 45, 46]. Currently, no EPA and DHA values are available for the patients of the GISSI Prevention Study. It is, however, well accepted that the risk of sudden cardiac death is reduced when the EPA+DHA level is increased. In the Physicians' Health Study, baseline whole blood levels of long-chain ω-3 fatty acids were inversely associated with the risk of sudden cardiac death [2]. As compared with men whose whole blood levels of long-chain ω-3 fatty acids were in the lowest quartile (2.12–4.32%), the relative risk of sudden cardiac death was significantly lower among men with levels in the third quartile (5.20–6.07%; adjusted relative risk, 0.19) and the fourth quartile (6.08–10.2%; adjusted relative risk, 0.10) [2]. Also the study of Siscovick et al. shows a risk reduction for primary cardiac arrest when the EPA+DHA level is increased [72]. Compared with a red blood cell membrane EPA+DHA level of 3.3% (the mean of the lowest quartile), a level of 5.0% (the mean of the third quartile) was associated with a 75% reduction in the risk of primary cardiac arrest [72]. Based on the data of the Physicians' Health Study [2] and the study of Siscovick et al. [72] and our own data on the EPA+DHA level after 1 g/day Omacor®, the reduction of sudden death risk observed in the GISSI Prevention Study [23, 45, 46] can be attributed to an increase in the EPA+DHA level leading to about 6% EPA+DHA (Figure 6). It can also be deduced from these data, that 4 g/day Omacor® would not be required which is expected to increase EPA+DHA to about 10% [15].

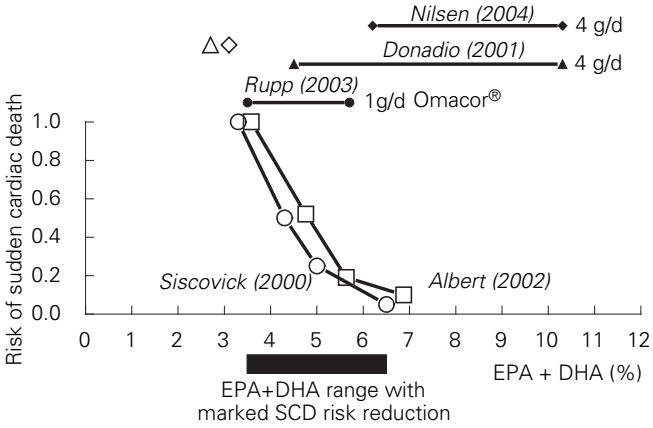


Figure 6. Interrelationship between EPA+DHA level and risk of sudden cardiac death (SCD). Data are adapted from the epidemiologic studies of (□) Albert et al. [2] and (○) Siscovick et al. [72]. Interventional studies with Omacor® were performed with 1 g/d (Rupp et al., data presented at the ESC Meeting, Vienna, Austria, 2003) and 4 g/d (Nilsen et al. [57] and Donadio et al. [15]). The data of Albert et al. [2] include also docosapentaenoic acid and are, therefore, by approximately 0.98 percentage points too high. Included are also data points representing controls in the epidemiologic studies of (△) Guallar et al. [26] and (◇) Leng et al. [43] which are similar to the baseline values of our study carried out in Marburg, Germany. It should be noted that as in our study, whole blood was used in the study of Albert et al. [2].

Abbildung 6. Zusammenhang zwischen dem EPA+DHA-Spiegel und dem Risiko eines plötzlichen Herztodes (SCD). Daten wurden aus den epidemiologischen Studien (□) von Albert et al. [2] und (○) Siscovick et al. [72] übernommen. Interventionelle Studien erfolgten mit 1 g/Tag Omacor® (Rupp et al., Daten auf dem ESC-Kongress, Wien, Österreich, 2003, vorgestellt) und 4 g/Tag Omacor® (Nilsen et al. [57] und Donadio et al. [15]). Die Daten von Albert et al. [2] schließen die Docosapentaensäure mit ein und sind folglich um ca. 0,98 Prozentpunkte bei der gewählten x-Achse (nur EPA+DHA) zu hoch. Dargestellt sind auch Datenpunkte von Kontrollpersonen in den epidemiologischen Studien von (△) Guallar et al. [26] und (◇) Leng et al. [43], die den Ausgangswerten unserer Studie ähneln. Wie in unserer Studie wurde auch von Albert et al. [2] Vollblut verwendet.

Intake of EPA and DHA Triacylglycerols from Fish and Fish Oil

Since in the GISSI Prevention Study 840 mg EPA and DHA was administered corresponding to 1 g/day Omacor®, we addressed the question to what extent dietary fish intake could contribute to the desirable EPA and DHA intake. We analyzed the EPA and DHA content of fish dishes prepared at the cafeteria of the Philipps University Hospital of Marburg (Table 1). The most often served fish dish was Alaska Pollock containing 125 ± 70 mg/100 g EPA+DHA while the warm-water fish Tilapia contained only 23 ± 5 mg/100 g. Taking into account that the average daily fish intake in Northern Germany is 18 g and 13 g in Southern Germany [55], it can be concluded that the dietary fish intake is too low for providing 840 mg EPA and DHA per day. This conclusion holds also for the eastern part of Germany with 19 g/day fish consumption [51]. Fish consumption in Germany appears to be comparable to that reported in the GISSI Prevention Study where at the beginning of the study 73.2% consumed fish once per week and 87.6% at the end of the study [23, 45, 46].

Although fish consumption cannot provide the EPA and DHA intake achieved in the GISSI Prevention Study, it has various beneficial effects as demonstrated in epidemiologic studies. Mortality from coronary heart disease was lower among those who consumed at least 30 g of fish per day than among those who did not eat fish [39]. Among adults, modest consumption of tuna or other broiled or baked fish, but not fried fish or fish sandwiches, was associated with lower mortality risk of ischemic heart disease, especially arrhythmic death [53]. In a prospective study of diabetic women, an inverse association between fish and long-chain ω-3 fatty acid consumption and risk of coronary heart disease and total mortality was observed [29]. In intervention trials involving intake of EPA and DHA triacylglycerols, cardiovascular effects were less pronounced. Thus, 6 g/day of EPA and DHA triacylglycerols for 3 months followed by 3 g/day for 21 months only modestly mitigated the course of coronary atherosclerosis [69]. Based on the current evidence derived from epidemiologic and intervention studies, it was pointed out in a scientific statement of the American Heart Association that patients with documented coronary heart disease should consume approximately 1 g/d EPA+DHA, while persons without documented coronary heart disease should eat (preferably) oily fish at least twice a week [38]. This recommendation is wider than the current indication for Omacor® which is a prescription drug in Germany for postmyocardial infarction patients in addition to standard therapy. In the guidelines of the European Society of Cardiology for the management of ST elevation myocardial infarction, supplementation with 1 g fish oil ω-3 polyunsaturated fatty acids was rated as class I recommendation, level of evidence was B (because of one randomized study) [74].

To compare the intake of EPA and DHA triacylglycerols from fish dishes with that of fish oils, we analyzed the EPA and DHA content of various brands of fish oil capsules. Although the capsules differed markedly with respect to their EPA and DHA content per capsule (Table 2), the EPA and DHA content calculated for 1 g capsule content was very similar with the excep-

Table 1. Fatty acid composition of baked fish dishes. n refers to the number of different fish dishes, whereby the number of different samples analyzed for a given dish is given in brackets. The dishes were prepared at the cafeteria of the Philipps University Hospital of Marburg, Germany. DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid.

Tabelle 1. Fettsäurenzusammensetzung von zubereitetem Fisch. DHA: Docosahexaensäure; EPA: Eicosapentaensäure. n bezieht sich auf die Anzahl verschiedener Fischgerichte; die Zahl in Klammern gibt die Proben an, die bei einem Gericht entnommen wurden. Die Fischgerichte wurden in der Cafeteria des Klinikums der Philipps-Universität Marburg zubereitet.

	n	EPA (%)	DHA (%)	AA (%)	EPA+DHA (mg/100 g)
Alaska Pollock	12 (1)	5.1 ± 3.4	16.4 ± 13.1	0.6 ± 0.5	125 ± 70
Shell	2 (3)	13.0 ± 3.6	16.6 ± 2.7	2.2 ± 1.2	396 ± 147
Hoki	2 (3)	5.9 ± 2.6	26.4 ± 12.2	2.6 ± 1.3	86 ± 76
Crab	1 (3)	16.1 ± 5.3	12.8 ± 1.2	5.1 ± 1.9	79 ± 30
Red snapper	1 (3)	3.4 ± 0.4	24.6 ± 3.5	7.6 ± 0.9	136 ± 13
Cat fish	1 (3)	10.1 ± 1.6	13.6 ± 8.4	3.4 ± 1.5	206 ± 34
Tilapia	1 (3)	0.1 ± 0.1	1.2 ± 0.4	1.4 ± 0.6	23 ± 5

Table 2. EPA, DHA and AA (arachidonic acid) content of fish oil capsules. The EPA+DHA content per 1 g of capsule content was calculated from the actual EPA+DHA content of a capsule and the weight of the capsule content. The fish oil #1 contained halibut oil, while the others appear to be derived from salmon.

Tabelle 2. EPA, DHA und AA (Arachidonsäure) in Fischölkapseln. Der EPA+DHA-Gehalt pro Gramm Kapselinhalt wurde aus dem EPA+DHA-Gehalt einer Kapsel und dem Gewicht des Kapselinhalts berechnet. Das Fischöl #1 enthielt Heilbuttöl, während alle anderen vermutlich aus Lachsöl hergestellt wurden.

Oil	EPA (%)	DHA (%)	AA (%)	EPA+DHA (mg/capsule)	EPA+DHA (mg/g)
# 1	0.5	0.5	0.1	3	11
# 2	26.9	15.9	2.2	89	298
# 3	17.5	15.8	1.3	124	249
# 4	26.4	18.0	1.6	145	289
# 5	23.6	14.4	1.5	159	292
# 6	23.0	15.2	1.4	200	294
# 7	26.9	15.8	1.8	276	282
# 8	26.7	17.6	1.6	303	301

tion of one brand containing halibut oil. This suggests that the fish oils used for manufacturing the capsules were very similar and appeared to be based on salmon oil. One might argue that the amount of daily consumed fish or fish oil could be raised to achieve a sufficiently high EPA and DHA intake which might then be associated with a more marked protective action. A problem associated with a high intake of fish or fish oils relates to their variable contamination with methylmercury and environmental pollutants including dioxins and polychlorinated biphenyls. Thus, the Food and Drug Administration advised pregnant women and children not to exceed three to four fish servings per week and to avoid certain predatory fish [38]. Of particular relevance is mercury which was associated with the risk of myocardial infarction [27]. Since DHA was inversely associated with the risk, it was concluded that high mercury levels may diminish protective effects of DHA. For minimizing the amount of methylmercury and other lipid soluble contaminants, a highly purified preparation involving also a transesterification step is required as carried out for Omacor® which contains ≤ 0.2 µg/capsule mercury (Solvay Pharmaceuticals, data on file).

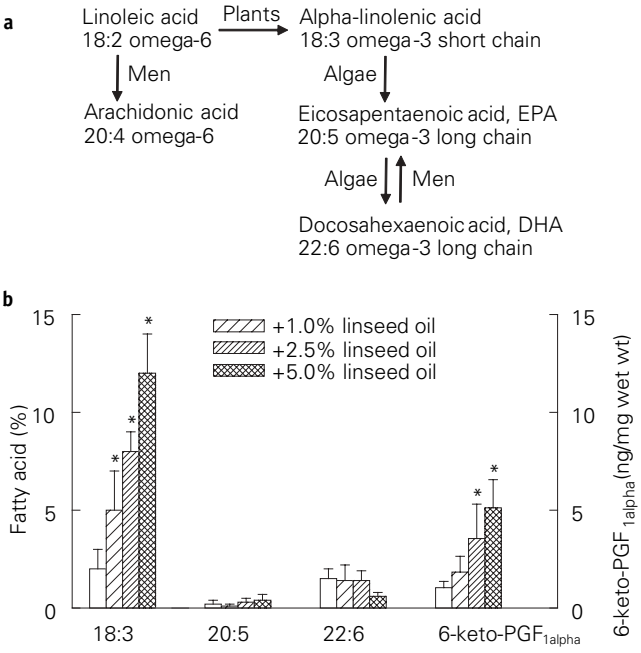
EPA and DHA Conversion from α-Linolenic Acid

It has been argued that the short-chain ω-3 fatty acid α-linolenic acid derived from plants could be consumed for increasing the amount of EPA and DHA in the body (Figure 7a). Of particular interest are plant oils with a high proportion of α-linolenic acid such as linseed oil (approximately 62%), perilla oil (approximately 54%) and canola oil (approximately 10%). We have shown for the rat that feeding increasing amounts of linseed oil does not increase the content of EPA and DHA in the aorta [65] (Figure 7b). However, the enhanced α-linolenic acid intake reduced the AA level and enhanced production of $PGF_{1\alpha}$ which is the stable degradation product of prostacyclin. Thus, although an increased α-linolenic acid intake did not raise the EPA+DHA level, it resulted in an enhanced production of prostacyclin which, in addition to various cytoprotective actions, is a potent inhibitor of platelet aggregation.

In humans with a background diet high in saturated fat, the conversion rate of α-linolenic acid was found to be 6% for EPA and 3.8% for DHA [22]. However, with a diet rich in ω-6 polyunsaturated fatty acids, conversion was reduced by 40–50% [22]. It can thus be concluded that α-linolenic acid cannot be converted into amounts of EPA and DHA which have been used in the GISSI Prevention Study [23, 45, 46]. Of great interest is, however, that any administered DHA can be retroconverted to EPA [70].

Mechanisms of Antiarrhythmogenic Action of EPA and DHA Free Fatty Acids

In animal studies, fish oil feeding reduced the incidence of arrhythmias and fibrillation [40, 50]. The EPA and DHA dosage used was, however, high and typically in-



Figures 7a and 7b. a) Biosynthesis of polyunsaturated fatty acids in plants, marine algae and men. EPA and DHA are produced by marine algae which are taken up by fish. When referring to an "ω-3 level", one has to include also the short-chain ω-3 fatty acid α-linolenic acid as well as the long-chain ω-3 fatty acids EPA and DHA. b) Levels of α-linolenic acid (18:3), EPA (20:5) and DHA (22:6) in the aorta after an increased dietary intake of α-linolenic acid. Spontaneously hypertensive rats were fed 1%, 2.5% and 5% linseed oil (62% α-linolenic acid) diets for 15–16 weeks. Although α-linolenic acid was significantly increased, EPA and DHA were not altered. However, the prostacyclin formation increased as deduced from the 6-keto-$PGF_{1\alpha}$ formation and the AA level was reduced. *$p < 0.05$ versus rats fed a control chow. Data are adapted from [65].

Abbildungen 7a und 7b. a) Biosynthese der mehrfach ungesättigten Fettsäuren bei Landpflanzen, Meeresalgen und Menschen. EPA und DHA werden von Algen produziert, die durch Fische aufgenommen werden. Verwendet man die Bezeichnung „ω-3-Spiegel", so müssen auch die kurzkettige ω-3-Fettsäure α-Linolensäure und die langkettigen ω-3-Fettsäuren EPA und DHA erfasst werden. b) Spiegel von α-Linolensäure (18:3), EPA (20:5) und DHA (22:6) in der Aorta nach einer gesteigerten Aufnahme von α-Linolensäure. Spontan hypertensive Ratten wurden mit Diäten mit 1%, 2,5% und 5% Leinöl (62% α-Linolensäure) über 15–16 Wochen gefüttert. Obgleich die α-Linolensäure erheblich erhöht wurde, waren EPA und DHA nicht signifikant verändert. Die Bildung von Prostacyclin war aber gesteigert.

volved 10% fish oil feeding. Within this dose range, also anti-inflammatory actions and lipid lowering have been observed as seen in humans using a 3–4 g/day EPA and DHA dose. Only recently, it has been shown that already < 0.5% of dietary DHA but apparently not EPA reduced the incidence of arrhythmic events [50]. Based on animal experiments, it might, therefore, have been unexpected that 1 g/day of EPA and DHA ethyl ester reduced the risk of sudden cardiac death in postmyocardial infarction patients [23, 45, 46]. Since the incidence of the second myocardial infarction was not reduced significantly, it appears that mechanisms potentially related to plaque stabilization were not involved.

It has been thought that the antiarrhythmogenic effects of EPA and DHA arise from their incorporation into membrane phospholipids thereby altering the membrane fluidity. However, in the context of studies on effects of ω-3 fatty acids on sarcoplasmic reticulum Ca^{2+} uptake [68], cardiac hypertrophy and dilatation [5, 32] and reperfusion-induced arrhythmias [4], no significant alterations in the Na^+ channel activity of papillary muscles of fish oil-fed rats was found by the loose patch clamp technique [17]. It was at that time not known that free fatty acids of EPA and DHA were involved in the antiarrhythmogenic action. In a series of detailed studies by Leaf et al. (reviewed in [42]), it has been shown that the free fatty acids of EPA and DHA but not other fatty acids inhibit the Na^+ channel activity. In addition, the L-type Ca^{2+} channel which has been inferred particularly in afterdepolarizations was inhibited [42]. For explaining inhibitory effects also on other channels like K^+ channels [42], one has to infer inhibitory effects which are specific for EPA and DHA but not for a particular ion channel. The inhibitory effects of EPA and DHA have been attributed to the non-covalent incorporation of the free fatty acids into the microenvironment of ion channels and the ensuing conformational change [42] (Figure 1). A consequence of this mechanism is that a critical concentration of free EPA and DHA has to be reached before an adequate number of channels are inhibited and antiarrhythmogenic effects ensue.

It can be deduced that EPA and DHA should have antiarrhythmogenic effects whenever the myocardial free fatty acids are raised. Thus, after myocardial infarction, sympathetic activity is increased due to the impaired heart performance leading to a rise in myocardial free fatty acids. The release of EPA and DHA is amplified by the preferred release of fatty acids from the inner position of phospholipids by PLA_2 (Figure 2). Such a mechanism explains also the lack of effect on Na^+ channel current determined in isolated papillary muscle [17]. Furthermore, no [11] or only moderate effects are expected for patients with implantable cardioverter defibrillators which terminate ventricular tachyarrhythmias rapidly and most probably before fatty acids are released to a greater extent. In accordance with this contention

would be the finding that sustained ventricular tachycardia can be reduced by infusion of 3.8 g ω-3 marine triacylglycerols in patients with implanted cardioverter defibrillators [71]. The potential contributions arising from an increased heart rate variability after EPA and DHA intake [60], an improved postischemic recovery [61] or a reduced heart rate [64] to antiarrhythmogenic effects remains to be assessed further.

Risk Stratification by the EPA+DHA Level and the EPA/AA Ratio

Taken together, there is increasing evidence that the long-chain ω-3 fatty acids EPA and DHA exhibit various protective actions. In particular, a low EPA+DHA level appears as a risk for sudden cardiac death. Based on the currently accessible data, the following risk stratification appears plausible:

Antiarrhythmogenic Effects: Based on the studies of Albert et al. [2] and Siscovick et al. [72] demonstrating a markedly reduced risk of sudden cardiac death in persons who exhibit about 3% more EPA+DHA compared with the quartile with the lowest EPA+DHA level, it appears that about 6% blood EPA+DHA represents a target level for prevention of sudden cardiac death (Figure 6). This target level is based also on our own data showing that 1 g/day Omacor® raises the EPA+DHA level from 3.5% to 5.7% of total fatty acids. The 1 g/day Omacor® dose equals the dose used in the GISSI Prevention Study where the risk of total mortality, cardiovascular mortality and of sudden death was significantly reduced [23, 45, 46]. It appears, however, that an upper limit of EPA+DHA concentrations exists above which further coronary heart disease benefit may not occur [57]. This seems to be the case with the 6.2–7.4% range of EPA+DHA in serum phospholipids since an increase to 10.3% by administering 4 g/day Omacor® had no further significant effect on the prognosis of cardiac events despite significant triacylglycerol lowering [56]. This intriguing study by Nilsen et al. was performed in Norway where the baseline EPA+DHA level was higher than in other countries [57] including our data (3.7% EPA+DHA). It can thus be concluded that an EPA+DHA level of 8–10% is not required for protection from sudden cardiac death.

In addition to the levels of incorporated EPA and DHA, one should take into account that the type of ester of administered EPA and DHA is of functional relevance. Compared with triacylglycerols present in fish and fish oils, Omacor® contains ethyl esters which provide a prolonged uptake. Taking into account that antiarrhythmogenic effects require the free fatty acids EPA and DHA, a therapeutic intervention which provides a sustained increase in plasma EPA and DHA levels should be advantageous.

Anti-Inflammatory Effects: For a rational interpretation of reduced pro-inflammatory effects by EPA and DHA, the level of AA should be taken into account. EPA and AA have opposite effects on various pro-inflammatory mediators. There is also increasing evidence that adrenergic influences can increase the membrane level of AA. Based on the data of Donadio et al. showing that 4 g/day Omacor® raise the EPA/AA ratio from 0.09 to 0.47 which was associated with clinical improvement in patients with severe IgA nephropathy, therapeutic approaches targeted at a competition of EPA with AA can be based on 4 g/day Omacor®. This does, however, not imply that lower doses of 2–3 g/day, do not reduce pro-inflammatory reactions. Even with 1 g/day Omacor®, the EPA/AA ratio was increased from 0.05 to 0.14 which demonstrates a reduced probability for AA release. In favor of the concept that less pro-inflammatory processes can be observed already at lower EPA/AA ratios is the finding that 1.4 g of EPA and DHA triacylglycerols reduced the incidence of plaque rupture [73]. It has also been shown that 900 mg/day of EPA ethyl ester reduced the plasma β-thromboglobulin level and the pressor responsiveness to infused angiotensin II [77].

Triacylglycerol Lowering: EPA and DHA have a long-standing clinical use for lowering plasma triacylglycerols [8]. Although serum triacylglycerols are reduced approximately in parallel with the unsaturation index of fatty acids [68], a more specific action has been described for EPA. An important mechanism relates to the inhibitory effects on the triacylglycerol synthesis from diacylglycerols and stimulation of fatty acid oxidation [7]. Although a moderate triacylglycerol lowering effect was observed already with 1 g/day EPA and DHA ethyl esters in the GISSI Prevention Study [23, 45, 46], doses of 3–4 g/day are typically used for achieving a greater triacylglycerol lowering [8].

Clearly, further work is required to delineate the relevance of other fatty acids [76] not covered in the present overview. One should also try to dissect protective actions which are common for EPA and DHA and selective effects of EPA and DHA. In this respect, terms such as "ω-3 level" are inaccurate when referring to EPA+DHA, since it has to include by definition

the ω-3 α-linolenic acid as well as EPA and DHA. As shown in rats fed increasing amounts of α-linolenic acid, the "ω-3 level" was increased due to the incorporation of α-linolenic acid while the EPA+DHA level was not altered [65]. Furthermore, the term "ω-3 level" has to include the docosapentaenoic acid which was, however, not predictive of sudden cardiac death [2].

In view of the present evidence, it is suggested to include the determination of fatty acid profile in the list of investigated parameters, particularly in patients after myocardial infarction. This would strengthen the rationale of therapeutic regimens with EPA and DHA as specified in current guidelines [38, 74]. Since only 10 µl of whole blood are required for the determination of a profile involving more than 35 fatty acids, it does rarely require additional blood sampling. By monitoring the EPA+DHA level, patients could be identified who are at an increased risk of sudden cardiac death irrespective of their underlying disease. Furthermore, longitudinal changes in the EPA+DHA incorporation can be monitored and it can be assessed whether a required EPA+DHA level has been reached in a particular patient.

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