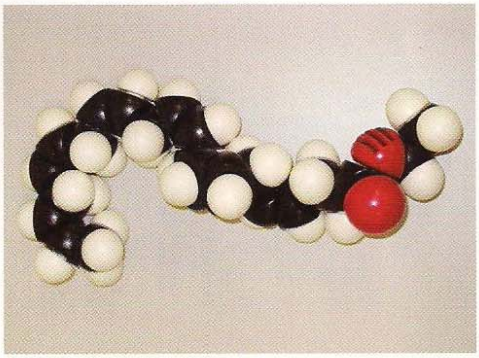
Herz Supplement

Cardiovascular Diseases 31. Jahrgang · Supplement III · Dezember 2006 · ISSN 0946–1299

Omega-3 Polyunsaturated Fatty Acids

Clinical Impact and Benefit of Use in Different Fields of Medicine



Organ des Bundesverbandes Niedergelassener Kardiologen (BNK)

URBAN & VOGEL
MEDIEN UND MEDIZIN VERLAGSGESELLSCHAFT

[15] C.M. Albert, C.H. Hennekens, C.J. O'Donnell et al. Fish consumption and risk of sudden cardiac death. JAMA 279 (1998) 23-28.
[16] M. de Lorgeril and P. Salen. Fish and N-3 fatty acids for the prevention and treatment of coronary heart disease: nutrition is not pharmacology. Am. J. Med. 112 (2002) 316-319.
[17] H.C. Bucher, P. Hengstler, C. Schindler et al. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am. J. Med. 112 (2002) 298-304.
[18] P.M. Kris-Etherton, W.S. Harris, L.J. Appel. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. Circulation 106 (2002) 2747-2757.
[19] W.E. Connor. Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr. 71 (2000) 171S-175S.
[20] R. De Caterina and P. Libby. Control of endothelial leukocyte adhesion molecules by fatty acids. Lipids 31 (1996) S57-S63.
[21] R. De Caterina, J.K. Liao, P. Libby. Fatty acid modulation of endothelial activation. Am. J. Clin. Nutr. 71 (2000) 213S-223S.
[22] Y. Abe, B. El-Masri, K.T. Kimball et al. Soluble cell adhesion molecules in hypertriglyceridemia and potential significance on monocyte adhesion. Arterioscler. Thromb. Vasc. Biol. 18 (1998) 723-731.
[23] I. Seljeflot, H. Arnesen, I.R. Brude et al. Effects of omega-3 fatty acids and/or antioxidants on endothelial cell markers. Eur. J. Clin. Invest. 28 (1998) 629-635.
[24] S. Endres and C. von Schacky. n-3 polyunsaturated fatty acids and human cytokine synthesis. Curr. Opin. Lipidol. 7 (1996) 48-52.
[25] P.M. Kris-Etherton, W.S. Harris, L.J. Appel. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106 (2002) 2747-2757.
[26] J.J. Agren, S. Vaisanen, O. Hanninen et al. Hemostatic factors and platelet aggregation after a fish-enriched diet or fish oil or docosahexaenoic acid supplementation. Prostaglandins Leukot. Essent. Fatty Acids 57 (1997) 419-421.
[27] T.A. Mori, L.J. Beilin, V. Burke et al. Interactions between dietary fat, fish, and fish oils and their effects on platelet function in men at risk of cardiovascular disease. Arterioscler. Thromb. Vasc. Biol. 17 (1997) 279-286.
[28] A. Leaf, J.X. Kang, Y.F. Xiao et al. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107 (2003) 2646-2652.
[29] S. Gudbjarnason and J. Hallgrimsson. The role of myocardial membrane lipids in the development of cardiac necrosis. Acta Med. Scand. Suppl. 587 (1976) 17-27.
[30] M.F. Murnaghan. Effects of fatty acids on the ventricular arrhythmia threshold in the isolated heart of the rabbit. Br. J. Pharmacol. 73 (1981) 909-915.
[31] P.L. McLennan, M.Y. Abeywardena, J.S. Charnock. Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. Am. Heart J. 116 (1988) 709-717.
[32] G.E. Billman, J.X. Kang, A. Leaf. Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. Circulation 99 (1999) 2452-2457.
[33] M.L. Burr, A.M. Fehily, J.F. Gilbert et al. Effect of changes in fat, fish and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 2 (1989) 757-761.
[34] M. de Lorgeril, P. Salen, J.L. Martin et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 99 (1999) 779-785.
[35] GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 354 (1999) 447-455.
[36] R. Marchioli, F. Barzi, E. Bomba et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105 (2002) 1897-1903.
[37] R.B. Singh, M.A. Niaz, J.P. Sharma et al. Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival-4. Cardiovasc. Drugs Ther. 11 (1997) 485-491.
[38] C.M. Albert, H. Campos, M.J. Stampfer et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N. Engl. J. Med. 346 (2002) 1113-1118.
[39] D. Mozaffarian, A. Ascherio, F.B. Hu et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation 111 (2005) 157-164.
[40] K. He, Y. Song, M.L. Daviglus et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation 109 (2004) 2705-2711.
[41] L. Djoussé, P.M. Rautaharju, P.N. Hopkins et al. Dietary linolenic acid and adjusted QT and JT intervals in the National Heart, Lung, and Blood Institute Family Heart study. J. Am. Coll. Cardiol. 45 (2005) 1716-1722.
[42] A. Leaf, C.M. Albert, M. Josephson et al. Prevention of fatal arrhythmias on high-risk patients by fish oil n-3 fatty acid intake. Circulation 112 (2005) 2762-2768.
[43] L. Calo, L. Bianconi, F. Colivicchi et al. N-3 fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized, controlled trial.. J. Am. Coll. Cardiol. 45 (2005) 1723-1728.
[44] J.N. Din, D.E. Newby, A.D. Flapan. Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328 (2004) 30-35.
[45] F.B. Hu, L. Bronner, W.C. Willett et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 287 (2002) 1815-1821.
[46] K. Yoshizawa, E.B. Rimm, J.S. Morris et al. Mercury and the risk of coronary heart disease in men. N. Engl. J. Med. 347 (2002) 1755-1760.
[47] E. Guallar, M.I. Sanz-Gallardo, P. van 't Veer et al. Mercury, fish oils and the risk of myocardial infarction. N. Engl. J. Med. 347 (2002) 1747-1754.
[48] FDA. What you need to know about mercury in fish and shellfish. http://www.cfsan.fda.gov/~dms/admehg3b.html (2004).
[49] C.N. Verboom, R. Marchioli, H. Rupp et al. Highly purified Omega-3 fatty acids for secondary prevention post-myocardial infarction. The Royal Society of Medicine Press Ltd. (2003) ISBN 1853155535.
[50] C.M. Albert, H. Campos, M.J. Stampfer et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N. Engl. J. Med. 346 (2002) 1113-1118.
[51] H. Rupp, D. Wagner, T. Rupp et al. 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. Herz 29 (2004) 673-685.
[52] R. Oh. Practical applications of fish oil (omega-3 fatty acids) in primary care. J. Am. Board Fam. Pract. 18 (2005) 28-36.

Correspondence to:
Heinz S. Weber
1st Medical Department
SMZ-Ost/Danube Hospital
Langobardenstrasse 122
A-1220 Vienna

Microdetermination of Fatty Acids by Gas Chromatography and Cardiovascular Risk Stratification by the "EPA+DHA Level"

Heinz Rupp, Thomas P. Rupp, Daniela Wagner, Peter Alter, Bernhard Maisch[1]

Abstract

The therapeutic options for interfering with the electrical instability of a pathologically remodeled or ischaemic heart remain limited. Of increasing importance become interventions which target the fatty acid composition of blood and membrane lipids. In particular, the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) provide parameters for stratification of

[1] Molecular Cardiology Laboratory, Department of Internal Medicine and Cardiology, Philipps University of Marburg, Germany

risks associated with severe arrhythmia disorders and sudden cardiac death. Since EPA and DHA appear to have their anti-arrhythmogenic actions when present as free fatty acids, the parameters which determine a critical free fatty acid concentration are of great interest. In the present study, conclusions on EPA and DHA incorporation in blood lipids are derived from the administration of Omacor® which contains highly purified (84%) EPA and DHA ethyl esters and reduced the risk of sudden cardiac death by 45% in post-myocardial infarction patients (GISSI-Prevention study). The "EPA+DHA level" is described as risk identifying parameter for severe arrhythmia disorders, particularly if they are associated with myocardial ischaemia. It appears essential not only to build up body stores for release of EPA and DHA but to provide also a sustained uptake of EPA and DHA in the form of ethyl esters. In contrast to more rapidly absorbed triacylglycerols from fish, ethyl esters are taken up slowly within 24 h. For the administration of 1 g/day Omacor® to healthy volunteers, it is shown that in whole blood EPA is increased from 0.6% to 1.4% within 10 days while DHA is increased from 2.9% to 4.3%. After withdrawal, the EPA and DHA levels 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 30 fatty acids. Evidence is summarized strengthening the concept that a low "EPA+DHA level" presents a risk for severe arrhythmia disorders and sudden cardiac death. The administration of 840 mg/day of EPA and DHA ethyl esters raises the "EPA+DHA level" to approximately 6% that is associated with protection from sudden cardiac death. The pharmacological effects of ethyl esters are compared with the naturally occurring EPA and DHA triacylglycerols present in fish or fish oils which are of interest in primary prevention of cardiovascular disorders.

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

Introduction

The stratification of risks for prevention of sudden cardiac death (SCD) remains a challenge particularly in aging populations [1]. In addition to psychological factors including depression [2, 3], a number of adverse structural and molecular alterations occur in the heart which increase the electrical instability often preceding SCD. Since left ventricular hypertrophy is a major predictor of SCD [4], an important contribution arises from the high prevalence of inadequately treated hypertension leading to pressure overloaded hypertrophied hearts. Also the well-established ECG parameters increased QRS duration and QT time [4] can be consequences of cardiac hypertrophy. Furthermore, the marked myocardial fibrosis is a characteristic of hypertensive heart disease [5, 6]. It promotes ischaemia and has an adverse influence on the conduction system, which often is also reflected in an asynchrony of ventricular excitation. A prolonged QT time can arise from defects in gene expression of components of cellular Ca^{2+} handling present in hypertrophied hearts involving the Ca^{2+} pump of sarcoplasmic reticulum (SERCA2a), various K^+ channels and the Na^+-Ca^{2+} exchanger. The development of drugs, which can up-regulate the expression of the SERCA2 gene and counteract the disturbed expression of associated genes has, therefore, emerged as a promising drug target [7, 8] for preventing pump failure [9] and sudden death. The risk of sudden cardiac death and ventricular tachyarrhythmias is increased further during deterioration of pump function. In patients with idiopathic dilated cardiomyopathy, parameters reflecting the risk of ventricular arrhythmias have recently been described in a prospective study [10]. Reduced left ventricular ejection fraction and lack of beta-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 [10].

Dilatation of the heart occurs in about one-third of post-myocardial infarction patients [11], which is reflected in a higher incidence of severe arrhythmias [12]. While hypertensive heart disease and its adverse consequences could be prevented by a more rigorous antihypertensive treatment, dilatation in post-myocardial infarction patients can often not be prevented due to the loss of viable myocardium. Irrespective of the aetiology, the dilatation of heart chambers increases the probability of the opening of stretch activated cation channels [13, 14]. This may further enhance the electric instability arising from fibrosis, which may come about from defects in Ca^{2+} handling genes and local ischaemia. Therapeutic options for preventing SCD remain, however, limited. As shown for patients with dilated cardiomyopathy [10], adequate beta-blockade should be part of the standard treatment. While implantable cardioverter defibrillators (ICDs) have proven useful, high costs are involved at least currently. The lay rescuer automated external defibrillator (AED) requires rescuers trained and equipped to recognize emergencies, activate the emergency medical services system and provide not only defibrillation but also cardiopulmonary resuscitation [15]. The impact of AED in "public access defibrillation" programs is, however, limited, since sudden cardiac arrest usually occurs at home (nearly 80% in Maastricht area) [16] and not in public places. Thus there is a clear need for

Omacor® is a registered trademark

developing alternative interventions, which can counteract adverse consequences of an electrical instability of the heart.

Of particular interest are in this respect factors related to the molecular constituents of lipids in the body. While the level of blood triacylglycerols and particularly LDL have been recognized as cardiovascular risk in various trials [17], the composition of blood lipids with respect to molecular components is still underrated. Blood and membrane lipids are composed of 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 long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which represent a small portion of total fatty acids, provide parameters for stratification of risks associated with severe arrhythmia disorders and SCD. It is also reviewed whether free fatty acids are involved and which parameters determine a critical free fatty acid concentration required for protective effects. The 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 [18, 19]. The latter two have been demonstrated to reduce the risk of SCD in post-myocardial infarction patients by 45% [20]. Since the incidence of the second myocardial infarction was not reduced significantly, plaque stabilization appears not to be primarily involved.

The "EPA+DHA level" is described as risk identifying parameter for severe arrhythmia disorders, particularly if they are associated with myocardial ischaemia. Since the determination of polyunsaturated fatty acids from small biological specimens (10 µl serum or whole blood) proved to be difficult to establish and to standardize, the procedures involved are described in detail. It was also found that the numerical integration of the areas of minor fatty acids required great attention and rules for the integration are given, which should permit the standardized determination also in laboratories without previous experience in lipid chemistry.

The question is also addressed, how the EPA+DHA level can be increased to a desired level in the body and whether the endogenous production from the short-chain omega-3 fatty acid alpha-linolenic acid has a role. The focus on the dosage of 1 g/day EPA and DHA ethyl esters does, however, not imply that anti-inflammatory and lipid-lowering actions, which are more pronounced with > 1 g/day EPA and DHA, are not of therapeutic relevance. The pharmacological effects of ethyl esters are compared with the naturally occurring EPA and DHA triacylglycerols present in fish and fish oils which have a long-standing interest in the primary prevention of cardiovascular disorders.

Microdetermination of Fatty Acids by Gas Chromatography

Extraction of Lipids

Although lipids can be extracted with a variety of organic solvents, the most commonly used procedure involves a mixture of methanol (MeOH) and chloroform ($CHCl_3$) introduced by Folch et al [21]. The extraction procedure for 10 samples and the transesterification step require 3.5 h and involve the following steps:

1. Prepare stock solution of 10% butylated hydroxytoluene (BHT) in MeOH, can be stored in the refrigerator.
2. Add 5 µl 10% BHT to 10 ml MeOH (final 0.005% BHT) in glass Erlenmeyer flask.
3. Prepare extraction solution according to Folch et al [21] by mixing 1 ml MeOH/0.005% BHT with 2 ml $CHCl_3$ ("Folch solution").
4. Add 30 x (w/v) "Folch solution" to tissue (contains 0.033 mg C17:0 internal standard) in 1.5 ml Eppendorf tube, vortex well. In the case of whole blood, use an ultrasonic bath with ice water for 5 min.
5. Extract for 45 min in ice water using a bench-top shaker and centrifuge at 4000 RPM in a table-top centrifuge for 15 min at 4 °C.
6. Transfer 200 µl of supernatant to a new Eppendorf tube and mix it with 40 µl 0.9% NaCl solution. Shake it for 5 min and centrifuge it at 4000 RPM for 15 min at 4 °C.
7. Recover the bottom phase by pipetting through the upper phase preferably with gentle positive pressure (gentle bubbling) thereby avoiding that the upper phase gets into the pipette tip. Do not withdraw more than 90% of bottom phase and do not withdraw the interface. Use a reaction vial with gas-tight Teflon® lined screw cap.
8. Evaporate the extract using a gentle stream of N2.

Transesterification of Triacylglycerols With Methanol

An often used method for producing methyl esters of fatty acids involves heating with a large excess of anhydrous methanol in the presence of the catalyst boron trifluoride (14% BF3, 86% MeOH) at 60–90 °C. It was, however, reported that a selective loss of polyunsaturated fatty acids and artifact peaks can occur [22, 23], which was observed also in our own experiments. We used, therefore, a base-catalyzed transesterification step which requires only mild heating conditions:

1. Prepare freshly 0.2 M KOH in dry MeOH.
2. Dissolve residue (of above step 8) in 750 µl of 1:1 MeOH:toluene.
3. Add 750 µl of 0.2 M KOH in MeOH.
4. Cap the vial and heat at 35 °C for 15 minutes.

5. Cool to room temperature and add 1.5 ml 4:1 hexane:CHCl3, mix.
6. Neutralize by adding approximately 100 µl 1 M acetic acid and monitor the pH by putting very small drops onto pH indicator paper.
7. Add 1.5 ml of quartz distilled water and shake until upper phase becomes clear.
8. Centrifuge at 2000 RPM in a table-top centrifuge for 5 min at room temperature.
9. Add upper phase to Eppendorf tube and let the solvent evaporate in a stream of N2 until it has nearly completely evaporated.
10. Use 1 µl for injection into the gas chromatograph.

Gas Chromatography

For gas chromatography, a model 8610C gas chromatograph from SRI Instruments (Torrance, CA, USA) and a Hewlett-Packard 5890 Series II gas chromatograph from Agilent Technologies (Palo Alto, CA, USA) were used. Both were equipped with a flame-ionization detector (FID) and used hydrogen as carrier gas. For safety reasons, the hydrogen gas was produced with H2-50XR Hydrogen Generators (50 ml/min of hydrogen gas at 30 psi) (SRI Instruments) separately for each gas chromatograph. For data acquisition and integration, the Peak Simple Chromatography Data System (SRI Instruments) with Model 302 (for up to six detectors) was used.

Methyl esters of fatty acids were separated on the SP-2560 fused-silica capillary column (100 m x 0.25 mm x 0.2 µm film thickness) of Supelco (Sigma-Aldrich, St. Louis, MO, USA) for which a standard with 37 fatty acid methyl esters is available (Supelco F.A.M.E. Mix C4-C24, no. 18919-1AMP). Mead's acid (C20:3n-9) was identified with the cis-5,8,11-eicosatrienoic acid methyl ester standard from Sigma (no. E6013).

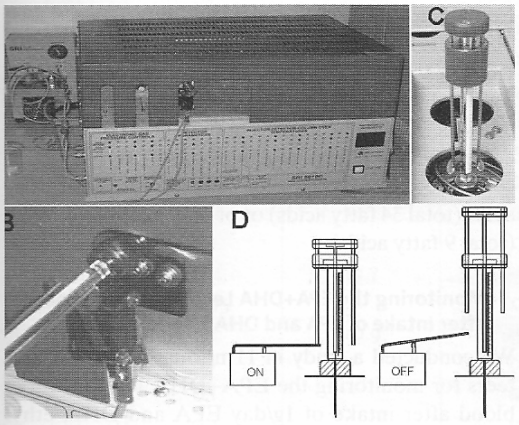


Figure 1: Gas chromatograph model 8610C from SRI Instruments (Torrance, CA, USA) equipped with a flame-ionization detector (FID). The hydrogen for the FID and for the carrier gas was produced by a H2-50XR Hydrogen Generator (50 ml/min of hydrogen gas at 30 psi) in a small quantity avoiding any risk of explosion. The model 8610C from SRI (A) and the Hewlett-Packard 5890 Series II gas chromatograph (not shown) from Agilent Technologies (Palo Alto, CA, USA) were connected to the Peak Simple chromatography data system from SRI Instruments and a personal computer. For synchronizing the data acquisition with the injection, a device (manufactured together with E. Schüler, technical development plant of Medizinische Forschungseinheiten) was used which incorporates a microswitch mounted in front of the sample injection port (B, SRI 8610C; C, Agilent 5890 II), whereby the lever of the switch was extended in a u-shaped manner (D). This u-shaped lever was pressed down during sample injection resulting in contact closure triggering the start of data acquisition. For this purpose, the syringe was incorporated in a guidance device with two thin steel rods which moved together with the needle and closed the u-shaped lever of the microswitch. This synchronization was found to be a prerequisite for reproducibly identifying fatty acid peaks based on the 37 fatty acid standard.

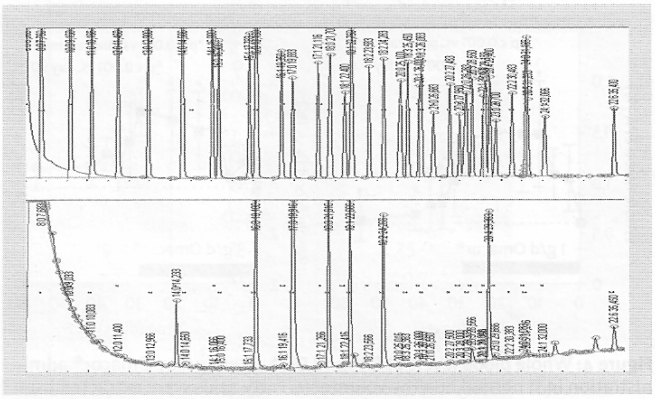


Figure 2: (Top) Gas chromatogram with 37 resolved fatty acids of a fatty acid standard (Supelco F.A.M.E. Mix C4-C24, no. 18919-1AMP). Arachidic acid (C20:0), arachidonic acid (C20:4n-6, cis-5,8,11,14), behenic acid (C22:0), butyric acid (C4:0), capric acid (C10:0), caproic acid (C6:0), caprylic acid (C8:0), cis-13,16-docosadienoic acid (C22:2), cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n-3), cis-11,14-eicosadienoic acid (C20:2n-6), cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n-3), cis-8,11,14-eicosatrienoic acid (C20:3n-6), cis-11,14,17-eicosatrienoic acid (C20:3n-3), cis-11-eicosenoic acid (C20:1), elaidic acid (C18:1, trans-9), erucic acid (C22:1, cis-13), heneicosanoic acid (C21:0), heptadecanoic acid (C17:0), cis-10 heptadecenoic acid (C17:1), lauric acid (C12:0), lignoceric acid (C24:0), linoleic acid (C18:2n-6 cis-9,12), linolelaidic acid (C18:2, trans-9,12), γ-linolenic acid (C18:3n-6, cis-6,9,12), linolenic acid (C18:3n-3, cis-9,12,15), myristic acid (C14:0), myristoleic acid (C14:1, cis-9), nervonic acid (C24:1, cis-15), oleic acid (C18:1n-9, cis-9), palmitic acid (C16:0), palmitoleic acid (C16:1, cis-9), pentadecanoic acid (C15:0), cis-10 pentadecenoic acid (C15:1), stearic acid (C18:0), tricosanoic acid (C23:0), tridecanoic acid (C13:0), undecanoic acid (C11:0). (Bottom) Using this fatty acid standard, fatty acids are identified as shown for a representative fatty acid profile of blood cells after clotting.

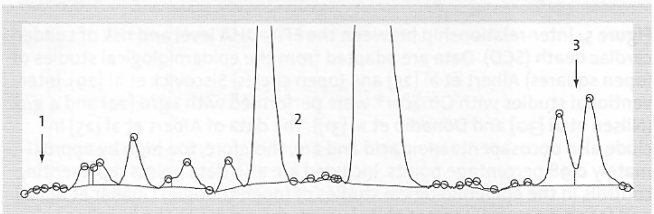


Figure 3A: Procedures for integration of fatty acids using the Peak Simple program. The arrows refer to rules described in the text.

Chromatographic conditions: column oven, 140 °C for 5 min, increase to 240°C at a rate of 4 °C/min, hold at 240 °C for 20 min; injector, 260 °C; detector, 260 °C; carrier gas, hydrogen at 1 ml/min; split 1:10; fuel gas, hydrogen at 30 ml/min, synthetic air at 300 ml/min; total duration of run 45 min. Data acquisition was synchronized with the sample injection by using a mechanical device which couples the injection with pushing the lever back of a microswitch resulting in its closure and the start signal (Figure 1). Gas chromatograms of the fatty acid standard, and blood cells after clotting are shown in Figure 2. The areas of the fatty acid components were calculated based on the following rules (Figure 3A):

(1) Try to draw the baseline as a continuum and as straight as possible, whereby the smallest peaks/elevations are located on the baseline or touch it continuously (arrow 1).

(2) In case the baseline is not a clear horizontal line, draw the baseline to follow the smallest peaks/elevations (arrow 2).

(3) In case of overlapped peaks, draw a perpendicular dropline from the valley to the baseline (arrow 3). Do not pass the baseline through the valley points.

Following these rules, the variability between two observers became small (Figure 3B). It should be noted that the actual percentages of fatty acids are influenced by the number of fatty acids included in the analysis. These differences in fatty acid composition are shown in Table 1 for blood, serum and blood cells after clotting for the inclusion of minor fatty acids (total 34 fatty acids) or of only major fatty acids (total 9 fatty acids).

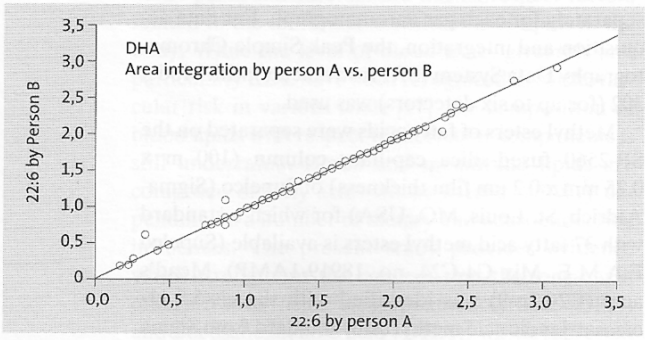


Figure 3B: Interobserver variabilities of percentage values of fatty acids determined independently by two persons.

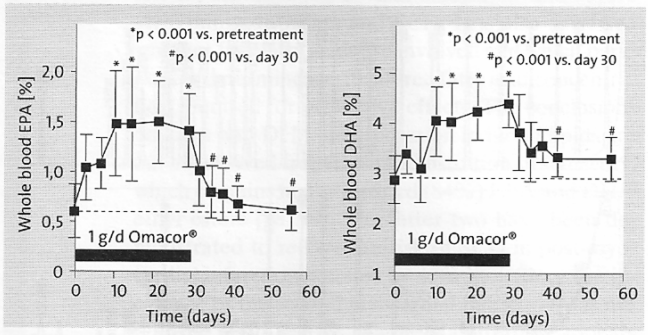


Figure 4: Whole blood levels of EPA and DHA after 1 g/day Omacor® administration in 11 normal healthy volunteers. Fatty acids were extracted from 10 µl whole blood. Omacor® was purchased. Statistical analysis was performed by repeated measures analysis of variance and the Tukey-Kramer multiple comparisons test using the "GraphPad InStat" package (San Diego, USA). The data are based on 11 persons during the administration of Omacor® and 9 persons after Omacor® withdrawal (from [24]).

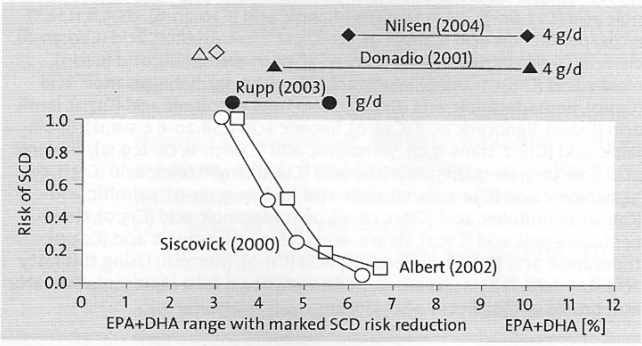


Figure 5: Inter-relationship between the EPA+DHA level and risk of sudden cardiac death (SCD). Data are adapted from the epidemiological studies of (open squares) Albert et al [25] and (open circles) Siscovick et al [29]. Interventional studies with Omacor® were performed with 1 g/d [24] and 4 g/d (Nilsen et al [30] and Donadio et al [31]). The data of Albert et al [25] include also docosapentaenoic acid and are, therefore, too high by approximately 0.98 percentage points. Included are also data points representing controls in the epidemiological studies of (open triangle) Guallar et al [32] and (open diamond) Leng et al [33] which are similar to the baseline values of our study carried out in Marburg. It should be noted that as in our study, whole blood was used in the study of Albert et al [25]. Figure from [24].

Monitoring the EPA+DHA Level in Whole Blood after Intake of EPA and DHA Ethyl Esters

We conducted a study in 11 normal healthy volunteers for monitoring the EPA+DHA level in whole blood after intake of 1g/day EPA and DHA ethyl esters (Omacor®) [24]. Whole blood had previously been used in the Physicians' Health Study examining the inter-relationship between risk of SCD and the level of omega-3 fatty acids [25]. The EPA concentration increased from 0.6% to 1.4% within 10 days leading to a plateau value (Figure 4). DHA values increased from 2.9% to 4.3%. After Omacor® discontinuation, the values approached the pre-study 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 are formed in the body, which could maintain the blood EPA+DHA level after discontinuation of Omacor® intake.

The Whole Blood EPA+DHA Level and Risk of SCD

The intake of 1 g/day Omacor® raises the whole blood EPA+DHA level from 3.5 to 5.7%. This increase is

associated with protective effects, which can be inferred from previous epidemiological studies in populations with a variable EPA and DHA blood content and a link can be provided with the data of the GISSI-Prevention study [20, 26, 27]. While no EPA and DHA values are available for the patients of the GISSI-Prevention study, it has been reported for Italian healthy volunteers that 1 g/day Omacor® raises EPA and DHA to levels which may explain their beneficial effects against cardiovascular diseases [28]. It is well-established that the risk of SCD is reduced when the EPA+DHA level is increased (Figure 5). In the Physicians' Health Study, base-line whole blood levels of long-chain omega-3 fatty acids were inversely associated with the risk of SCD [25]. As compared with men whose whole blood levels of long-chain omega-3 fatty acids were in the lowest quartile (2.12–4.32%), the relative risk of SCD 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) [25]. Also the study of Siscovick et al shows a risk reduction for primary cardiac arrest when the EPA+DHA level is increased [29]. 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

	Whole blood (34 fatty acids)	Whole blood (9 fatty acids)	Serum (34 fatty acids)	Serum (9 fatty acids)	Blood cells (34 fatty acids)	Blood cells (9 fatty acids)
8:0	ND		<0.05		0.1	
10:0	0.1		0.1		0.2	
11:0	0.3		ND		0.1	
12:0	0.7		0.2		0.1	
13:0	<0.05		<0.05		<0.05	
14:0	1.4		1.1		0.8	
14:1	<0.05		<0.05		<0.05	
15:0	0.3		0.2		0.3	
15:1	0.1		<0.05		<0.05	
16:0	27.9	29.8	28.0	29.4	23.7	25.0
16:1	1.0	1.1	1.3	1.3	0.7	0.7
17:1	1.0		0.1		0.1	
18:0	13.2	14.1	9.7	10.2	14.9	15.8
18:1n9t	0.1		0.1		0.3	
18:1n9c	18.4	19.7	21.4	22.4	18.5	19.6
18:2n6t	<0.05		0.1		<0.05	
18:2n6c	19.6	20.9	25.3	26.6	18.3	19.4
20:0	0.1		0.2		0.2	
18:3n6	0.1		0.2		0.2	
20:1	0.2		0.2		0.2	
18:3n3	0.3	0.3	0.3	0.3	0.2	0.2
21:0	0.2		<0.05		<0.05	
20:2	0.2		0.1		0.3	
20:3n9	<0.05		<0.05		<0.05	
22:0	0.1		0.2		0.1	
20:3n6	1.4		1.5		1.7	
22:1n9	<0.05		<0.05		0.1	
20:3n3	<0.05		<0.05		<0.05	
20:4n6	9.8	10.5	6.0	6.4	13.8	14.6
23:0	ND		ND		ND	
22:2	<0.05		0.2		0.3	
24:0	0.1		<0.05		0.2	
20:5n3	1.0	1.1	1.4	1.5	1.4	1.5
24:1	0.1		0.1		0.1	
22:6n3	2.4	2.6	1.8	1.9	3.1	3.3
EPA+DHA	3.4	3.7	3.2	3.4	4.5	4.8

Table 1: Differences in fatty acid composition (%) of blood arising from area calculations including also minor fatty acids (total 34 fatty acids) or only major fatty acids (total 9 fatty acids).

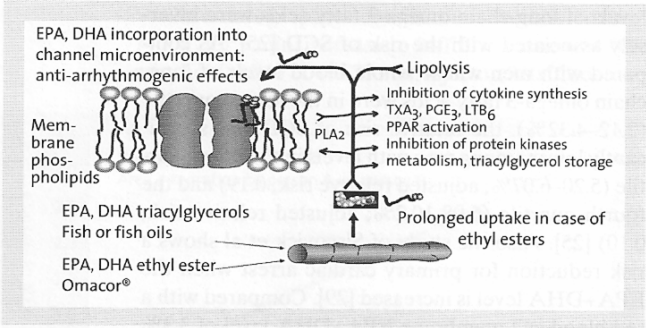


Figure 6A: Schematic representation of biological effects of EPA and DHA administered as triacylglycerols or ethyl esters. EPA attenuates some of the actions of arachidonic acid particularly via the synthesis of TXA_3 and LTB_5. The incorporation of EPA and DHA in the form of free fatty acids into the micro-environment of ion channels is based on studies of Leaf et al [38].

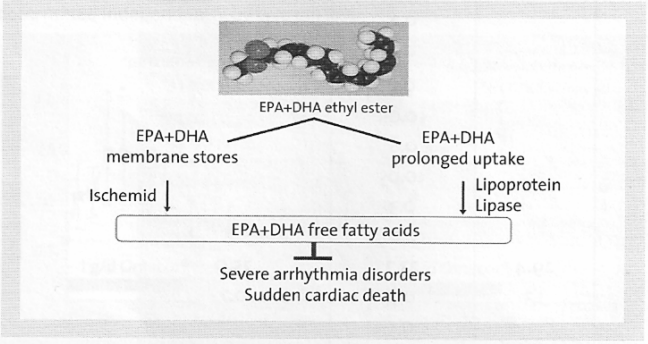


Figure 6B: Mechanisms contributing to a critical rise in EPA and DHA required for reducing the risk of severe arrhythmia disorders and sudden cardiac death. As a result of ischaemia, EPA and DHA are released from membrane phospholipids and contribute to the non-membrane bound EPA and DHA pool. In the case of ethyl ester administration, an EPA and DHA trough-to-peak ratio close to 1.0 is expected for the administration since they are absorbed in a sustained manner.

mean of the third quartile) was associated with a 75% reduction in the risk of primary cardiac arrest [29]. Based on the data of the Physicians' Health Study [25] and the study of Siscovick et al [29] 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 [20, 26, 27] can be attributed to an increase in the EPA+DHA level leading to about 6% EPA+DHA (Figure 5).

The Non-Membrane Bound EPA+DHA Level – Enhanced Release of Free Fatty Acids in Myocardial Infarction

Although it is well-known that EPA and DHA have to be present as free fatty acids before they can be converted into eicosanoids or can activate transcription factors such as PPARs [24] (Figure 6), it remains a misconception that anti-arrhythmogenic effects involve membrane bound phospholipids of EPA and DHA. Major sources for the release of EPA and DHA are phospholipid membranes (Figure 6). The polyunsaturated fatty acids EPA, DHA and arachidonic acid are incorporated to a greater extent into the inner position of membrane phospholipids [34]. A rise in sympathetic nervous system activity is associated with a raised phospholipase A2 activity and the subsequent release of fatty acids from membranes. Since phospholipase A2 mobilizes fatty acids from the inner position of phospholipids, an over-proportional increase of these polyunsaturated fatty acids is expected. This has been shown for pigs after coronary occlusion, which had been fed a diet enriched in EPA and DHA triacylglycerols [35]. Compared with pigs fed with saturated fat, an over-proportional increase of the EPA and DHA concentration was observed in the raised myocardial free fatty acids. The relevance of non-membrane bound EPA and DHA is demonstrated also in a study on dogs which had sustained a prior myocardial infarction [36]. The animals were tested during treadmill exercise and occlusion of the left circumflex artery. When 50-60 min prior to coronary occlusion, a 70% omega-3 fatty acids concentrate was administered intravenously, fibrillation did not occur. A similar infusion of a soy bean oil emulsion resulted as expected in prompt development of ventricular fibrillation. Since the 50–60 min were too short and the administered amount not enough for raising the whole body membrane EPA and DHA content appreciably, it can be deduced that EPA and DHA present in serum had a protective action [36]. In accordance with this contention would be the finding that sustained ventricular tachycardia can be reduced by infusion of 3.8 g omega-3 marine triacylglycerols in patients with ICDs [37].

Mechanisms of Anti-arrhythmogenic Action of EPA and DHA Free Fatty Acids

In the context of our studies on effects of omega-3 fatty acids on sarcoplasmic reticulum Ca^{2+} uptake [39], cardiac hypertrophy and dilatation [40, 41] and reperfusion-induced arrhythmias [42], no significant alterations in the Na^+ channel activity of papillary muscles of fish oil fed rats were found by the loose patch clamp technique [43] (Table 2). The Na^+ channel properties were not affected although the DHA content of phospholipids was increased from 9 to 28% and the number of ischaemia-reperfusion arrhythmias was reduced [42]. Thus, EPA and DHA bound to membrane phospholipids do not affect the properties of the Na^+ channel. At the time of these experiments we were not aware of findings that free fatty acids of EPA and DHA are involved in the anti-arrhythmogenic action. Free EPA and DHA were, therefore, not added during the electrophysiological experiments, which according to the later experiments of Leaf et al (reviewed in [38]) would have been required for observing inhibitory effects on ion channels.

In a series of detailed studies, it has been shown by Leaf et al that the free fatty acids of EPA and DHA but not other fatty acids inhibit the Na+ channel activity which occurs rapidly and can be washed out [44]. In addition, the cardiac Na^+-Ca^{2+} exchanger [45] and the L-type Ca^{2+} channel [46] which has been inferred particularly in after-depolarisations were inhibited. For explaining inhibitory effects also on other channels like the transient outward K^+ current [47] and the major voltage-dependent delayed rectifier current (Kv1.5) [48] one has to infer inhibitory effects which are specific for EPA and DHA but not for a particular ion channel. Also the Ca^{2+} release from intracellular Ca^{2+} stores, the sarcoplasmic reticulum, was inhibited [49, 50]. The inhibitory effects of EPA and DHA have been attributed to the non-covalent incorporation of free fatty acids into the micro-environment of ion channels and the ensuing conformational change [38] (Figure 6A). 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 become inhibited and anti-arrhythmogenic effects ensue. After myocardial infarction, sympathetic activity is increased due to the impaired pump 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 phospholipase A2.

It might thus not be unexpected that protective effects can be smaller in patients with ICD. In contrast to ischaemic events such as myocardial infarction which raise free EPA and DHA to levels required for their anti-arrhythmogenic action, the ICD is expected to terminate re-entrant ventricular tachycardias or ventricular fibrillation before marked sympathetic activation and release of EPA and DHA occurs. In the trial by Raitt et al [51], 1.8 g/day EPA and DHA ethyl esters did not reduce the risk of ventricular tachycardia or ventricular fibrillation in 100 patients with ICDs when compared with 100 patients on placebo (olive oil). The Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA) by Brouwer et al assessed effects of taking 2 g fish oil on life-threatening arrhythmias in ICD patients. At 12 months, 30% of the 273 patients in the fish oil group had experienced either life-threatening arrhythmia or death compared to 33% of the 273 patients in the placebo oil group (not statistically significant). Among the subgroup of 342 patients who previously had a myocardial infarction there was a trend towards a beneficial effect of fish oil (p=0.086). In this subgroup, 28% of the patients on fish oil experienced either life-threatening arrhythmia or death compared to 35% of the patients on placebo oil. (http://www.escardio.org/vpo/ESC_congress_information/ConferenceReleases/CPreleases/Brouwer.htm).

	Mackerel oil	Hydrogenated coconut oil	
Na^+ conductance (pS/µm²)	159 ± 31	94 ± 8	P = 0.06
Activation curves			
Hodgkin-Huxley "m" (mV)	−52 ± 2	−55 ± 1	N.S.
Steepness of "m" (mV)	11.1 ± 0.5	10.6 ± 0.4	N.S.
Steady state inactivation curve			
Midpoint (mV)	−79.7 ± 0.8	−78.4 ± 2.7	N.S.
Steepness (mV)	11.8 ± 0.8	10.2 ± 0.8	N.S.
Ischaemic zone (%)	36.8%	40.8%	N.S.
Arrhythmia incidence	0.4	3.6	P < 0.05
Cardiac DHA (%)	28.4 ± 1.8%	13.7 ± 0.8%	P < 0.05

Table 2: Lack of changes in steady state or kinetic parameters of the cardiac Na^+ current measured in isolated papillary muscles of rats fed 10% mackerel oil for 10–13 weeks. In open chest rats, the left anterior descending coronary artery was constricted for 40 min. This ischaemic period was followed by 60 min reperfusion. The arrhythmia incidence was calculated summing up the total counts of arrhythmias and dividing it by the number of animals; fatty acids were determined with gas chromatography. [37, 77]. Using the loose-patch-clamp technique [89], no significant differences in steady state or kinetic parameters of the cardiac Na^+ current were observed [43]. The reduced incidence of arrhythmias and fibrillation is in accordance with studies by McLennan et al [37, 90]. Recent data show that a much lower dose (< 0.5% of dietary DHA but apparently not EPA) is required for reducing the incidence of arrhythmic events in rats [90].

Although the study appears to be underpowered for the subgroup analysis, the trend would be in accordance with the GISSI-Prevention study which included 2835 post-myocardial infarction patients in the EPA and DHA ethyl ester group. In the study by Leaf et al [52] 402 ICD patients were randomized to 2.6 g EPA and DHA ethyl ester or olive oil as placebo for 12 months. Compliance with the double-blind treatment was similar in the two groups; however, the non-compliance rate was high (35% of all enrollees). This might not be surprising since four 1.0 g capsules had to be taken daily. The primary end-point, time to first ICD event for ventricular tachycardia or fibrillation confirmed by stored ECG or death from any cause was borderline significant (risk reduction of 28%; p = 0.057). For those who stayed on protocol for at least 11 months, the anti-arrhythmic benefit of EPA and DHA ethyl esters was improved for those with confirmed events (risk reduction of 38%; p = 0.034). Why in this study capsules with only 65% EPA and DHA instead of 84% as in the case of Omacor® were used, remains intriguing. The study again strengthens the fact that patient compliance is greatly reduced with daily four 1.0 g capsules.

Further support for anti-arrhythmogenic effects of omega-3 fatty acids was provided in the study of Calo et al [53]. Two 1 g capsules of EPA and DHA ethyl esters were administered during hospitalization in patients undergoing coronary artery bypass graft surgery (CABG). Postoperative atrial fibrillation devel-

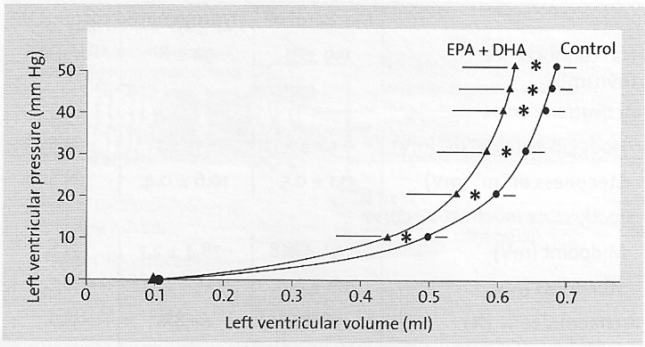


Figure 7: Attenuation of the adverse dilatation of pressure overloaded heart of spontaneously hypertensive rats by 10% fish oil feeding while the elastic material properties of the myocardium were not affected [41, 57]. The passive pressure volume curve of the left ventricle is obtained by stepwise filling/emptying the arrested ventricle with defined volumes of saline. Since in the untreated rats the curve is shifted to greater volumes, it can be concluded that the omega-3 fatty acid treatment counteracted the adverse dilatation of the pressure overloaded left ventricle

oped in 27 patients of the control group (33.3%) and in 12 patients of the EPA and DHA ethyl ester group (15.2%) ($p = 0.013$). There was no significant difference in the incidence of non-fatal postoperative complications, and postoperative mortality was similar in the EPA and DHA ethyl ester-treated patients (1.3%) versus controls (2.5%). After CABG, the EPA and DHA ethyl ester-treated patients were hospitalized for significantly fewer days than controls (7.3 ± 2.1 days vs. 8.2 ± 2.6 days, $p = 0.017$).

In addition to inhibitory effects on ion channels by EPA and DHA, the functional impact of various putative anti-arrhythmogenic mechanisms remains to be examined in greater detail. In particular, contributions arising from an increased heart rate variability [54], an improved postischaemic recovery [55] and a reduced heart rate [56] require further attention. It remains also an intriguing possibility that the process of adverse dilatation of the heart could be attenuated by EPA and DHA as shown in the pressure overloaded rat heart. When spontaneously hypertensive rats were treated with fish oil, the degree of left ventricular hypertrophy was not affected, however, the dilatation of the left ventricle was significantly reduced (Figure 7). Since cardiac dilatation is a major predisposing factor for SCD, it could be inferred from these animal experiments that a reduced adverse geometrical remodeling of the heart contributes to the observed protection of the GISSI-Prevention study.

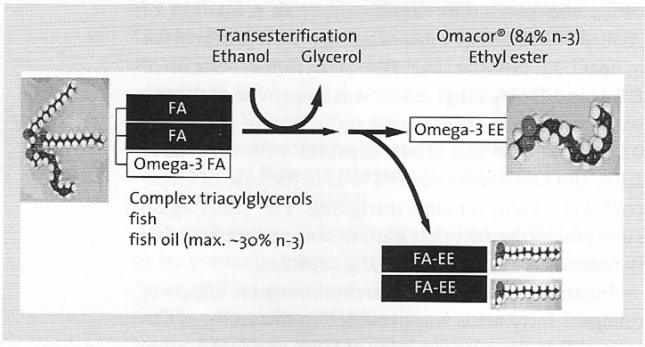


Figure 8A: Transesterification of triacylglycerols from fish leading to a highly purified EPA and DHA ethyl ester preparation. EPA and DHA are omega-3 fatty acids since the first double bond is at the position three when counting from the methyl end.

The EPA+DHA Level and the Sustained Uptake of EPA and DHA Ethyl Esters

Whether a required level of non-membrane bound EPA+DHA is reached, depends not only on the fatty acid release from tissue stores but also on the absorption of orally administered EPA and DHA. These fatty acids can be administered as the chemically prepared ethyl esters (Omacor®), chemically prepared free fatty acids (to our knowledge not used as commercially available compounds) or the naturally occurring triacylglycerols present in fish and fish oil. Initially, the rationale for ethyl esters was to provide a high-purity EPA and DHA preparation, which reduced the number of daily required capsules. In fish, the triacylglycerols contain on average one EPA or DHA and two saturated fatty acids per glycerol molecule. Thus, in fish oils approximately one-third of the fatty acids are EPA and DHA. For achieving a much higher concentration of EPA and DHA, one has to substitute the glycerol with an alcohol with only one hydroxyl group resulting in the individual fatty acid esters. Fish oils can be transesterified with alcohol such as methanol and ethanol. Methanol is used routinely during the derivatization of fatty acids for their gas chromatographic determination. Methylation is also used for the large-scale production of plant oil based fuel ("biodiesel") [58]. In the case of omega-3 fatty acid esters for human consumption, ethanol is used which results in a mixture of saturated and unsaturated ethyl esters. Based on the different physico-chemical properties arising from chain length and number of double bonds, nearly homogeneous EPA and DHA ethyl esters can be isolated and the respective saturated fatty acids are discarded in the following purification process (Figure 8A). This procedure obviously increases the production costs when compared with the simple extraction procedure used for fish oil. An important side-effect of this purification relates to reduction in environmental pollutants such as methyl-mercury to very low levels (see below).

A representative gas chromatogram of Omacor® injected directly without derivatization is shown in Figure 8B. A chromatogram is also given for a sample which was obtained by mixing 1:1 untreated Omacor® and Omacor® which had been transesteri-

fied with methanol. For this sample, 2 peaks corresponding to methyl and ethyl esters of EPA, DHA and other minor fatty acids were observed (Figure 8B). The procedure of transesterification has often been misinterpreted by stating that it represents just a "refinement" and ethyl esters of EPA and DHA are referred to as some kind of "highly purified fish oil" which considers, however, only one aspect of the production process. The reason why less attention has been given to the transesterification step relies probably in the fact that early comparative studies on triacylglycerols and ethyl esters have not been judged encouraging with respect to the absorption kinetics of ethyl esters. Since the GISSI-Prevention study was performed with EPA and DHA ethyl esters and a reduction in the risk of sudden death by 45% was observed, questions are raised on the biological relevance of conclusions drawn from the early studies, which judged ethyl esters as less favorable compared with triacylglycerols.

Lawson et al [59] studied in humans the plasma incorporation of EPA after administration of 1 g EPA either as ethyl ester or triacylglycerol during 8 h after intake. Ethyl esters were poorly absorbed and even triacylglycerols were not completely absorbed during this short time interval. It was concluded that ethyl esters are poor substrates for pancreatic lipase which is in accordance with in vitro studies. 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 [60]. Also el Boustani et al [61] reported a reduced absorption of ethyl esters within 12 h when compared with the free fatty acid or a glycerol ester. It was, therefore, an intriguing finding by Luley et al [62] that in healthy volunteers the long-term (after 7–28 days) bioavailability does not differ between ethyl esters and triacylglycerols. In particular, no differences were observed after the intake of 2 x 1 capsule of a 85% EPA and DHA ethyl ester preparation (1.70 g EPA+DHA) compared with 3 x 2 capsules of a 32% EPA and DHA triacylglycerol preparation (1.92 g EPA+DHA). These findings are noteworthy since a twice daily administration would in general be expected to result in a lower blood level compared with a three times daily administration. Since blood was drawn in the morning and no further information is given on the time in between capsule intake and blood sampling [62], no conclusions can, however, be drawn on the peak-to-trough values of EPA and DHA concentration in the blood after the intake of EPA and DHA in the form of triacylglycerols or ethyl esters.

The type of ester bond of EPA and DHA has thus consequences for the absorption kinetics of EPA and DHA and the duodenal uptake rates differ between triacylglycerols and ethyl esters. Triacylglycerols are rapidly degraded by pancreatic lipase and, in the case

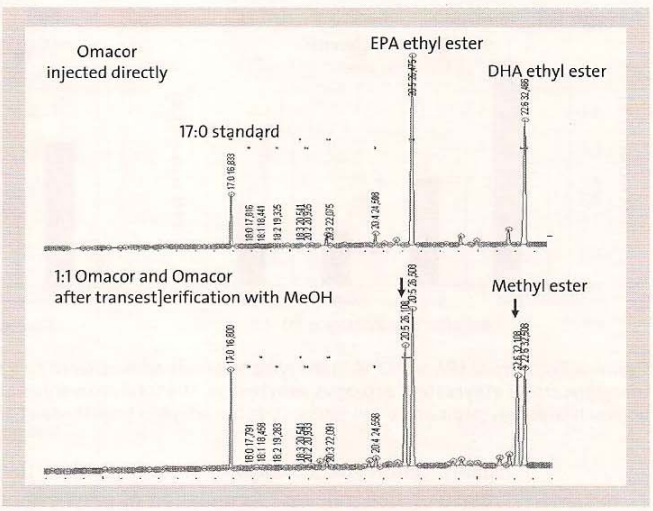


Figure 8B: A representative gas chromatogram of Omacor® injected without derivatization into the gas chromatograph. A chromatogram is also shown for a sample which was obtained by 1:1 mixing untreated Omacor® with Omacor® which has been transesterified with methanol. In this sample, 2 peaks corresponding to methyl and ethyl esters of EPA, DHA and also other minor fatty acids are observed.

of polyunsaturated fatty acids, particularly by carboxyl ester hydrolase. Compared with the corresponding triacylglycerols, the synthetic 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 and the recovery of the administered fatty acids was determined in the lymph [63]. Within 3 h after the administration, the recovery in the lymph of the respective fatty acids was greater in the case of triacylglycerols [63] (Figure 9). After 15 h, the recovery from ethyl esters was, however, 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 is of importance, since ventricular tachyarrhythmias are more abundant in the early morning hours [64]. 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 within a 24 h period seen in the rat appear to hold also for humans.

We examined in healthy volunteers whether 18 h after the intake of one capsule of Omacor®, increased concentrations of EPA and DHA occur in the serum. Concentrations were determined 18 h after intake of one capsule at days 1, 3, 7, 11, 15, 30 and 43. At day 1, 15, 30 and 43 concentrations were determined at 3, 6, 9, 18 and 24 h after the intake. EPA and DHA were increased in the serum after 3, 7, 11, 15, 30 and 43 days and the levels did not differ significantly at 3 vs. 18 h after capsule intake (H. Rupp et al, unpub-

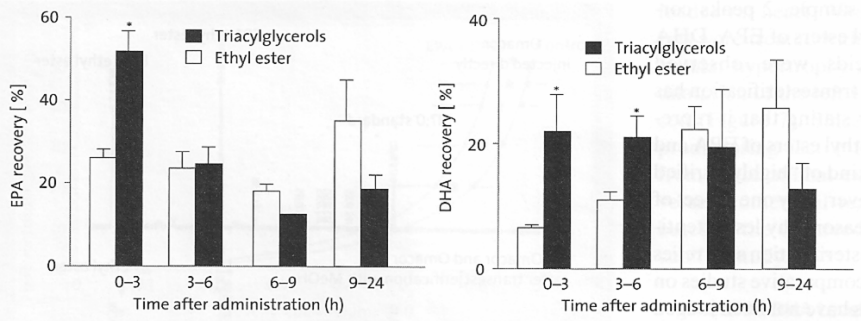


Figure 9: Recovery of EPA and DHA in the lymphe of rats administered by gavage EPA and DHA either as triacylglycerol or ethyl ester. *p<0.05 vs. ethyl esters. The total recovery after 24 h did not differ significantly between triacylglycerols and ethyl esters. Data are adapted from Ikeda et al [63].

lished). These data indicate, therefore, that the "retard or slow release" formulation of ethyl esters has the advantage of providing sustained increased non-membrane bound EPA and DHA levels (in the form of blood triacylglycerols and VLDL), which are expected to contribute to the critical rise in EPA and DHA required for an anti-arrhythmogenic action.

These considerations are based on a once daily administration which is relevant in patients after myocardial infarction being on standard therapy with a beta-blocker, ACE-inhibitor, anti-platelet drug and a statin. It remains to be assessed to what extent the 45% risk reduction of sudden death observed in the GISSI-Prevention study [20, 26, 27] arises from the administration of ethyl esters of EPA and DHA as compared with triacylglycerols. In this respect it should be pointed out that no interventional study comparable to the GISSI-Prevention study is currently available for EPA and DHA triacylglycerols of fish or fish oils.

Dietary Sources of Omega-3 Fatty Acids

EPA and DHA Triacylglycerols in Prepared and Frozen Fish

Since in the GISSI-Prevention study 840 mg EPA and DHA ethyl esters were administered corresponding to 1 g/day Omacor® in addition to regular fish consumption as might also be inferred from a Mediterranean lifestyle, we addressed the question to what extent dietary fish intake contributes 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 [24]. The most often served fish dish was Alaska pollock containing 125±70 mg/100 g EPA+DHA. In addition to EPA and DHA, fish contains the saturated fatty acids palmitic acid (C16:0), stearic acid (C18:0), the omega-9 monounsaturated fatty acid oleic acid (C18:1) and the omega-6 polyunsaturated fatty acid linoleic acid (C18:2) (Table 3). The variable content of EPA and DHA per 100 g wet weight depends on a number of factors. Omega-3 polyunsaturated fatty acids are produced by algae particularly in cold water and are taken up by fish via the food chain. It is, therefore, not unexpected that the fish Tilapia, which lives in a warm water sea, exhibits the lowest EPA and DHA content. Another influence arises from the fat content of fish as exemplified by the eel. Although the eel was caught in a river nearby Marburg, it has the highest EPA and DHA content of all fish analyzed. As expected, the locally caught non-oily/fatty fish pike exhibits a low content of EPA and DHA. From a comparison of frozen (Table 4) versus prepared (cooked/baked) fish, it can be deduced that the EPA and DHA content appears not to be reduced appreciably by the present food preparation.

Taking into account that the average daily fish intake in Northern Germany is 18 g and 13 g in Southern Germany [65], 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 [66]. 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 once per week fish and 87.6% at the end [20, 26, 27].

Although fish consumption cannot provide the EPA and DHA intake achieved in the GISSI-Prevention study, it appears to have various beneficial effects in primary prevention as demonstrated in epidemiological 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 [67]. 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 ischaemic heart disease, especially arrhythmic death [68]. In a prospective study in diabetic women, an inverse association between fish and long-chain omega-3 fatty acid consumption and risk of coronary heart disease and total mortality was observed [69]. A recent meta-analysis of 11 studies including 222,364 individuals with an average 11.8 years of follow-up showed that, compared with those who never consumed fish or ate fish less than once per month, individuals with a higher intake of fish had lower coronary heart disease mortality [70]. However, in intervention trials involving intake of EPA and DHA triacylglycerols, cardiovascular effects

	Tilapia	Crab	Zander	Hoki	Red snapper	Pollock	Cat fish	Shell	Common sole	Eel smoked
8:0	ND	0.4	4.5	ND	ND	3.7	0.7	5.1	12.0	0.1
10:0	1.5	1.0	2.2	ND	4.0	<0.05	2.2	17.1	8.8	0.2
11:0	0.4	ND	2.2	ND	0.6	<0.05	0.3	2.2	1.0	0.3
12:0	4.0	1.6	3.4	ND	7.9	0.5	3.7	23.8	16.6	5.5
13:0	0.5	ND	1.0	ND	0.4	<0.05	0.3	ND	2.5	<0.05
14:0	53.4	6.6	15.0	3.6	27.6	10.8	26.3	109.8	73.0	147.0
14:1	3.6	0.4	2.7	ND	1.6	0.2	1.5	4.5	0.8	4.5
15:0	5.3	2.7	5.0	0.8	4.3	1.1	4.1	20.8	13.2	13.2
15:1	0.1	<0.05	0.5	<0.05	ND	ND	<0.05	0.2	<0.05	0.3
16:0	395.2	54.0	133.6	156.1	170.0	353.4	159.5	443.4	306.7	748.9
16:1	70.7	6.9	11.0	9.8	19.1	4.9	48.1	109.7	67.8	354.3
17:1	ND	ND	3.5	ND	ND	8.9	0.6	2.8	7.7	11.8
18:0	99.0	22.3	36.3	34.6	54.7	50.5	39.7	117.0	91.8	148.9
18:1n9t	9.1	0.4	3.8	0.4	3.1	2.5	4.6	6.2	16.7	3.9
18:1n9c	478.1	18.8	169.6	93.5	102.9	438.4	175.6	173.9	198.1	947.1
18:2n6t	<0.05	0.1	1.0	ND	0.2	<0.05	0.2	ND	0.2	6.2
18:2n6c	234.5	2.4	101.1	10.7	14.5	117.0	13.0	34.6	17.1	98.1
20:0	3.7	0.2	4.1	ND	0.5	3.8	0.4	1.5	1.4	3.0
18:3n6	10.8	0.1	1.3	ND	0.8	0.4	0.3	0.8	9.8	2.7
20:1	22.9	0.7	5.9	3.4	1.9	6.8	50.6	34.2	7.6	23.3
18:3n3	16.4	1.0	5.1	1.6	3.2	0.7	9.4	38.0	6.1	88.1
21:0	0.6	<0.05	23.7	ND	0.2	12.2	2.6	20.5	4.0	0.7
20:2	13.9	1.1	1.1	0.8	1.2	0.2	5.5	25.7	6.3	14.0
20:3n9	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.6
22:0	1.2	0.3	2.4	ND	0.6	0.6	0.3	0.4	ND	0.7
20:3n6	13.4	0.6	0.6	0.4	2.1	0.6	0.5	2.0	17.4	7.3
22:1n9	1.0	3.9	1.0	0.2	0.5	1.4	2.5	0.5	4.6	0.9
20:3n3	2.4	0.4	4.0	0.1	0.6	0.5	ND	1.6	3.8	9.3
20:4n6	28.9	7.9	20.6	14.3	40.3	8.2	31.7	19.3	77.7	46.1
23:0	ND	ND	0.1	ND	0.2	ND	0.1	2.0	ND	1.5
22:2	ND	ND	2.2	ND	ND	2.0	0.3	3.4	12.4	12.1
24:0	2.7	ND	1.6	ND	ND	<0.05	ND	ND	0.9	ND
20:5n3	1.9	42.9	28.0	44.2	18.8	58.2	85.2	174.6	55.7	115.4
24:1	ND	ND	5.9	1.0	1.2	1.1	ND	0.1	ND	0.6
22:6n3	15.5	23.0	81.5	188.1	124.6	119.2	136.7	223.6	286.4	103.4
EPA+DHA	17.4	66.0	109.5	232.3	143.3	177.4	222.0	398.1	342.1	218.9

Table 3: Content (mg/100 g wet weight) of fatty acids in fish prepared at the cafeteria of the University Hospital (Lahnberge) Marburg.

were far 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 [71].

Based on the current evidence derived from epidemiological and interventional 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/day EPA and DHA while subjects without documented coronary heart disease should eat (preferably) oily fish at least twice a week [72]. This recommendation is wider than the current indication for Omacor®, which is a prescription drug in Germany

	Cod	Plaice	Pollock	Alaska Pollock	„Wild" Pollock	Pike	Eel
8:0	ND	ND	1.2	0.7	ND	14.9	9.5
10:0	ND	ND	0.1	< 0.05	0.2	4.3	0.1
11:0	ND	ND	< 0.05	< 0.05	0.3	0.7	2.7
12:0	ND	ND	0.3	0.2	0.9	1.9	18.3
13:0	ND	ND	< 0.05	<0.05	0.1	3.1	1.5
14:0	4.1	21.9	1.4	0.9	25.7	2.7	200.8
14:1	ND	1.4	< 0.05	< 0.05	0.2	2.8	7.0
15:0	1.2	4.9	0.5	0.2	2.4	3.1	27.1
15:1	0.1	1.4	< 0.05	< 0.05	< 0.05	3.0	2.3
16:0	80.3	154.9	31.0	29.1	90.5	52.0	1026.0
16:1	ND	76.8	1.1	1.6	22.2	9.2	627.7
17:1	0.8	3.9	0.3	0.1	2.4	2.7	33.0
18:0	14.5	24.8	8.9	4.7	46.8	18.7	203.3
18:1n9t	< 0.05	8.2	0.3	0.2	ND	2.0	20.6
18:1n9c	50.1	105.5	20.6	10.9	136.9	30.3	1999.3
18:2n6t	1.2	3.9	< 0.05	< 0.05	3.4	1.4	10.1
18:2n6c	2.3	6.2	1.9	5.2	9.0	11.7	163.1
20:0	0.2	0.8	0.1	0.2	5.2	10.1	42.1
18:3n6	1.5	3.9	2.0	3.8	0.1	3.5	41.9
20:1	0.7	9.3	6.6	0.8	74.2	3.7	53.3
18:3n3	2.2	9.2	< 0.05	ND	11.5	1.2	48.9
21:0	1.1	1.7	0.7	0.5	0.4	3.4	172.3
20:2	0.9	3.7	0.3	0.1	4.0	3.9	63.6
20:3n9	ND	ND	ND	ND	0.4	ND	ND
22:0	0.8	1.2	< 0.05	< 0.05	0.4	0.1	24.8
20:3n6	1.2	1.6	2.1	1.8	1.0	5.8	28.5
22:1n9	1.4	0.3	0.3	0.2	ND	2.0	6.6
20:3n3	0.4	0.1	< 0.05	< 0.05	ND	5.2	7.6
20:4n6	8.0	23.6	2.7	1.0	45.5	22.9	86.8
23:0	3.3	0.1	ND	ND	1.6	ND	ND
22:2	1.0	1.7	ND	< 0.05	5.8	8.0	4.2
24:0	0.2	0.1	0.7	0.3	0.1	6.2	53.2
20:5n3	36.1	112.4	10.8	12.1	52.7	24.3	292.1
24:1	ND	0.3	1.8	0.7	2.5	7.1	6.3
22:6n3	129.2	24.1	55.5	37.2	166.1	95.3	155.0
EPA+DHA	165.3	186.5	66.3	49.3	218.8	119.6	447.1

Table 4: Content (mg/100 g wet weight) of fatty acids in frozen fish.

for post-myocardial 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 omega-3 polyunsaturated fatty acids was rated as class I recommendation, level of evidence B (because of currently only one randomized study, i.e. GISSI-Prevention study) [73].

EPA and DHA Triacylglycerols in Fish Oils

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 11 over-the-counter brands of fish oil capsules available in Germany. Using the C17:0 fatty acid standard, the amount of EPA and DHA per capsule was determined and compared with the specified content. The capsules differed markedly

	Fish oil 1	Fish oil 2	Fish oil 3	Fish oil 4	Fish oil 5	Fish oil 6	Fish oil 7	Fish oil 8	Fish oil 9	Fish oil 10	Fish oil 11	Halibut liver oil	Cod liver oil	Omacor®
8:0	0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.4
10:0	<f0.05	<0.05	1.5	0.1	0.1	ND	ND	ND	ND	ND	ND	ND	<0.05	0.8
11:0	<0.05	<0.05	<0.05	<0.05	<0.05	ND	ND	ND	ND	ND	ND	ND	0.1	0.1
12:0	1.1	0.7	1.3	0.7	0.5	ND	ND	ND	ND	ND	ND	ND	0.5	0.4
13:0	0.4	0.2	0.4	0.2	0.2	ND	ND	ND	ND	ND	ND	ND	0.3	<0.05
14:0	66.3	32.6	62.1	34.9	34.4	38.4	40.7	49.7	27.7	65.3	90.9	1.4	66.6	0.3
14:1	0.3	0.5	0.3	0.3	0.1	0.3	0.3	0.5	0.3	0.6	0.8	<0.05	1.6	0.1
15:0	4.7	2.3	4.8	3.3	2.4	3.1	3.2	3.2	1.9	4.4	5.9	0.2	5.9	0.1
15:1	0.1	<0.05	0.2	0.4	0.3	<0.05	0.4	0.1	<0.05	<0.05	0.1	<0.05	1.0	0.2
16:0	136.2	73.9	143.3	79.8	75.2	92.1	87.1	119.2	54.2	160.3	178.0	17.0	203.0	0.4
16:1	73.4	35.6	75.2	36.8	42.8	46.4	47.4	49.3	28.4	70.0	94.3	2.4	142.0	1.4
17:1	10.0	3.9	10.2	4.7	6.0	7.1	6.7	7.8	5.1	11.0	15.4	0.2	17.6	<0.05
18:0	26.3	13.0	26.3	16.3	15.8	25.7	22.4	44.8	16.1	64.3	46.6	5.1	27.6	0.7
18:1n9t	1.2	0.6	11.8	4.9	6.6	0.8	0.5	1.0	0.4	0.9	1.9	0.1	53.9	0.2
18:1n9c	71.3	44.6	84.8	56.3	57.6	73.8	45.1	67.7	32.8	62.2	91.0	182.0	432.1	1.2
18:2n6t	0.7	0.2	2.7	1.9	0.8	9.2	0.6	ND	0.4	1.0	1.3	0.3	3.8	<0.05
18:2n6c	29.8	10.0	17.2	6.1	8.6	16.5	6.7	7.4	3.7	10.9	14.2	54.8	28.0	1.2
20:0	9.9	1.3	1.2	1.0	1.1	1.2	1.3	2.0	0.8	2.3	2.8	1.7	2.3	0.3
18:3n6	3.3	0.8	4.6	0.6	1.5	1.0	1.5	1.2	0.7	2.4	2.2	1.7	3.9	ND
20:1	9.3	1.6	14.1	11.0	10.4	1.6	0.5	0.8	0.6	1.1	1.5	1.7	ND	1.0
18:3n3	8.8	11.5	10.8	3.4	1.2	5.2	4.4	5.7	2.5	6.2	9.1	24.1	234.9	0.4
21:0	0.4	0.1	1.3	0.3	0.7	1.3	ND	ND	ND	ND	ND	ND	ND	ND
20:2	24.0	0.9	30.0	17.2	11.8	13.3	17.6	19.4	8.9	23.5	35.5	5.8	ND	16.2
20:3n9	4.1	12.2	0.6	0.6	0.4	ND	1.3	ND	ND	ND	ND	ND	ND	ND
22:0	1.4	0.6	1.1	0.3	0.2	0.2	0.2	0.2	0.2	0.4	0.3	0.1	ND	5.5
20:3n6	1.7	0.5	2.7	1.2	0.7	0.3	0.8	0.9	ND	1.0	1.5	0.9	ND	1.1
22:1n9	5.3	8.7	ND	ND	ND	0.5	0.5	0.1	ND	0.1	0.1	0.1	ND	0.3
20:3n3	1.3	0.7	ND	2.0	4.9	0.3	0.4	0.6	0.7	0.7	0.9	<0.05	ND	0.2
20:4n6	6.8	3.0	22.4	6.1	5.2	4.8	5.2	6.1	4.5	7.6	11.9	1.8	165.5	29.0
23:0	ND	ND	0.5	0.9	0.2	<0.05	ND	ND	0.1	0.1	0.1	1.5	12.3	ND
22:2	0.3	3.9	9.2	ND	ND	5.9	4.5	4.4	2.4	6.9	8.8	0.3	ND	0.3
24:0	0.1	<0.05	ND	ND	ND	0.5	0.2	<0.05	0.1	<0.05	0.2	<0.05	ND	2.8
20:5n3	147.8	58.3	140.4	63.7	67.7	65.0	87.0	98.8	55.9	120.0	173.6	1.4	175.9	490.8
24:1	<0.05	1.8	4.8	ND	ND	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	3.4	9.2
22:6n3	101.1	40.4	87.4	67.1	43.3	58.6	59.3	60.5	33.0	80.0	102.0	1.5	207.8	391.4
Capsule content (mg)	991	499	845	502	498	498	501	544	299	680	980	275	1000	996
EPA+DHA	248.9	98.7	227.8	130.7	111.0	123.6	146.3	159.3	88.9	200.0	275.6	2.9	383.8	882
specified EPA+DHA	297.4	120	255	150 n3	150 n3	120	NA	150 n3	NA	180 n3	309 n3	NA	NA	840

Table 5: Fatty acid content (mg per capsule) of 11 over-the-counter fish oil brands (labeled as of Pollock origin), halibut liver oil, cod liver oil and Omacor®.

with respect to their EPA and DHA content per capsule (Table 5). The EPA and DHA content calculated for 1 g capsule content was comparable within the brands containing oil from pollock approaching 300 mg/g EPA and DHA [24]. This might suggest that the fish oils used for producing the capsules were similar, however, the oils differed actually with respect to the composition of other fatty acids. Major fatty acids were the saturated fatty acids palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and arachidonic acid (C20:4) reflecting the composition of fish. Liver oils from halibut and cod are characterized by greater amounts of oleic acid and alpha-linolenic acid. In contrast to cod liver oil, the halibut oil capsules contained only a small amount of EPA and DHA.

	Linseed oil	Canola/ rape seed oil	Walnut oil	Sunflo-wer oil	Olive oil	Perilla oil
8:0	ND	ND	<0.05	ND	ND	<0.05
10:0	< 0.05	<0.05	<0.05	<0.05	<0.05	0.1
11:0	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
12:0	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
13:0	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
14:0	<0.05	0.1	<0.05	0.1	<0.05	<0.05
14:1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
15:0	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
15:1	<0.05	ND	<0.05	<0.05	<0.05	<0.05
16:0	5.3	4.6	7.5	6.4	12.5	13.6
16:1	0.1	0.2	0.2	0.1	0.9	0.2
17:1	ND	<0.05	<0.05	<0.05	0.1	<0.05
18:0	1.2	0.7	3.9	4.6	1.5	1.6
18:1n9t	8.9	ND	ND	<0.05	ND	<0.05
18:1n9c	13.0	64.6	15.2	30.0	77.4	14.3
18:2n6t	2.9	0.5	ND	ND	ND	<0.05
18:2n6c	12.0	19.9	59.3	55.8	5.5	12.8
20:0	ND	0.7	0.4	1.5	0.8	0.1
18:3n6	ND	0.2	0.3	ND	ND	0.1
20:1	ND	ND	6.5	0.1	ND	ND
18:3n3	56.1	8.0	6.5	0.5	1.0	56.6
21:0	ND	ND	0.2	<0.05	0.1	ND
20:2	0.2	0.1	0.1	<0.05	<0.05	0.2
20:3n9	0.2	0.3	<0.05	0.5	0.1	0.2
22:0	<0.05	ND	<0.05	<0.05	<0.05	ND
20:3n6	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
22:1n9	<0.05	ND	<0.05	0.1	ND	<0.05
20:3n3	<0.05	<0.05	<0.05	<0.05	0.1	<0.05
20:4n6	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
23:0	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
22:2	ND	0.2	<0.05	<0.05	<0.05	<0.05
24:0	<0.05	ND	<0.05	0.2	<0.05	<0.05
20:5n3	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
24:1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
22:6n3	<0.05	<0.05	<0.05	0.1	<0.05	<0.05

Table 6: Fatty acid composition (%) of plant oils as potential sources of alpha-linolenic acid which is the precursor fatty acid of EPA and DHA.

Mercury, Dioxins and PCBs in Fish Products

One might argue that the amount of daily consumed fish or fish oil could be raised in general. A problem associated with a high intake of fish relates to the contamination with methyl-mercury and various environmental pollutants including dioxins and polychlorinated biphenyls (PCBs). According to the US Environmental Protection Agency, the maximum safe level of mercury in edible fish is 0.55 ppm which was exceeded in a recent study in 4 out of 5 fish in California waters, whereby the maximum content was 7.5 ppm [74]. It is, therefore, not unexpected that patients in San Francisco with a history of fish consumption had a mercury blood level of 15 µg/l (women) and 13 µg/l (men) [75] which correspond to 0.015 and 0.013 ppm. The mean level for women in this survey was 10 times that of mercury levels found in a recent population survey by the U.S. Centers for Disease Control and Prevention. Some children were > 40 times the national mean. Mercury levels declined after stopping fish consumption. Although symptoms of mercury poisoning

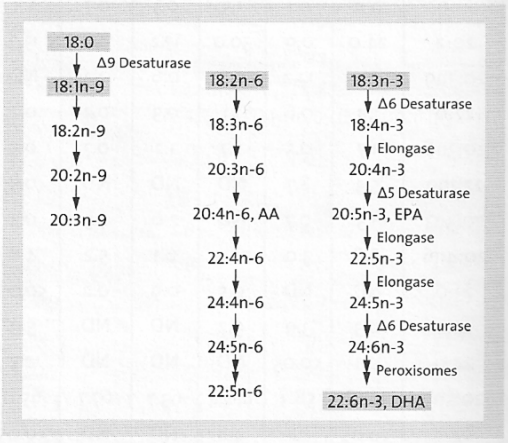


Figure 10: Biosynthesis of unsaturated fatty acids. Oleic acid (18:1n-9) can be produced by humans while linoleic acid (18:2n-6) and linolenic acid (18:3n-6) are essential fatty acids and are produced mainly by plants. EPA and DHA are produced by marine algae which are taken up by fish via the food chain. When referring to an "omega-3 level", one has to include also the short-chain omega-3 fatty acid alpha-linolenic acid as well as the long-chain omega-3 fatty acids EPA and DHA. When 24:5n-6 (6,9,12,15,18-24:5) and 24:6n-3 (6,9,12,15,18,21-24:6) are produced in the endoplasmic reticulum, they move to peroxisomes for conversion to 22:5n-6 (4,7,10,13,16-22:5) and 22:6n-3 (4,7,10,13, 6, 19-22:6) [83].

apparently arising from consuming tuna steak almost every night were observed as having trouble concentrating, feeling sluggish and experiencing hand tremors, they appear not to be experienced by all subjects [76]. In preschool Inuit children from Canada, tremor amplitude was related to blood mercury concentrations, which corroborates an effect already reported among adults [77]. In accordance, the Food and Drug Administration advised pregnant women and children not to exceed 3 to 4 fish servings per week and to avoid certain predatory fish [72].

Although PCBs have various negative health consequences [76], specific adverse actions on the cardiovascular system have been reported for mercury. The mercury level in the toenail which represents a long-term storage site for heavy metals was directly associated with the risk of myocardial infarction while, as expected, the adipose-tissue DHA level was inversely associated with the risk [78]. After adjustment for the DHA level and coronary risk factors, the mercury levels in patients were 15% higher than those in controls. The risk-factor-adjusted odds ratio for myocardial infarction associated with the highest as compared with the lowest quintile of mercury was 2.16 (P for trend = 0.006). After adjustment for the mercury level, the DHA level was inversely associated with the risk of myocardial infarction (P for trend = 0.02). A high mercury content may, therefore, diminish the cardioprotective effect of fish intake. It was concluded that substitution of fish with high methyl-mercury concentrations with fish containing less methyl-mercury among women of childbearing age yield substantial developmental benefits and few negative impacts [79]. If women instead decrease fish consumption, countervailing risks substantially reduce net benefits. If other adults reduce their fish consumption, the net public health impact is expected to be negative [79].

The question arises, therefore, whether fish oil capsules provide a substitute of fish intake in primary prevention with the suggested two fish meals per week. The mercury content of 3 over-the-counter fish oil supplements was < 6 µg/l, in one brand 10 µg/l and in another brand 12 µg/l [80]. By contrast, the amount of mercury was specified as low as ≤ 0.2 µg per 1 g capsule for Omacor®. In fish oils, the amount of PCBs and organochlorine pesticides was less than the Food and Drug Administration daily recommended limits [80]. However, in an extensive study of the UK Food Standards Agency, in 12 of the 33 samples of fish oil supplements, the concentration of dioxins exceeded the European Commission's limit for dioxins which compares with 10 out of 15 samples in an Irish study (http://www.food.gov.uk/multimedia/pdfs/26diox.pdf). Manufacturers were asked to withdraw batches of two products which, on their own, could result in intakes of dioxins and dioxin like PCBs that would exceed twice the Tolerable Daily Intake (TDI). While not stated in guidelines, it appears that patients with coronary heart disease should have a much lower intake of toxins particularly mercury since they have been advised to consume 1 g EPA and DHA per day. In particular, a daily 1 g EPA and DHA dosage is advised for post-myocardial infarction patients who have already a markedly increased risk for coronary events. EPA and DHA supplements for post-myocardial infarction patients should, therefore, have toxin levels which are as low as technically achievable. For minimizing the amount of methyl-mercury and other lipid soluble environmental toxins, various purification steps including the transesterification procedure appear to be needed. While over-the-counter dietary supplements are currently not oblig-

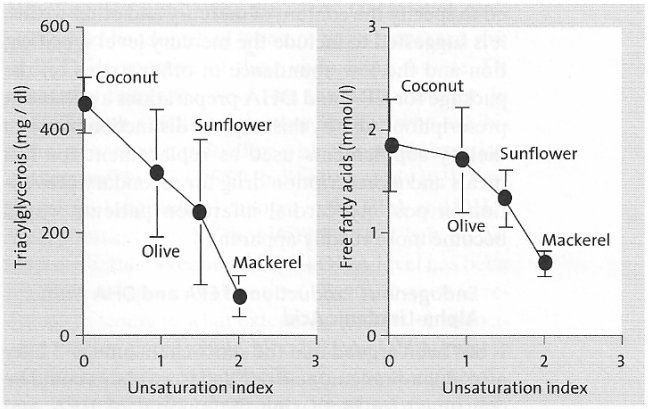


Figure 11: The inter-relationship between the serum concentration of triacylglycerols or free fatty acids and the unsaturation index in rats fed 6 weeks diets which contained 10% coconut fat, 10% olive oil, 10% sunflower oil or 10% mackerel oil. The unsaturation index was calculated by multiplying the percent proportion of each fatty acid with the number of double bonds contained in that fatty acid. The values thus obtained were summed over all the fatty acids present. The average number of double bonds per molecule was obtained by referring the number of double bonds to 100%. Data are adapted from Rupp et al [39].

	Control	1%	2.5%	5%
18:3n3	2.3 ± 0.6	4.7 ± 1.6*	7.6 ± 0.9*	11.7 ± 2.2*
20:4n6	14.4 ± 3.4	12.2 ± 2.5	11.8 ± 3.6	10.4 ± 3.1*
20:5n3	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.4 ± 0.3
22:6n3	1.5 ± 0.5	1.4 ± 0.8	1.4 ± 0.5	0.6 ± 0.2
N6/n3	6.4 ± 1.3	4.7 ± 1.1*	3.1 ± 0.3*	2.3 ± 0.2*
PGF_{1alpha}	1.0 ± 0.3	1.8 ± 0.8	3.6 ± 1.8*	5.1 ± 1.4*

Table 7: Effect of an increased dietary alpha-linolenic acid intake on the fatty acid composition and prostanoids of the aorta of spontaneously hypertensive rats. Rats were fed diets containing 1%, 2.5% and 5% linseed oil for 15–16 weeks. Prostanoids were determined with HPLC and electrochemical detection (as 2,4-dimethoxyanilides after derivatization). TXB_2, PGE_2 and PGF_{2alpha} were not altered. Systolic and diastolic blood pressure measured by radio telemetry was reduced by 6 mm Hg (in sleeping phase) after 5% linseed oil feeding for 7 weeks. Data are from Rupp et al [81].

ed to specify the content of mercury and other toxins, it is suggested to include the mercury level specification and the low abundance of other toxins on the package for EPA and DHA preparations available at prescription. Also by this way, the distinction between dietary supplements used as replacement for fish meals and a prescription drug for secondary prevention in post-myocardial infarction patients would become more readily apparent.

Endogenous Production of EPA and DHA from Alpha-Linolenic Acid

It has been argued that the short-chain omega-3 fatty acid alpha-linolenic acid derived from plants could be consumed for increasing the amount of EPA and DHA in the body by the enzymes Δ-6 desaturase and Δ-5 desaturase (Figure 10). Of particular interest are plant oils with a high proportion of alpha-linolenic acid such as linseed oil, canola oil/rapeseed oil and walnut oil (Table 6). For the rat we have shown that feeding increasingly greater amounts of linseed oil does not increase the content of EPA and DHA in the aorta [81] (Table 7). However, the enhanced alpha-linolenic acid intake reduced the arachidonic acid level and enhanced the production of PGF_{1alpha} which is the stable degradation product of prostacyclin. Thus, although an increased alpha-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. The increased prostacyclin production is expected to contribute to the improvement in endothelial function when the alpha-linolenic intake is increased [82].

A further beneficial action of polyunsaturated fatty acids relates to their triacylglycerol-lowering. As shown in rat experiments, a correlation exists between the serum triacylglycerol and free fatty acid concentration and the unsaturation index of fatty acids [39] (Figure 11). For omega-3 fatty acids, additional specific actions have to be inferred. EPA but not DHA inhibited the diacylglycerol acyltransferase, thereby reducing the biosynthesis of triacylglycerols [84]. If intracellular EPA becomes elevated it is expected to activate PPARalpha which is a major determinant for the gene expression of enzymes involved in fatty acid oxidation. PPARalpha is down regulated in failing heart [85] and by this mechanism also the fatty acid profile is expected to be altered (Rupp et al, unpublished).

In humans with a background diet high in saturated fat, the conversion rate of alpha-linolenic acid was found to be 6% for EPA and 3.8% for DHA [86]. However, with a diet rich in omega-6 polyunsaturated fatty acids, conversion was reduced by 40 to 50% [86]. In women of reproductive age, an increased conversion of alpha-linolenic acid into EPA (21%) and DHA (9%) was found which is higher than in men [87]. The higher conversion capacity appears to be important for meeting the demands of the fetus and neonate during pregnancy and lactation [87]. In this respect it is of great interest that any administered DHA can be retroconverted to EPA [88]. As regards secondary prevention of myocardial infarction, alpha-linolenic acid cannot be converted into amounts of EPA and DHA which have been used in the GISSI-Prevention study [20, 26, 27]. There is also increasing evidence that the capacity for EPA and DHA production becomes reduced in patients with cardiac dysfunction (Rupp et al, unpublished).

It is of particular interest to compare the risk reduction by omega-3 fatty acids and established prescription drugs in patients with coronary heart disease as done in a recent meta-analysis on antilipidemic interventions including 97 studies with 137,140 individuals in intervention and 138,976 individuals in control groups [89]. Compared with control groups, risk ratios for overall mortality were 0.87 for statins, 1.00 for fibrates, 0.84 for resins, 0.96 for niacin, 0.77 for omega-3 fatty acids, and 0.97 for diet [89]. The mortality risk was reduced significantly only by statins and omega-3 fatty acids. Although this meta-analysis included also presumable effects of alpha-linolenic acid, a major contribution is expected to arise from EPA and DHA. Also based on the evaluation of primary and secondary prevention studies, it was concluded that the "Omega-3 Index" (red blood cell EPA+DHA) may represent a novel, physiologically relevant, easily modified, independent, and graded risk factor for death from coronary heart disease that could have significant clinical utility [90].

Conclusion

Taken together, there is increasing evidence that omega-3 fatty acids exhibit various protective actions in the cardiovascular system. A protection has been inferred from primary prevention studies based on the intake of fish. Stronger evidence comes from interventional studies with EPA and DHA ethyl ester administration involving post-myocardial infarction patients [20] and ICD patients [52]. Best characterized is currently the link between a low EPA+DHA level and the risk for SCD. Based on the studies of Albert et al [25] and Siscovick et al [29] demonstrating a markedly reduced risk of SCD in persons who exhibit about 3% more EPA and 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 SCD (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 regimen of the GISSI-Prevention study where the risk of total mortality, cardio-

vascular mortality and sudden death was significantly reduced [20, 26, 27]. It appears, however, that an upper limit of EPA and DHA concentrations exists above which further coronary heart disease benefit may not occur [30]. 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 [91]. This intriguing study by Nilsen et al was performed in Norway where the baseline EPA+DHA level was much higher than in other countries [30] including our own data. Whether adverse effects of mercury intake arising from fish might have interfered with protective actions of EPA and DHA remains unresolved.

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 anti-arrhythmogenic effects appear to 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. An ethyl ester preparation has also the advantage of only one capsule per day. As exemplified in the study of Leaf et al [52], the non-compliance rate is increased to 35% of ICD patients on four 1 g capsules per day.

Clearly, further work is required to delineate the relevance of other fatty acids [92] mentioned only briefly 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 "omega-3 level" are inaccurate when referring to EPA+DHA, since it has to include by definition the short-chain omega-3 alpha-linolenic acid as well as the long-chain EPA and DHA. As shown in rats fed increasing amounts of alpha-linolenic acid, the "omega-3 level" was increased due to the incorporation of alpha-linolenic acid while the EPA+DHA level was not altered [81]. Furthermore, the term "omega-3 level" has to include docosapentaenoic acid which was, however, not predictive of SCD in the study of Albert et al [25].

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 pa-tients after myocardial infarction. This would strengthen the rationale of therapeutic regimens with EPA and DHA as specified in current guidelines [72, 73]. 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 SCD irrespective of their underlying disease. Of particular relevance would be the determination in patients with a reduced ejection fraction who exhibited a greater risk reduction with the administration of Omacor® in the GISSI-Prevention study [93]. Furthermore, longitudinal changes in the EPA and DHA incorporation can be monitored and it can be assessed whether a required EPA+DHA level has been reached in a particular patient. Further work is required to assess to what extent the protection associated with a given whole blood EPA+DHA level is influenced by the pathophysiology of severe arrhythmia disorders. In particular, to what extent differences occur between re-entry tachyarrhythmias in the absence of a severe ischaemic event and tachyarrhythmias arising from myocardial infarction linked with sympathetic nervous system activation and release of polyunsaturated fatty acids.

References

[1] S.S. Chugh, J. Jui, K. Gunson et al. Current burden of sudden cardiac death: multiple source surveillance versus retrospective death certificate-based review in a large U.S. community. J. Am. Coll. Cardiol. 44 (2004) 1268-1275.
[2] N. Frasure-Smith, F. Lespérance, M. Talajic. Depression and 18-month prognosis after myocardial infarction. Circulation 91 (1995) 999-1005.
[3] Y. Osher, Y. Bersudsky, R.H. Belmaker. Omega-3 eicosapentaenoic acid in bipolar depression: report of a small open-label study. J. Clin. Psychiatry 66 (2005) 726-729.
[4] M.T. Kearney, K.A. Fox, A.J. Lee et al. Predicting sudden death in patients with mild to moderate chronic heart failure. Heart 90 (2004) 1137-1143.
[5] C.G. Brilla, R.C. Funck, H. Rupp. Lisinopril-mediated regression of myocardial fibrosis in patients with hypertensive heart disease. Circulation 102 (2000) 1388-1393.
[6] C.G. Brilla, H. Rupp, B. Maisch. Effects of ACE inhibition versus non-ACE inhibitor antihypertensive treatment on myocardial fibrosis in patients with arterial hypertension. Retrospective analysis of 120 patients with left ventricular endomyocardial biopsies. Herz 28 (2003) 744-753.
[7] M. Turcani and H. Rupp. Etomoxir improves left ventricular performance of pressure-overloaded rat heart. Circulation 96 (1997) 3681-3686.
[8] H. Rupp, W. Schulze, R. Vetter. Dietary medium-chain triglycerides can prevent changes in myosin and SR due to CPT-1 inhibition by etomoxir. Am. J. Physiol. 269 (1995) R630-R640.
[9] H. Rupp, T.P. Rupp, B. Maisch. Fatty acid oxidation inhibition with PPARalpha activation (FOXIB/PPARalpha) for normalizing gene expression in heart failure? Cardiovasc. Res. 66 (2005) 423-426.
[10] W. Grimm, M. Christ, J. Bach et al. Noninvasive arrhythmia risk stratification in idiopathic dilated cardiomyopathy: results of the Marburg Cardiomyopathy Study. Circulation 108 (2003) 2883-2891.
[11] P.J. de Kam, G.L. Nicolosi, A.A. Voors et al. Prediction of 6 months left ventricular dilatation after myocardial infarction in relation to cardiac morbidity and mortality. Application of a new dilatation model to GISSI-3 data. Eur. Heart J. 23 (2002) 536-542.
[12] P. Gaudron, I. Kugler, K. Hu et al. Time course of cardiac structural, functional and electrical changes in asymptomatic patients after myocardial infarction: their inter-relation and prognostic impact. J. Am. Coll. Cardiol. 38 (2001) 33-40.
[13] M.R. Franz, R. Cima, D. Wang et al. Electrophysiological effects of myocardial stretch and mechanical determinants of stretch-activated arrhythmias. Circulation 86 (1992) 968-978.
[14] Z. Wang, L.K. Taylor, W.D. Denney et al. Initiation of ventricular extrasystoles by myocardial stretch in chronically dilated and failing canine left ventricle. Circulation 90 (1994) 2022-2031.
[15] M.F. Hazinski, A.H. Idris, R.E. Kerber et al. Lay rescuer automated external defibrillator ("public access defibrillation") programs: lessons learned from an international multicenter trial: advisory statement from the American Heart Association Emergency Cardiovascular Committee; the Council on Cardiopulmonary, Perioperative, and Critical Care; and the Council on Clinical Cardiology. Circulation 111 (2005) 3336-3340.
[16] J.J. de Vreede-Swagemakers, A.P. Gorgels, W.I. Dubois-Arbouw et al. Out-of-hospital cardiac arrest in the 1990's: a population-based study in the Maas-

tricht area on incidence, characteristics and survival. J. Am. Coll. Cardiol. 30 (1997) 1500-1505.
[17] A. Dotevall, S. Johansson, L. Wilhelmsen et al. Increased levels of triglycerides, BMI and blood pressure and low physical activity increase the risk of diabetes in Swedish women. A prospective 18-year follow-up of the BEDA*study. Diabet. Med. 21 (2004) 615-622.
[18] C.N.Verboom, R. Marchioli, H. Rupp et al. Highly purified omega-3 fatty acids for secondary prevention of post-myocardial infarction. Royal Society of Medicine Press, London (2003).
[19] H. Rupp, C.N. Verboom, B. Jaeger. Saving lives post-MI: highly purified omega-3 PUFAs for the prevention of sudden death. J. Clin. Basic Cardiol. 5 (2002) 209-214.
[20] GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 354 (1999) 447-455.
[21] J. Folch, M. Lees, G.H. Sloane Stanley. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226 (1957) 497-509.
[22] W.K. Fulk and M.S. Shorb. Production of an artifact during methanolysis of lipids by boron trifluoride-methanol. J. Lipid Res. 11 (1970) 276-277.
[23] C. Stavarache, M. Vinatoru, Y. Maeda. Ultrasonic versus silent methylation of vegetable oils. Ultrason Sonochem 13 (2006) 401-407.
[24] H. Rupp, D. Wagner, T. Rupp et al. B. 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. Herz 29 (2004) 673-685.
[25] C.M. Albert, H. Campos, M.J. Stampfer et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N. Engl. J. Med. 346 (2002) 1113-1118.
[26] R. Marchioli, F. Avanzini, F. Barzi et al. Assessment of absolute risk of death after myocardial infarction by use of multiple-risk-factor assessment equations: GISSI-Prevenzione mortality risk chart. Eur. Heart J. 22 (2001) 2085-2103.
[27] R. Marchioli, F. Barzi, E. Bomba et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105 (2002) 1897-1903.
[28] D. Di Stasi, R. Bernasconi, R. Marchioli et al. Early modifications of fatty acid composition in plasma phospholipids, platelets and mononucleates of healthy volunteers after low doses of n-3 polyunsaturated fatty acids. Eur. J. Clin. Pharmacol. 60 (2004) 183-190.
[29] D.S. Siscovick, T.E. Raghunathan, I. King et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA 274 (1995) 1363-1367.
[30] D.W. Nilsen and W.S. Harris. n-3 Fatty acids and cardiovascular disease. Am. J. Clin. Nutr. 79 (2004) 166.
[31] J.V. Donadio Jr, T.S. Larson, E.J. Bergstralh et al. A randomized trial of high-dose compared with low-dose omega-3 fatty acids in severe IgA nephropathy. J. Am. Soc. Nephrol. 12 (2001) 791-799.
[32] E. Guallar, C.H. Hennekens, F.M. Sacks et al. A prospective study of plasma fish oil levels and incidence of myocardial infarction in U.S. male physicians. J. Am. Coll. Cardiol. 25 (1995) 387-394.
[33] G.C. Leng, G.S. Taylor, A.J. Lee et al. Essential fatty acids and cardiovascular disease: the Edinburgh Artery Study. Vasc. Med. 4 (1999) 219-226.
[34] P.J. Robinson, J. Noronha, J.J. DeGeorge et al. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: review and critical analysis. Brain Res. Brain Res. Rev. 17 (1992) 187-214.
[35] S.S. Nair, J. Leitch, J. Falconer et al. Cardiac (n-3) non-esterified fatty acids are selectively increased in fish oil-fed pigs following myocardial ischemia. J. Nutr. 129 (1999) 1518-1523.
[36] G.E. Billman, H. Hallaq, A. Leaf. Prevention of ischemia-induced ventricular fibrillation by omega 3 fatty acids. Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 4427-30.
[37] R. Schrepf, T. Limmert, P. Claus Weber et al. Immediate effects of n-3 fatty acid infusion on the induction of sustained ventricular tachycardia. Lancet 363 (2004) 1441-1442.
[38] A. Leaf, Y.F. Xiao, J.X. Kang et al. Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. Pharmacol. Ther. 98 (2003) 355-377.
[39] H. Rupp, R. Wahl, M. Hansen. Influence of diet and carnitine palmitoyltransferase I inhibition on myosin and sarcoplasmic reticulum. J. Appl. Physiol. 72 (1992) 352-360.
[40] D. von Au, M. Brändle, H. Rupp et al. Influence of a diet rich in fish oil on blood pressure, body weight and cardiac hypertrophy in spontaneously hypertensive rats. Eur. J. Appl. Physiol. Occup. Physiol. 58 (1988) 97-99.
[41] R. Jacob, M. Brändle, B. Dierberger et al. Antihypertensive und kardioprotektive Effekte verschiedener Öldiäten. In: D. Ganten, G. Mall (Eds): Herz-Kreislauf-Regulation, Organprotektion und Organschäden. Schattauer Verlag, Stuttgart (1991) 25-46.
[42] S. al Makdessi, M. Brändle, M. Ehrt et al. Myocardial protection by ischemic preconditioning: the influence of the composition of myocardial phospholipids. Mol. Cell. Biochem. 145 (1995) 69-73.
[43] R. Eickhorn, H. Isensee, R. Jacob et al. No obvious influence of dietary fatty acid intake on cardiac sodium current of the rat. Pflugers Arch. 420 (suppl. 1) (1992) 334.
[44] Y.F. Xiao, J.X. Kang, J.P. Morgan et al. Blocking effects of polyunsaturated fatty acids on Na+ channels of neonatal rat ventricular myocytes. Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 11000-11004.
[45] Y.F. Xiao, Q. Ke, Y. Chen et al. Inhibitory effect of n-3 fish oil fatty acids on cardiac Na+/Ca2+ exchange currents in HEK293t cells. Biochem. Biophys. Res. Commun. 321 (2004) 116-123.
[46] Y.F. Xiao, A.M. Gomez, J.P. Morgan et al. Suppression of voltage-gated L-type Ca2+ currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 4182-4187.
[47] K.Y. Bogdanov, H.A. Spurgeon, T.M. Vinogradova et al. Modulation of the transient outward current in adult rat ventricular myocytes by polyunsaturated fatty acids. Am. J. Physiol. 274 (1998) H571-H579.
[48] E. Honore, J. Barhanin, B. Attali et al. External blockade of the major cardiac delayed-rectifier K+ channel (Kv1.5) by polyunsaturated fatty acids. Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 1937-1941.
[49] S.C. O'Neill, M.R. Perez, K.E. Hammond et al. Direct and indirect modulation of rat cardiac sarcoplasmic reticulum function by n-3 polyunsaturated fatty acids. J. Physiol. 538 (2002) 179-184.
[50] J.S. Swan, K. Dibb, N. Negretti et al. Effects of eicosapentaenoic acid on cardiac SR Ca(2+)-release and ryanodine receptor function. Cardiovasc. Res. 60 (2003) 337-346.
[51] M.H. Raitt, W.E. Connor, C. Morris et al. Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrillators: a randomized controlled trial. JAMA 293 (2005) 2884-2891.
[52] A. Leaf, C.M. Albert, M. Josephson et al. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112 (2005) 2762-2768.
[53] L. Calo, L. Bianconi, F. Colivicchi et al. N-3 fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized, controlled trial. J. Am. Coll. Cardiol. 45 (2005) 1723-1728.
[54] C. Pater, D. Compagnone, J. Luszick et al. Effect of Omacor on HRV parameters in patients with recent uncomplicated myocardial infarction - A randomized, parallel group, double-blind, placebo-controlled trial: study design [ISRCTN75358739]. Curr. Control Trials Cardiovasc. Med. 4 (2003) 2.
[55] S. Pepe and P.L. McLennan. Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function. Circulation 105 (2002) 2303-2308.
[56] D. Rousseau, D. Moreau, D. Raederstorff et al. Is a dietary n-3 fatty acid supplement able to influence the cardiac effect of the psychological stress? Mol. Cell. Biochem. 178 (1998) 353-366.
[57] M. Brändle and R. Jacob. Effects of a diet rich in Q-3 fatty acids on left ventricular geometry and dynamics in spontaneously hypertensive rats. Eur. J. Appl. Physiol. Occup. Physiol. 61 (1990) 177-181.
[58] M. Kaieda, T. Samukawa, A. Kondo et al. Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various lipases in a solvent-free system. J. Biosci. Bioeng. 91 (2001) 12-15.
[59] L.D. Lawson and B.G. Hughes. Human absorption of fish oil fatty acids as triacylglycerols, free acids, or ethyl esters. Biochem. Biophys. Res. Commun. 152 (1988) 328-335.
[60] L.D. Lawson and B.G. Hughes. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. Biochem. Biophys. Res. Commun. 156 (1988) 960-963.
[61] S. el Boustani, C. Colette, L. Monnier et al. Enteral absorption in man of eicosapentaenoic acid in different chemical forms. Lipids 22 (1987) 711-714.
[62] C. Luley, H. Wieland, J. Grünwald. Bioavailability of omega-3 fatty acids: ethylester preparations are as suitable as triglyceride preparations. Akt. Ernähr-Med. 15 (1990) 123-125.
[63] I. Ikeda, Y. Imasato, H. Nagao et al. Lymphatic transport of eicosapentaenoic and docosahexaenoic acids as triglyceride, ethyl ester and free acid, and their effect on cholesterol transport in rats. Life Sci. 52 (1993) 1371-1379.
[64] M. Kozak, L. Krivan, B. Semrad. Circadian variations in the occurrence of ventricular tachyarrhythmias in patients with implantable cardioverter defibrillators. Pacing Clin. Electrophysiol. 26 (2003) 731-735.
[65] Die Nationale Verzehrsstudie. Bremerhaven: Wirtschaftsverlag NW (1991).
[66] G.B. Mensink, M. Thamm, K. Haas. [Nutrition in Germany 1998]. Gesundheitswesen 61 (1999) S200-S206.
[67] D. Kromhout, E.B. Bosschieter, C. de Lezenne Coulander. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312 (1985) 1205-1209.
[68] D. Mozaffarian, R.N. Lemaitre, L.H. Kuller et al. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. Circulation 107 (2003) 1372-1377.
[69] F.B. Hu, E. Cho, K.M. Rexrode et al. Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. Circulation 107 (2003) 1852-1857.
[70] K. He, Y. Song, M.L. Daviglus et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation 109 (2004) 2705-2711.
[71] C. von Schacky, P. Angerer, W. Kothny et al. The effect of dietary omega-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann. Intern. Med. 130 (1999) 554-562.
[72] P.M. Kris-Etherton, W.S. Harris, L.J. Appel. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106 (2002) 2747-2757.

[73] F. van de Werf, D. Ardissino, A. Betriu et al. Management of acute myocardial infarction in patients presenting with ST-segment elevation. The Task Force on the Management of Acute Myocardial Infarction of the European Society of Cardiology. Eur. Heart J. 24 (2003) 28-66.
[74] S. Yoon, A.E. Albers, A.P. Wong et al. Screening mercury levels in fish with a selective fluorescent chemosensor. J. Am. Chem. Soc. 127 (2005) 16030-16031.
[75] J.M. Hightower and D. Moore. Mercury levels in high-end consumers of fish. Environ. Health Perspect. 111 (2003) 604-608.
[76] J.F. Wilson. Balancing the risks and benefits of fish consumption. Ann. Intern. Med. 141 (2004) 977-980.
[77] C. Despres, A. Beuter, F. Richer et al. Neuromotor functions in Inuit preschool children exposed to Pb, PCBs, and Hg. Neurotoxicol. Teratol. 27 (2005) 245-257.
[78] E. Guallar, M.I. Sanz-Gallardo, P. van 't Veer et al. Mercury, fish oils, and the risk of myocardial infarction. N. Engl. J. Med. 347 (2002) 1747-1754.
[79] J.T. Cohen, D.C. Bellinger, W.E. Connor et al. A quantitative risk-benefit analysis of changes in population fish consumption. Am. J. Prev. Med. 29 (2005) 325-334.
[80] S.F. Melanson, E.L. Lewandrowski, J.G. Flood et al. Measurement of organochlorines in commercial over-the-counter fish oil preparations: implications for dietary and therapeutic recommendations for omega-3 fatty acids and a review of the literature. Arch. Pathol. Lab. Med. 129 (2005) 74-77.
[81] H. Rupp, M. Turcani, T. Ohkubo et al. Dietary linolenic acid-mediated increase in vascular prostacyclin formation. Mol. Cell. Biochem. 162 (1996) 59-64.
[82] E. Ros, I. Nunez, A. Perez-Heras et al. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. Circulation 109 (2004) 1609-1614.
[83] H. Sprecher. An update on the pathways of polyunsaturated fatty acid metabolism. Curr. Opin. Clin. Nutr. Metab. Care 2 (1999) 135-138.
[84] R.K. Berge, L. Madsen, H. Vaagenes et al. In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. Biochem. J. 343 (1999) 191-197.
[85] M.N. Sack, T.A. Rader, S. Park et al. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation 94 (1996) 2837-2842.
[86] H. Gerster. Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)? Int. J. Vitam. Nutr. Res. 68 (1998) 159-173.
[87] G.C. Burdge and S.A. Wootton. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br. J. Nutr. 88 (2002) 411-420.
[88] C. von Schacky and P.C. Weber. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. J. Clin. Invest. 76 (1985) 2446-2450.
[89] M. Studer, M. Briel, B. Leimenstoll et al. Effect of different antilipidemic agents and diets on mortality: a systematic review. Arch. Intern. Med. 165 (2005) 725-730.
[90] W.S. Harris and C. von Schacky. The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev. Med. 39 (2004) 212-220.
[91] D.W. Nilsen, G. Albrektsen, K. Landmark et al. Effects of a high-dose concentrate of n-3 fatty acids or corn oil introduced early after an acute myocardial infarction on serum triacylglycerol and HDL cholesterol. Am. J. Clin. Nutr. 74 (2001) 50-56.
[92] P. Yli-Jama, H.E. Meyer, J. Ringstad et al. Serum free fatty acid pattern and risk of myocardial infarction: a case-control study. J. Intern. Med. 251 (2002) 19-28.
[93] A. Macchia, G. Levantesi, M.G. Franzosi et al. Left ventricular systolic dysfunction, total mortality, and sudden death in patients with myocardial infarction treated with n-3 polyunsaturated fatty acids. Eur. J. Heart Fail. 7 (2005) 904-909.

Correspondance to:
Prof. Dr. Heinz Rupp
Molecular Cardiology Laboratory
Department of Internal Medicine and Cardiology
Philipps University of Marburg,
Karl-von-Frisch-Straße 1
D-35033 Marburg