Prostaglandins, Leukotrienes and Essential Fatty Acids 92 (2015) 41–47

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Omega-3 fatty Acids in cardiovascular disease – An uphill battle

Clemens von Schacky [a,b,*]

[a] Medizinische Klinik I, Department Preventive Cardiology, Ludwig-Maximilians-University, Ziemssenstrasse 1, D-80336 Munich, Germany
[b] Omegametrix, Am Klopferspitz 19, 82152 Martinsried, Germany[1]

ABSTRACT

In cardiology, results of recent large intervention trials with eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) supplements were neutral. In contrast, in epidemiologic studies, an inverse relation between clinical events and intake of EPA+DHA was found which was steeper for higher levels of EPA+DHA. A standardized way of determining levels is the Omega-3 Index, which is the percentage of EPA+DHA of a total of 26 fatty acids measured in erythrocytes. According to current criteria, a low Omega-3 Index is a cardiovascular risk factor.

What can explain this contradiction? Trial participants were recruited irrespective of their baseline status in EPA+DHA – an important predictor of events. Levels of EPA+DHA have a statistically normal distribution; together with the large inter-individual variability of levels' responding to increased intake, this created a large overlap of EPA+DHA levels between intervention and control groups. Moreover, trial participants were advised to take EPA+DHA supplements with breakfast, frequently a low fat meal, resulting in poor bioavailability. As a result, there is an urgent need for new intervention trials in cardiology, for which participants with a low baseline omega-3 index are recruited, and then treated with individually tailored doses of EPA+DHA to a prespecified target range.

1. Introduction

Neutral results of recent large intervention trials, their meta-analyses, and guidelines of cardiac societies do not compel clinicians to use eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplements in the treatment of their patients with cardiovascular conditions, heart rhythm issues or congestive heart failure. This contrasts with rather consistent results of epidemiologic studies, animal studies and mechanisms of action (the latter two reviewed elsewhere, Ref. [1]). In the present review, this state of the evidence is reported, a change in perspective is suggested from focusing on intake to focusing on levels, it is delineated why assessing levels with the HS-Omega-3 Index methodology has certain advantages, reasons for the contrasting results of epidemiologic studies and intervention trials are discussed, and a Omega-3 Index-based design of intervention trials is proposed.

1.1. Current state of the large intervention trials, their meta-analyses, and of guidelines

A series of intervention trials with clinical endpoints investigating EPA+DHA supplements in persons at risk for a myocardial infarction reported neutral results on total mortality and major adverse cardiac events [2–7]. Not surprisingly, in recent meta-analyses and systematic reviews, also including earlier large trials with more positive results, an overall neutral effect was seen [8–12]. This was in contrast to epidemiologic observations, based mostly on intake of EPA+DHA in food, where risk for e.g. total coronary artery disease events was some 50% lower in individuals with a high intake of EPA+DHA (e.g. [13,14]). The contrast to epidemiologic observations, based on levels of EPA+DHA, was even stronger: risk for total coronary artery disease events was some 80% lower with high than with low levels (Hedge g effect size -0.19 $p < 0.01$, 95% confidence interval -0.06 to -0.33), as found in a meta-analysis of 19 studies [15]. In cardiovascular prevention guidelines, the American Heart Association (AHA) endorsed use of EPA+DHA, while the European Society for Cardiology (ESC) did so more reluctantly [16,17].

Similarly, intervention trials in patients at risk for supraventricular or ventricular rhythm disturbances also reported neutral results on prevention of ventricular or atrial fibrillation [1,18,19]. Again, this is in contrast to epidemiologic studies: an inverse

* Correspondence address: Medizinische Klinik I, Department Preventive Cardiology, Ludwig-Maximilians-University, Ziemssenstrasse 1, D-80336 Munich, Germany. Tel.: +49 89 5160 2165; fax: +49 89 5160 2194.
E-mail addresses: ehavio.vonschacky@med.uni-muenchen.de, c.vonschacky@omegametrix.eu, clemens.vonschacky@med.uni-muenchen.de
[1] Tel.: +49 89 4400 52165; fax: +49 89 4400 52194.

http://dx.doi.org/10.1016/j.plefa.2014.05.004
0952-3278/

relation between risk for sudden cardiac death, the worst outcome of ventricular rhythms disturbances, and intake, and even more pronouncedly, levels of EPA+DHA in erythrocytes or in whole blood: in the highest quartiles, risk was 10% of what it was in the lowest quartiles [20,21]. High intake or high levels of EPA and DHA are also inversely related to development of atrial fibrillation [1,22,23]. Current pertinent AHA/ESC guidelines state that "n-3 polyunsaturated fatty acid supplementation may be considered for patients with ventricular arrhythmias and underlying CHD" (a Class IIb, level of evidence B recommendation, Ref. [24]) but in connection with atrial fibrillation EPA+DHA are not mentioned [25,26].

However, a large intervention trial in patients with congestive heart failure demonstrated a small positive effect of 1 g EPA+DHA ethyl-ester on total mortality and rehospitalizations (a combined endpoint, Ref. [27]). This is supported by epidemiologic evidence demonstrating that high levels of EPA+DHA in plasma phospholipid fatty acids are inversely related to development of congestive heart failure [28], and by epidemiologic evidence relating high levels EPA+DHA in plasma phospholipid fatty acids to survival in patients with heart failure [29]. Yet, current AHA guidelines do not recommend EPA+DHA in heart failure [30], while the ESC states "n-3 PUFA preparation may be considered to reduce the risk of death and the risk of cardiovascular hospitalization in patients treated with an ACE inhibitor (or ARB), beta-blocker, and an MRA (or ARB) (a Class IIb, level of evidence B recommendation, Ref. [31])".

Taken together, translating the epidemiologic evidence into positive trial results largely failed. Guidelines are not consistent with results of meta-analyses, and differ between Europe and the USA. Moreover, epidemiologic evidence based on dietary intake of EPA+DHA tended to show weaker correlations than epidemiologic evidence based on their levels.

1.2. How to assess levels of EPA+DHA

Levels of EPA+DHA can be assessed by fatty acid analysis in all sorts of compartments, each with specific advantages and disadvantages, from plasma free fatty acids, plasma phospholipids, to tissue levels that are reflected by erythrocyte levels. This topic is discussed in detail elsewhere [32]. Traditionally, fatty acid analysis was and still is being performed in individual laboratories using individual analytical methods. Because sample preparation and gas-chromatographic analysis requires multiple steps, each impacting on the results, individual laboratories report individual results [32]. Not surprisingly, when a single sample was sent to 5 laboratories, each using an individual analytical method, results for erythrocyte EPA+DHA varied by a factor of 3.5 [32]. Such an individual analytical approach can provide internal consistency within one study, but precluded, and still precludes, comparing results across laboratories. Moreover, a standardized analytical method is a clear prerequisite for the clinical application of a laboratory parameter, as mandated by pertinent statements by the American Heart Association and the US Preventive Services Task Force [33,34].

1.3. The Omega-3 Index

Against this background, the Omega-3 Index was defined as "the content of EPA+DHA in red blood cell membranes (expressed as a percentage of total FA measured)", i.e. the content of eicosapentaenoic acid plus docosahexaenoic acid in erythrocyte membranes [35]. An integral part of the definition was a highly standardized analytical method subjected to and conforming to the rules and regulations of Clinical Chemistry (constancy checks, plausibility testing, proficiency testing, asf). To highlight this specific analytical method, the Omega-3 Index was trademarked, "HS-Omega-3 Index®". Of note, pre-analytical concerns are minor.

Table 1
Fatty acid spectrum reported with the Omega-3 Index.

Saturated fatty acids
Myristinic C14:0
Palmitinic C16:0
Sterarinic C18:0
Arachinic 20:0
Behenic 22:0
Lignocerinic C24:0
Monounsaturated fatty acids
Palmitoneinic 16:1ω-7
Oleic C18:1ω-9
Gondonic C20:1ω-9
Nervonic C24:1ω-9
ω-6 fatty acids
Linoleic (LA) C18:2ω-6
γ-linolenic (GLA) C18:3ω-6
Dihomo-γ-linolenic (DGLA) C20:3ω-6
Arachidonic (AA) C20:ω-6
Docosatetraenoic (DTA) C22:4ω-6
Eicosadienoic C22:2ω-6
Docosapentaenoic C22:5ω-6
ω-3 fatty acids
α-linolenic (ALA) C18:3ω-3
Eicosapentaenoic (EPA) C20:5ω-3
Docosapentaenoic (DPA) C22:5ω-3
Docosahexaenoic (DHA) C22:6 ω-3
Trans fatty acids
Palmitolenic C16:1ω-7t
Elaidinic C18:1ω-9t
C18:2ω-6tt
C18:2ω-6ct
C18:2ω-6tc

At ambient temperature, samples are stable for at least seven days, which makes it possible to send samples with regular mail in most cases. If frozen at −80 °C, samples are stable for many years [36]. However, when frozen at −20 °C, levels of EPA+DHA in erythrocytes are not stable [35]. The results of an erythrocyte fatty acid analysis according to this method include 26 fatty acids, as listed in Table 1. Erythrocytes were chosen because early work had indicated that they incorporate dietary EPA and DHA in a dose- and time-dependent manner [37]. Incorporation kinetics indicated a very limited exchange of EPA and DHA between plasma and erythrocytes, and that their fatty acid composition was defined to a considerable extent during cell formation [37]. From an analytical point of view, the erythrocyte membrane had the advantage of being composed almost exclusively of phospholipids, making purification steps unnecessary [32]. Subsequent work demonstrated a low analytical variability of the Omega-3 Index method (3.9 relative %) and a low biological variability of the erythrocyte fatty acid composition (1.3 relative %), both much lower than of plasma phospholipid or whole blood fatty acid compositions [38]. Neither acute intake of omega-3 fatty acids, nor severe clinical events impact on the Omega-3 Index [38-40]. Thus, the Omega-3 Index qualified as a "low-noise" parameter, which suits epidemiologic studies. If no fish oil supplementation was started, the Omega-3 Index was stable through years [41]. It was also found that erythrocyte EPA+DHA correlated to EPA+DHA in cardiac tissue [42], and, in the experimental animal, to other tissues like lung, brain, kidney and others [43]. We concluded that the Omega-3 Index reflected an individual's status in EPA+DHA [44].

1.4. Determinants of the Omega-3 Index

All levels are determined by the equilibrium of inflow and outflow. A detailed discussion of all known determinants of the Omega-3 Index is outside the scope of this review. Chronic intake of EPA+DHA, as assessed with dietary questionnaires, although

the main predictor of the Omega-3 Index, explains only some 20% of its variability [45–47]. Other determinants are heritability (25%), and fish oil supplementation (15%) [47]. This indicated that dietary intake of omega-3 fatty acids qualifies only as one, minor, determinant of levels of EPA+DHA in erythrocytes. In keeping, in 40 individuals with a baseline Omega-3 Index $<5\%$, who ingested 0.5 g EPA+DHA per day for 8 weeks in 200 ml of a convenience drink, the response in erythrocyte EPA+DHA varied by a factor of up to 13 inter-individually [48].

2. Epidemiology of the Omega-3 Index and cardiac Diseases

2.1. Sudden cardiac death

As yet, the relation of the Omega-3 Index to the occurrence of sudden cardiac death has not yet been directly investigated. However, during the acute phase of a myocardial infarction, a 1% increase of the Omega-3 Index correlated with a 58% (95% CI: 0.25–0.76%) lower risk for ventricular fibrillation [49,50]. Since ventricular fibrillation during a myocardial infarction is the predominant cause of sudden cardiac death, the data just mentioned indirectly support a steep relation between the Omega-3 Index and sudden cardiac death.

2.2. Total mortality and cardiovascular morbidity

The pertinent data are reported in Table 2.

Atrial Fibrillation and Ventricular Arrhythmias have not yet been investigated with the exception just mentioned.

Congestive Heart Failure: as mentioned, individuals with low levels of EPA+DHA are at risk for developing heart failure [28,59,60], and to die from it [29]. We found a low mean Omega-3 Index in 847 patients with heart failure ($3.74 \pm 0.97\%$, unpublished). Whether a low Omega-3 Index impacts on survival is currently investigated.

2.3. The Omega-3 Index as a cardiovascular risk factor

Criteria for a cardiovascular risk factor have been proposed by American Heart Association and United States-Preventive Services Task Force [33,34]. These can be summarized as follows:

- Standardized analytical procedure: as already discussed, the Omega-3 Index is determined with a strictly standardized analytical procedure. This is in contrast to biomarkers already in clinical use, like assessment of carotid intima-media thickness with ultrasound, lipoprotein (a), and others.
- Incremental information to risk assessment based on conventional risk factors. This was calculated with c-statistics using data obtained from the "Heart and Soul" and "Triumph" studies, and in a smaller sample in Korea [52,61,62]. The Omega-3 Index provided additional information to conventional risk scoring systems, like the Framingham or GRACE scores for predicting fatal events [52,61,62].
- Correct reclassification of individuals from intermediate risk into high or low risk categories. This has been demonstrated for the Omega-3 Index [52,61,63].
- Therapeutic consequence. According to the current cardiovascular prevention guidelines of the European Society for Cardiology, causality between a beneficial change in the risk factor and clinical outcome needs to be demonstrated, ultimately in form of a large trial with clinical endpoints. According to the European Society for Cardiology, this criterion is not fulfilled by any new biomarker, including C-reactive protein and LpPLA2 [16]. Many established parameters, like triglycerides, HDL, and others also do not fulfil this criterion, but are in clinical use. However, results from studies in experimental animals, and in humans using surrogate and intermediate parameter are promising: in experimental animals, effects of increasing the Omega-3 Index were largely positive: heart rate was decreased and heart rate variability increased, inflammation was suppressed, and cardiac remodelling and dysfunction and other, similar effects were prevented, whereas ischemia-induced ventricular fibrillation and arrhythmia susceptibility in both non-infarcted and low risk post-MI dogs were not decreased [63–68]. In one animal study, an antidepressant effect was seen [69]. Surrogate parameters were measured in humans. Increasing the Omega-3 Index decreased heart rate, increased heart rate variability, reduced blood pressure, decreased platelet reactivity, lowered triglycerides, increased large LDL-particles and decreased small ones, increased large buoyant HDL2, decreased VLDL1+2, reduced some inflammatory cytokines (tumour necrosis factor alpha, interleukin-1ß, interleukins-6,8,10 and monocyte chemoattractant protein-1), increased vitamin D and fetuin-A, and affected some aspects of gene expression and oxylipin response [70–90].

In a randomized, controlled double-blind intervention trial on the intermediate parameter coronary lesions, an increase in

Table 2

Akronym	Design	Disease	*n*	years	Criterion	Comparison	Result
Total mortality							
Heart and Sou [51]	Cohort	stable CAD	956	5.9	Total mortality	HS-Omega-3 Index $\geq 4.6\%$ vs. $<4.6\%$	HR 0.73; 95% CI, 0.56–0.94
Triumph [52]	Cohort	recent MI	1144	2	Total mortality	EPA in red cells tertiles	EPA $<0.25\%$ total mortality 26%, $0.25<$ EPA $\leq 0.8\%$ tot. mort. 13%, EPA $>0.80\%$ total mortality 7%
Triumph [53]	Cohort	Recent MI	1424	1	Total mortality	HS-Omega-3 Index $<4\%$ vs. $\geq 4.0\%$	HR 2.0; 95% CI 1.2–3.3
Racs* [54]	Cohort	Recent ACS	460	2	Total mortality	HS-Omega-3 Index in quartiles	n.s.
Cardiac morbidity							
[55]	Case-control	ACS	94/94 cases/controls		ACS	Whole blood EPA+DHA in quintile	OR 1.0–0.2 (95% CI not reported), OR 0.67 (95% CI 0.46–0.98) per, 1 standard deviation increase EPA+DHA
[56]	Case-control	ACS	768/768 cases/controls		ACS	HS-Omega-3 Index in tertiles	OR 1.0–0.31 (95% CI 0.14–0.67), across tertiles
[57]	Case-control	ACS	50/50 cases/controls		ACS	HS-Omega-3 Index in tertiles	OR 1.0–0.08 (95% CI 0.02–0.38), across tertiles,
[58]	Case-control	ACS	24/68 cases/controls		STEMI	HS-Omega-3 Index, in quartiles	OR 6.38 (95% CI 1.02–39.85) – 1.0, across quartiles

Abbreviations: Coronary artery disease: CAD; HR: hazard ratio; MI: myocardial infarction; EPA: eicosapentaenoic acid; ACS: acute coronary syndrome; SCD: sudden cardiac death; DHA: docosahexaenoic acid; OR: odds ratio; STEMI: ST-elevation myocardial infarction. *No case estimate was reported in Racs. Therefore, by definition, it is unclear, whether the discriminatory power of the Omega-3 Index was too small, or the study was too small to detect the discriminatory power.

erythrocyte EPA+DHA was associated with less progression and more regression [91]. The evidence just mentioned supports the notion of causality between a high Omega-3 Index and the absence of cardiovascular events. A large intervention trial based on the Omega-3 Index with clinical endpoints remains to be performed.

3. Translating epidemiology into results of trials with clinical endpoints

How can it be that, so far, it was impossible to translate the rather consistent epidemiologic findings mentioned, impossible even to translate an improvement of a cardiovascular risk factor into a reduction of clinical events in large trials with clinical endpoints?

3.1. Issues in study design

Up to now, participants of intervention trials were recruited irrespective of their baseline status of omega-3 fatty acids (e.g. [2–7]). As to be expected from a biomarker, the Omega-3 Index had a statistically normal distribution in every population studied so far [44]. Clearly, in trial participants with a high Omega-3 Index at baseline and presumably throughout the study, few, if any, events are to be expected, whereas they are more likely in individuals with a low Omega-3 Index. Thus, by not focusing on study participants with low baseline levels, detecting a therapeutic effect was more difficult. Conversely, if a trial happened to focus on a disease associated with a low Omega-3 Index, like congestive heart failure, detecting an effect of EPA+DHA becomes more likely.

As mentioned, in terms of the Omega-3 Index, the response to a given dose of EPA+DHA can vary up to a factor of 13 from individual to individual [48]. Together with the statistically normal distribution of baseline levels, this leads to a substantial proportion of trial participants in both intervention and control groups having comparable levels – the opposite of what is aimed for in a clinical trial: the biggest possible difference between intervention and control groups in terms of the intervention. This phenomenon generated a strong tendency towards a neutral result.

3.2. Issues of bioavailability

Bioavailability of omega-3 fatty acids was recently reviewed in this journal [92]. In short, differences in bioavailability of different chemical forms of EPA+DHA supplements exist. Under otherwise identical conditions, the bioavailability of EPA+DHA in phospholipids≫recombined triglycerides≫triglycerides≫free fatty acids≫ethyl-ester, but varies overall by a maximum factor of approx. 2 [76,79,92–94].

More importantly, however, bioavailability of an EPA+DHA ethyl-ester – the chemical form that was predominantly used in large intervention trials – was found to be 13 fold higher with a high fat meal, as compared to a low fat meal [95]. According to personal information from the first authors, in SU.FOL.OM3, alpha–omega, OMEGA, DOIT and ORIGIN (all trials with a neutral result), study participants were advised to take their study capsules with breakfast – in many countries a low fat meal, if any. Thus, poor bioavailability of EPA+DHA contributed to the neutral results of the studies mentioned [2–6].

Taken together, frequently the least bioavailable formulation of EPA+DHA was used, and capsule ingestion was poorly timed, which resulted in poor bioavailability of EPA+DHA. Moreover, study participants were recruited irrespective of their baseline levels in EPA+DHA, and treated with fixed doses, ignoring the large inter-individual variability in uptake of EPA+DHA. At least in parts, the factors mentioned may explain a tendency towards neutral results of previous intervention trials.

3.3. Design of future intervention studies

To demonstrate an effect of increased intake of EPA+DHA, it seems logical to recruit study participants with low levels of EPA+DHA, e.g. an Omega-3 Index $<5\%$. Only if an effect could be demonstrated in study participants with low levels would it make sense, as a second step, to recruit populations irrespective of their baseline levels of EPA+DHA.

Due to the issues of bioavailability mentioned, it appears prudent to advise study participants to ingest EPA+DHA with the main meal of the day, usually containing sufficient fat to trigger fat digestion and absorption. By use of an emulsified ethyl-ester, bioavailability can be improved by a factor of up to 21 in comparison to an unemulsified ethyl-ester [96]. Bioavailability of triglycerides can also be improved [97]. The variability in response of the Omega-3 Index to increased intake of EPA+DHA already discussed [48] suggests aiming for a target range for the Omega-3 Index (e.g. the suggested target range of 8–11%) with variable doses of EPA+DHA, instead of using one fixed dose of EPA+DHA for study participants in the intervention group. Blinding can be maintained by also adjusting the dose of placebo. This way, the difference between the intervention and control groups in terms of the Omega-3 Index can be maximized, an overlap of levels avoided, and the tendency towards neutral results minimized. Clearly, this kind of study design is not restricted to the cardiovascular area, but can be also used in other areas to make intervention trials more efficient and to provide clearer results than previously obtained.

4. Safety issues and limitations

While the European Food Safety Authority (EFSA) considers a daily intake of up to 5 g/day long-chain omega-3 fatty acids (i.e. EPA and DHA) safe (http://www.efsa.europa.eu/en/press/news/120727.htm), the US-American Food and Drug Administration (FDA) considers a daily intake of up to 3 g EPA+DHA safe (*Docket* No. 91N-0103). As mentioned above, the relation between intake of EPA+DHA and the Omega-3 Index is loose.

At present, it is unclear, whether the Omega-3 Index can be so high as to be unsafe. A relation between bleeding and the Omega-3 Index has not been found in patients with acute myocardial infarction, a situation where a maximal bleeding tendency is generated by use of pertinent drugs [98]. A bleeding tendency has also not been reported from individuals with an Omega-3 Index $>16\%$, when questioned on the telephone by the author. This issue remains to be investigated systematically.

In Japan and Korea, countries, in which a Omega-3 Index in the target range of 8–11% was found in various populations [32], life expectancy is longer than in countries, in which a lower Omega-3 Index was found, e.g. USA or Germany. Rates of cardiovascular diseases are lower in Japan and Korea than in Western countries, as are rates for some malignancies, like prostate, breast and colon cancer [99]. In general, risk for prostate, breast and colon cancers has been found to be lower with higher proportions of EPA+DHA in erythrocytes [100–107]. This argues against increased risk for the three cancers mentioned with an Omega-3 Index in the proposed target range of 8–11%, but remains to be demonstrated directly.

Taken together, safety issues with an Omega-3 Index in the proposed target range of 8–11% remain to be reported.

Clearly, many health issues and diseases are independent of omega-3 fatty acids. Therefore, the Omega-3 Index has only diagnostic value in the health issues where omega-3 fatty acids play a role. However, many ongoing and future research projects will not only add precision to the current application, but also define new ones, like trans fatty acids, for which erythrocytes are thought to be a suitable biomarker for long-term intake.

5. Ethical considerations

Any large intervention trial aims to assess risks and benefits of a therapeutic intervention. As just discussed, current state of the evidence and government agencies clearly indicate that up to 3–53–5 g/day, EPA+DHA are safe. Therefore, an Omega-3 Index-based large intervention trial with EPA+DHA in cardiology would be unlikely to uncover safety issues, but would rather evaluate a possible benefit of a targeted approach in the use of EPA+DHA in cardiology.

As reviewed in more detail elsewhere in this issue, a number of brain functions depend on supply with omega-3 fatty acids, or a high Omega-3 Index. A number of meta-analyses and more recent trial results attest to the therapeutic value of EPA+DHA e.g. in pregnancy, attention deficit-hyperkinetic syndrome, depression, cognitive decline, and others [108–113]. In other words: a high Omega-3 Index helps to maintain uncompromised brain function. Currently, however, a general agreement on this issue has not yet been reached.

For the design of an Omega-3 Index-based large intervention trial with EPA+DHA in the cardiovascular field two options exist: a comparison with placebo or a comparison with untargeted supplementation (the design of the previous trials mentioned, Ref. [2–12]). A comparison with placebo would mean maintaining the Omega-3 index of the control- or placebo group in a low range, e.g. $<5\%$; this would preclude access of the placebo group to EPA+DHA necessary to maintain uncompromised brain function. Thus, in order to not become an ethical problem, such a trial would need to be performed before a general agreement on the beneficial effects of EPA+DHA on maintaining brain function is reached. However, a comparison with an untargeted supplementation would be more likely not to raise ethical concerns now or later. Otherwise, a large trial with clinical endpoints might not be ethically feasible. The worst possibility would be not to conduct a large Omega-3 Index-based intervention trial, which would mean that the neutral results of the current intervention trials and their meta-analyses will remain unchallenged – and leave the likely benefits of omega-3 fatty acids in cardiology unrealized.

6. Conclusion

Baseline levels of EPA+DHA, bioavailability issues, and individual factors used to be ignored in the design of large intervention trials in cardiology. This introduced a strong tendency towards neutral results most likely responsible for the failure of trial results to reflect the inverse relation of clinical events to intake, and, more steeply, to levels of EPA+DHA. For a number of reasons, these levels are frequently assessed with the Omega-3 Index, a standardized fatty acid analysis of the percentage of EPA+DHA in erythrocytes. A low HS-Omega-3 Index is a cardiovascular risk factor, and also makes impairments of complex brain functions more likely. In future trial design, recruiting trial participants with a low Omega-3 Index, treating them to a target range (e.g. 8–11%), and compare this to either placebo or an untargeted approach will make trials more effective, and neutral results less likely. However, in light of the many other positive actions already demonstrated for a high Omega-3 Index, and to avoid ethical problems, large trials need to be conducted soon.

References

[1] C. von Schacky, Omega-3 fatty acids: anti-arrhythmic, pro-arrhythmic, or both? Front. Cardiac Electrophysiol. 3 (2012) 98.

[2] P. Galan, E. Kesse-Guyot, S. Czernichow, S. Briancon, J. Blacher, S. Hercberg, SU.FOL.OM3 Collaborative Group, Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial, Br. Med. J. 341 (2010) c6273.

[3] B. Rauch, R. Schiele, S. Schneider, F. Diller, N. Victor, H. Gohlke, OMEGA Study Group, et al., OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction, Circulation 122 (2010) 2152–2159.

[4] G. Einvik, T.O. Klemsdal, L. Sandvik, E.M. Hjerkinn., A randomized clinical trial on n-3 polyunsaturated fatty acids supplementation and all-cause mortality in elderly men at high cardiovascular risk, Eur. J. Cardiovasc. Prev. Rehabil. 17 (2010) 588–592.

[5] D. Kromhout, E.J. Giltay, J.M. Geleijnse, Alpha Omega Trial Group, n-3 fatty acids and cardiovascular events after myocardial infarction, N. Engl. J. Med. 363 (2010) 2015–2026.

[6] J. Bosch, H.C. Gerstein, G.R. Dagenais, R. Díaz R, L. Dyal, H. Jung, et al., ORIGIN Trial Investigators, N-3 fatty acids and cardiovascular outcomes in patients with dysglycemia, N. Engl. J. Med. 367 (2011) 319–328.

[7] M.C. Roncaglioni, M. Tombesi, F. Avanzini, S. Barlera, V. Caimi, P. Longoni, et al., Risk and Prevention Study Collaborative Group, n-3 fatty acids in patients with multiple cardiovascular risk factors, N. Engl. J. Med. 368 (2013) 1800–1808 (Erratum in N. Engl. J. Med. 368 (2013) 2146).

[8] R. Chowdhury, S. Stevens, D. Gorman, A. Pan, S. Warnakula S, S. Chowdhury, et al., Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis, Br. Med. J. 345 (2012) e6698.

[9] S. Kotwal, M. Jun, D. Sullivan, V. Perkovic, B. Neal, Omega 3 fatty acids and cardiovascular outcomes: systematic review and meta-analysis, Circ. Cardiovasc. Qual. Outcomes 5 (2012) 808–818.

[10] E.C. Rizos, E.E. Ntzani, E. Bika, M.S. Kostapanos, M.S. Elisaf, Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis, J. Am. Med. Assoc. 308 (2012) 1024–1033.

[11] S.M. Kwak, S.K. Myung, Y.J. Lee, H.G. Seo, Korean Meta-analysis Study Group, Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials, Arch. Intern. Med. 172 (2012) 686–694.

[12] J. Delgado-Lista, P. Perez-Martinez, J. Lopez-Miranda, F. Perez-Jimenez., Long chain omega-3 fatty acids and cardiovascular disease: a systematic review, Br. J. Nutr. 107 (Suppl. 2) (2012) S201–S213.

[13] F.B. Hu, L. Bronner, W.C. Willett, M.J. Stampfer, K.M. Rexrode, C.M. Albert, et al., Fish and omega-3 fatty acid intake and risk of coronary heart disease in women, J. Am. Med. Assoc. 287 (2002) 1815–1821.

[14] D. Mozaffarian, E.B. Rimm., Fish intake, contaminants, and human health: evaluating the risks and the benefits, J. Am. Med. Assoc. 296 (2006) 1885–1899.

[15] W.S. Harris, W.C. Poston, C.K. Haddock, Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events, Atherosclerosis 193 (2007) 1–10.

[16] J. Perk, G. De Backer, H. Gohlke, I. Graham, Z. Reiner, M. Verschuren, European Association for Cardiovascular Prevention & Rehabilitation (EACPR), ESC Committee for Practice Guidelines (CPG), et al., European guidelines on cardiovascular disease prevention in clinical practice (version 2012): the Fifth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Fifth Joint Task Force of the European Society of Cardiology; European Association of Echocardiography; European Association of Percutaneous Cardiovascular Interventions; European Heart Rhythm Association; Heart Failure Association; European Association for Cardiovascular Prevention & Rehabilitation; European Atherosclerosis Society; International Society of Behavioural Medicine; European Stroke Organisation; European Society of Hypertension; European Association for the Study of Diabetes; European Society of General Practice/Family Medicine; International Diabetes Federation Europe; European Heart Network, Eur. J. Prev. Cardiol. 19 (2012) 585–667.

[17] S.C. Smith Jr., E.J. Benjamin, R.O. Bonow, L.T. Braun, M.A. Creager, B.A. Franklin BA, et al.; World Heart Federation and the Preventive Cardiovascular Nurses Association. AHA/ACCF Secondary Prevention and Risk Reduction Therapy for Patients with Coronary and other Atherosclerotic Vascular Disease: 2011 update: a guideline from the American Heart Association and American College of Cardiology Foundation. Circulation. 124 (2011) 2458-2473.

[18] I.A. Brouwer, M.H. Raitt, C. Dullemeijer, D.F. Kraemer DF, P.L. Zock, C. Morris, et al., Effect of fish oil on ventricular tachyarrhythmia in three studies in patients with implantable cardioverter defibrillators, Eur. Heart J. 30 (2009) 820–826.

[19] D. Mozaffarian, J.H. Wu, M.C. de Oliveira Otto, C.M. Sandesara, R.G. Metcalf, R. Latini, et al., Fish oil and post-operative atrial fibrillation: a meta-analysis of randomized controlled trials, J. Am. Coll. Cardiol. 61 (2013) 2194–2196.

[20] D.S. Siscovick, T.E. Raghunathan, I. King, S. Weinmann, K.G. Wicklund, J. Albright, et al., Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the riskof primary cardiac arrest, J. Am. Med. Assoc. 275 (1995) 836–837.

[21] C.M. Albert, H. Campos, M.J. Stampfer, P.M. Ridker, J.E. Manson, W.C. Willett, J. Ma, Blood levels of long-chain n-3 fatty acids and the risk of sudden death, N. Engl. J. Med. 346 (2002) 1113–1118.

[22] N.N. Gronroos, A. Alonso., Diet and risk of atrial fibrillation – epidemiologic and clinical evidence, Circ. J. 74 (2010) 2029–2038.

[23] J.H. Wu, R.N. Lemaitre, I.B. King, X. Song, F.M. Sacks, E.B. Rimm, Association of plasma phospholipid long-chain omega-3 Fatty acids with incident atrial fibrillation in older adults: the cardiovascular health study, Circulation 125 (2012) 1084–1093.

[24] D.P. Zipes, A.J. Camm, M. Borggrefe, A.E. Buxton, B. Chaitman, M. Fromer, American College of Cardiology/American Heart Association Task Force; European Society of Cardiology Committee for Practice Guidelines; European Heart Rhythm Association; Heart Rhythm Societyet al.; ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (writing committee to develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. Circulation. 114 (2006) e385-e484.

[25] A.J. Camm, G.Y. Lip, R. De Caterina, I. Savelieva, D. Atar, S.H. Hohnloser, et al., 2012 focused update of the ESC guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation. Developed with the special contribution of the European Heart Rhythm Association, Eur. Heart J. 33 (2012) 2719–2747.

[26] L.S. Wann, A.B. Curtis, C.T. January, K.A. Ellenbogen, J.E. Lowe JE, N.A. Estes 3rd, ACCF/AHA Task Force Members, et al., 2011 ACCF/AHA/HRS focused update on the management of patients with atrial fibrillation (updating the 2006 guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, Circulation 123 (2011) 104–123.

[27] Gissi-H.F. Investigators, L. Tavazzi, A.P. Maggioni, R. Marchioli, S. Barlera, M. G. Franzosi, R. Latini, et al., Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial, Lancet 372 (2008) 1223–1230.

[28] D. Mozaffarian, R.N. Lemaitre, I.B. King, X. Song, D. Spiegelman, F.M. Sacks, et al., Circulating long-chain ω-3 fatty acids and incidence of congestive heart failure in older adults: the cardiovascular health study: a cohort study, Ann. Intern. Med. 155 (2011) 160–170.

[29] S. Masson, R. Marchioli, D. Mozaffarian, R. Bernasconi, V. Milani, L. Dragani, et al., Plasma n-3 polyunsaturated fatty acids in chronic heart failure in the GISSI-Heart Failure Trial: relation with fish intake, circulating biomarkers, and mortality, Am. Heart J. 165 (2013) 208–215.

[30] M. Jessup, W.T. Abraham, D.E. Casey, A.M. Feldman, G.S. Francis, T.G., et al., 2009 focused update: ACCF/AHA guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation, Circulation 119 (2009) 1977–2016.

[31] J.J. McMurray, S. Adamopoulos, S.D. Anker, A. Auricchio, M. Bohm, K. Dickstein, ESC Committee for Practice Guidelines, et al., ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC, Eur. Heart J. 33 (2012) 1787–1847.

[32] W.S. Harris, C. von Schacky, Y. Park, Standardizing methods for assessing omega-3 fatty acid biostatus, in: R.K. McNamara (Ed.), The Omega-3 Fatty Acid Deficiency Syndrome, Nova Science Publishers, Hauppauge, NY, USA, 2013 (chapter XIX).

[33] M.A. Hlatky, P. Greenland, D.K. Arnett, C.M. Ballantyne, M.H. Criqui, M. S. Elkind, et al., American Heart Association Expert Panel on subclinical atherosclerotic diseases and emerging risk factors and the stroke council, Circulation 119 (2009) 2408–2416.

[34] M. Helfand, D.I. Buckley, M. Freeman, R. Fu, K. Rogers, C. Fleming, L. L. Humphrey, Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force, Ann. Intern. Med. 151 (2009) 496–507.

[35] W.S. Harris, C. von Schacky, The Omega-3 Index: a new risk factor for death from CHD? Prev. Med. 39 (2004) 212–220.

[36] J.V. Pottala, M.A. Espeland, J. Polreis, J. Robinson, W.S. Harris, Correcting the effects of −20 °C storage and aliquot size on erythrocyte fatty acid content in the women's health initiative, Lipids 47 (2012) 835–846.

[37] C. von Schacky, S. Fischer, P.C. Weber, Long term effects of dietary marine II-3 fatty acids upon plasma- and cellular lipids, platelet function and eicosanoid formation in humans, J. Clin. Invest. 76 (1985) 1626–1631.

[38] W.S. Harris, R.M. Thomas, Biological variability of blood omega-3 biomarkers, Clin. Biochem. 43 (2010) 338–340.

[39] G.C. Shearer, J. Chen, Y. Chen, W.S. Harris, Myocardial infarction does not affect fatty acid profiles in rats, Prostaglandins Leukot. Essent. Fatty Acids 81 (2009) 411–416.

[40] W.S. Harris, S.A. Varvel, J.V. Pottala, G.R. Warnick, J.P. McConnell., The comparative effects of an acute dose of fish oil on omega-3 fatty acid levels in red blood cells versus plasma: implications for clinical utility, J. Clin. Lipidol. (2014) (in press).

[41] W.S. Harris, J.V. Pottala, R.S. Vasan, M.G. Larson, S.J. Robins, Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in Older Americans, J. Nutr. 142 (2012) 1297–1303.

[42] W.S. Harris, S.A. Sands, S.L. Windsor, H.A. Ali, T.L. Stevens, A. Magalski, et al., Omega-3 fatty acid levels in transplanted human hearts: effect of supplementation and comparison with erythrocytes, Circulation 110 (2004) 1645–1649.

[43] C. Arnold, M. Markovic, K. Blossey, G. Wallukat, R. Fischer, R. Dechend, et al., Arachidonic acid-metabolizing cytochrome P-450 enzymes are targets of omega-3 fatty acids, J. Biol. Chem. 285 (2010) 32720–32733.

[44] C. von Schacky, The Omega-3 Index as a risk factor for cardiovascular diseases, Prostaglandins Other Lipid Mediators 96 (2011) 94–98.

[45] S.O. Ebbesson, R.B. Devereux, S. Cole, L.O. Ebbesson, R.R. Fabsitz, K. Haack, et al., Heart rate is associated with red blood cell fatty acid concentration: the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) study, Am. Heart J. 159 (2010) 1020–1025.

[46] A. Sala-Vila, W.S. Harris, M. Cofán, A.M. Pérez-Heras, X. Pintó, R.M. Lamuela-Raventós, et al., Determinants of the omega-3 index in a Mediterranean population at increased risk for CHD, Br. J. Nutr. 106 (2011) 425–431.

[47] W.S. Harris, J.V. Pottala, S.M. Lacey, R.S. Vasan, M.G. Larson, S.J. Robins, Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study, Atherosclerosis 225 (2012) 425–431.

[48] A. Kohler, D. Bittner, A. Low, C. von Schacky, Effects of a convenience drink fortified with n-3 fatty acids on the n-3 index, Br. J. Nutr. 104 (2010) 729–736.

[49] H. Aarsetoey, V. Ponitz, O.B. Nilsen, H. Grundt, W.S. Harris, D.W. Nilsen, Low levels of cellular omega-3 increases the risk of ventricular fibrillation during the acute ischaemic phase of a myocardial infarction, Resuscitation 78 (2008) 258–264.

[50] H. Aarsetøy, R. Aarsetøy, T. Lindner, H. Staines, W.S. Harris, D.W.T. Nilsen., Low levels of the Omega-3 Index are associated with sudden cardiac arrest and remain stable in survivors in the subacute phase, Lipids 46 (2011) 151–161.

[51] J.V. Pottala, S. Garg, B.E. Cohen, M.A. Whooley, W.S. Harris WS, Blood eicosapentaenoic and docosahexaenoic acids predict all-cause mortality in patients with stable coronary heart disease: the heart and soul study, Circ. Cardiovasc. Qual. Outcomes 3 (2010) 406–412.

[52] W.S. Harris, K.F. Kennedy, J.H. O'Keefe Jr, J.A. Spertus, Red blood cell fatty acid levels improve GRACE score prediction of 2-yr mortality in patients with myocardial infarction, Int. J. Cardiol. 168 (2013) 53–59.

[53] M. Abuannadi, J.H. O'Keefe, J.A. Spertus, K.F. Kennedy, W.S. Harris,et al., Omega-3 Index: an independent predictor of 1 year all cause mortality in myocardial infarction (MI) patients, in: Poster 174, AHA QCOR Meeting, ⟨http://my.americanheart.org/idc/groups/ahamah-public/@wcm/@sop/@scon/documents/downloadable/ucm_319392.pdf⟩, May 19–21, 2010 (accessed 13.03.13).

[54] H. Aarsetoey, V. Ponitz, H. Grundt, H. Staines, W.S. Harris WS, D.W. Nilsen, (n-3) Fatty acid content of red blood cells does not predict risk of future cardiovascular events following an acute coronary syndrome, J. Nutr. 139 (2009) 507–513.

[55] W.S. Harris, K.J. Reid, S.A. Sands, J.A. Spertus, Blood omega-3 and trans fatty Acids in middle-aged acute coronary syndrome patients, Am. J. Cardiol. 99 (2007) 154–158.

[56] R.C. Block, W.S. Harris, K.J. Reid, S.A. Sands, J.A. Spertus, EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls, Atherosclerosis 197 (2008) 821–828.

[57] Y. Park, J. Lim, J. Lee, S.G. Kim, Erythrocyte fatty acid profiles can predict acute non-fatal myocardial infarction, Br. J. Nutr. 102 (2009) 1355–1356.

[58] Y.J. Kim, D.W. Jeong, J.G. Lee, H.C. Lee, S.Y. Lee, Y.J. Kim, et al., Omega-3 index and smoking in patients with acute ST-elevation myocardial infarction taking statins: a case-control study in Korea, Lipids Health Dis. 11 (2012) 43.

[59] L. Djoussé, A.O. Akinkuolie, J.H. Wu, E.L. Ding, J.M. Gaziano, Fish consumption, omega-3 fatty acids and risk of heart failure: a meta-analysis, Clin. Nutr. 31 (2012) 846–853.

[60] J.B. Wilk, M.Y. Tsai, N.Q. Hanson, J.M. Gaziano, L. Djoussé, Plasma and dietary omega-3 fatty acids, fish intake, and heart failure risk in the Physicians' Health Study, Am. J. Clin. Nutr. 96 (2012) 882–888.

[61] G.C. Shearer, J.V. Pottala, J.A. Spertus, W.S. Harris, Red blood cell fatty acid patterns and acute coronary syndrome, PloS One 4 (2009) e5444, http://dx.doi.org/10.1371/journal.pone.0005444.

[62] Y. Park, S. Park, H. Yi, H.Y. Kim, S.J. Kang, J. Kim, H. Ahn, Low level of n-3 polyunsaturated fatty acids in erythrocytes is a risk factor for both acute ischemic and hemorrhagic stroke in Koreans, Nutr. Res. 29 (2009) 825–830.

[63] M.K. Duda, K.M. O'Shea, A. Tintinu, W. Xu, R.J. Khairallah, B.R. Barrows, et al., Fish oil but not flaxseed oil decreases inflammation and prevents pressure-overload induced cardiac dysfunction, Cardiovasc. Res. 81 (2009) 319–327.

[64] G.E. Billman, Y. Nishijima, A.E. Belevych, D. Terentyev, Y. Xu, K.M. Haizlip, et al., Effects of dietary omega-3 fatty acids on ventricular function in dogs with healed myocardial infarctions: in vivo and in vitro studies, Am. J. Physiol. Heart Circ. Physiol. 298 (2010) H1219–H1228.

[65] R. Yang, W.S. Harris, K. Vernon, A.M. Thomas, N. Qureshi, D.C. Morrison, C.W. van Way 3rd, Pre-feeding with omega-3 fatty acids suppresses inflammation following hemorrhagic shock, J. Parenter. Enter. Nutr. 34 (2010) 496–502.
[66] J. Chen, G.C. Shearer, Q. Chen, C.L. Healy, A.J. Beyer, V.B. Nareddy, et al., Omega-3 fatty acids prevent pressure overload-induced cardiac fibrosis through activation of cGMP/PKG ehaviour in cardiac fibroblasts, Circulation 123 (2011) 584–593.
[67] G.E. Billman, W.S. Harris WS, Effect of dietary omega-3 fatty acids on the heart rate and the heart rate variability response to myocardial ischemia or submaximal exercise, Am. J. Physiol. Heart Circ. Physiol. 300 (2011) H2288–H2299.
[68] G.E. Billman, C.A. Carnes, P.B. Adamson, E. Vanoli, P.J. Schwartz, Dietary omega-3 fatty acids and susceptibility to ventricular fibrillation: lack of protection and a proarrhythmic effect, Circ. Arrhythm. Electrophysiol. 5 (2012) 553–560.
[69] Y. Park, H.M. Moon, S.H. Kim, N-3 polyunsaturated fatty acid consumption produces neurobiological effects associated with prevention of depression in rats after the forced swimming test, J. Nutr. Biochem. 23 (2012) 924–928.
[70] W.S. Harris, M. Gonzales, N. Laney, A. Sastre, A.M. Borkon, Effects of omega-3 fatty acids on heart rate in cardiac transplant recipients, Am. J. Cardiol. 98 (2006) 1393–1395.
[71] W.S. Harris, J.V. Pottala, S.A. Sands, P.G. Jones, Comparison of the effects of fish and fish oil capsules on the n-3 fatty acid content of blood cells and plasma phospholipids, Am. J. Clin. Nutr. 86 (2007) 1621–1625.
[72] M.K. Larson, J.H. Ashmore, K.A. Harris, J.L. Vogelaar, J.V. Pottala JV, M. Sprehe, W.S. Harris, Effects of omega-3 acid ethyl esters and aspirin, alone and in combination, on platelet function in healthy subjects, Thromb. Haemost. 100 (2008) 634–641.
[73] W.S. Harris, S.L. Lemke, S.N. Hansen, D.A. Goldstein, M.A. DiRienzo, A. Su, et al., Stearidonic acid-enriched soybean oil increased the Omega-3 Index, an emerging cardiovascular risk marker, Lipids 43 (2008) 805–811.
[74] R.M. Carney, K.E. Freedland, P.K. Stein, B.C. Steinmeyer, W.S. Harris, E.H. Rubin, et al., Effect of omega-3 fatty acids on heart rate variability in depressed patients with coronary heart disease, Psychosom. Med. 72 (2010) 748–754.
[75] S.L. Lemke, J.L. Vicini, H. Su, D.A. Goldstein, M.A. Nemeth, E.S. Krul, W. S. Harris, Dietary intake of stearidonic acid-enriched soybean oil increases the omega-3 index: randomized, double-blind clinical study of efficacy and safety, Am. J. Clin. Nutr. 92 (2010) 766–775.
[76] J. Neubronner, J.P. Schuchardt, G. Kressel, M. Merkel, C. von Schacky, A. Hahn, Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters, Eur. J. Clin. Nutr. 65 (2011) 247–254.
[77] A.C. Skulas-Ray, P.M. Kris-Etherton, W.S. Harris, J.P. vanden Heuvel, P. R. Wagner, S.G. West, Dose response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy people with moderate hypertriglyceridemia, Am. J. Clin. Nutr. 93 (2011) 243–252.
[78] Y. Park, M. Kim., Serum 25-hydroxyvitamin D concentrations are associated with erythrocyte levels of n-3 PUFA but not risk of CVD, Br. J. Nutr. 106 (2011) 1529–1534.
[79] J.P. Schuchardt, J. Neubronner, G. Kressel, A. Merkel, C. von Schacky, A. Hahn, Moderate doses of EPA and DHA from re-esterified triacylglycerols but not from ethyl-esters lower fasting serum triacylglycerols in statin-treated dyslipidemic subjects: results from a six month randomized controlled trial, Prostaglandins Leukot. Essent. Fatty Acids 85 (2011) 381–386.
[80] K.C. Maki, H.E. Bays, M.R. Dicklin, S.L. Johnson, M. Shabbout M, Effects of prescription omega-3-acid ethyl esters, coadministered with atorvastatin, on circulating levels of lipoprotein particles, apolipoprotein CIII, and lipoprotein-associated phospholipase A2 mass in men and women with mixed dyslipidemia, J. Clin. Lipidol. 5 (2011) 483–492.
[81] A. Dewell, F.F. Marvasti, W.S. Harris, P. Tsao, C.D. Gardner., Dose-dependent effects of plant and marine omega-3 fatty acids on inflammatory markers in insulin resistant adults. A randomized controlled trial, J. Nutr. 141 (2011) 2166–2171.
[82] E.S. Krul, S.L. Lemke, R. Mukherjea, M.L. Taylor, D.A. Goldstein DA, H. Su, et al., Effects of duration of treatment and dosage of eicosapentaenoic acid and stearidonic acid on red blood cell eicosapentaenoic acid content, Prostaglandins Leukot. Essent. Fatty Acids 86 (2012) 51–59.
[83] W.S. An, S.M. Lee, Y.K. Son, S.E. Kim, K.H. Kim, J.Y. Han, et al., Effect of omega-3 fatty acids on the modification of erythrocyte membrane fatty acid content including oleic acid in peritoneal dialysis patients, Prostaglandins Leukot. Essent. Fatty Acids. 86 (2012) 29–34.
[84] R.C. Block, U. Dier, P. Calderonartero, G.C. Shearer, L. Kakinami L, M.K. Larson, et al., The effects of EPA+DHA and aspirin on inflammatory cytokines and angiogenesis factors, World J. Cardiovasc. Dis. 2 (2012) 14–19.
[85] S. Schmidt, F. Stahl, K.O. Mutz, T. Scheper, A. Hahn, J.P. Schuchardt, Transcriptome-based identification of antioxidative gene expression after fish oil supplementation in normo- and dyslipidemic men, Nutr. Metab. 9 (1) (2012) 45.
[86] A.C. Skulas-Ray, P.M. Kris-Etherton, W.S. Harris, S.G. West, Effects of marine-derived omega-3 fatty acids on systemic hemodynamics at rest and during stress: a dose-response study, Ann. Behav. Med. 44 (2012) 301–308.
[87] W.S. An, S.M. Lee, Y.K. Son, S.E. Kim, K.H. Kim, J.Y. Han, et al., Omega-3 fatty acid supplementation increases 1,25-dihydroxyvitamin D and fetuin-A levels in dialysis patients, Nutr. Res. 32 (2012) 495–502.
[88] S. Schmidt, F. Stahl, K.O. Mutz, T. Scheper, A. Hahn, J.P. Schuchardt, Different gene expression profiles in normo- and dyslipidemic men after fish oil supplementation: results from a randomized controlled trial, Lipids Health Dis. 11 (2012) 105.
[89] J.P. Schuchardt, S. Schmidt, G. Kressel, H. Dong, I. Willenberg, B.D. Hammock, et al., Comparison of free serum oxylipin concentrations in hyper- vs. normolipidemic men, Prostaglandins Leukot. Essent. Fatty Acids. 89 (2013) 19–29.
[90] T. Metkus, J. Timpone, D. Leaf, M. Bidwell Goetz, W.S. Harris, T. Brown, Omega-3 fatty acid therapy reduces triglycerides and interleukin-6 in hypertriglyeridemic HIV patients, HIV Med. 14 (2013) 530–539.
[91] C. von Schacky, P. Angerer, W. Kothny, K. Theisen, H. Mudra., 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.
[92] J.P. Schuchardt, A. Hahn, Bioavailability of long-chain omega-3 fatty acids, Prostaglandins Leukot. Essent. Fatty Acids 89 (2013) 1–8.
[93] J. Dyerberg, P. Madsen, J.M. Møller, I. Aardestrup, E.B. Schmidt, Bioavailability of marine n-3 fatty acid formulations, Prostaglandins Leukot. Essent. Fatty Acids. 83 (2010) 137–141.
[94] S.M. Ulven, B. Kirkhus, A. Lamglait, S. Basu, E. Elind, T. Haider, et al., Metabolic effects of krill oil are essentially similar to those of fish oil but at lower dose of EPA and DHA, in healthy volunteers, Lipids 46 (2011) 37–46.
[95] M.H. Davidson, J. Johnson, M.W. Rooney, M.L. Kyle, D.F. Kling., A novel omega-3 free fatty acid formulation has dramatically improved bioavailability during a low-fat diet compared with omega-3-acid ethyl esters: the ECLIPSE (Epanova(®) compared to Lovaza(®) in a pharmacokinetic single-dose evaluation) study, J. Clin. Lipidol. 6 (2012) 573–584.
[96] E.K. Hussey, S. Portelli, M.J. Fossler, F. Gao, W.S. Harris, R.A. Blum, Relative bioavailability of an emulsion formulation for omega-3-acid ethyl esters compared to thecommercially available formulation: a randomized, parallel-group, single-dose study followed by repeat dosing in healthy volunteers, Clin. Pharm. Drug Develop. 1 (2012) 14–23.
[97] I.J. Haug, L.B. Sagmo, D. Zeiss, I.C. Olsen, K.I. Draget, T. Sternes, Bioavailability of EPA and DHA delivered by gelled emulsions and soft gel capsules, Eur. J. Lipid Sci. Technol. 113 (2011) 137–145.
[98] A.C. Salisbury, W.S. Harris, A.P. Amin, K.J. Reid, J.H. O'Keefe Jr., J.A. Spertus, Relation between red blood cell omega-3 fatty acid index and bleeding during acute myocardial infarction, Am. J. Cardiol. 109 (2012) 13–18.
[99] S.C. Larsson, M. Kumlin, M. Ingelman-Sundberg, A. Wolk, Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms, Am. J. Clin. Nutr. 79 (2004) 935–945.
[100] J. Shannon, I.B. King, R. Moshofsky, J.W. Lampe, D.L. Gao, R.M. Ray, D. B. Thomas, Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China, Am. J. Clin. Nutr. 85 (2007) 1090–1097.
[101] K. Kuriki, K. Hirose, K. Wakai, K. Matsuo, H. Ito, T. Suzuki, et al., Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids in Japanese, Int. J. Cancer 121 (2007) 377–385.
[102] A.E. Norrish, C.M. Skeaff, G.L. Arribas, S.J. Sharpe, R.T. Jackson, Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study, Br. J. Cancer 81 (1999) 1238–1242.
[103] P.A. Godley, M.K. Campbell, P. Gallagher, F.E. Martinson, J.L. Mohler, R. S. Sandler, Biomarkers of essential fatty acid consumption and risk of prostatic carcinoma, Cancer Epidemiol. Biomarkers Prev. 5 (1996) 889–895.
[104] C.R. Ritch, R.L. Wan, L.B. Stephens, J.B. Taxy, D. Huo, E.M. Gong, et al., Dietary fatty acids correlate with prostate cancer biopsy grade and volume in Jamaican men, J. Urol. 177 (2007) 97–101.
[105] J. Shannon, J. O'Malley, M. Mori, M. Garzotto, A.J. Palma, I.B. King, Erythrocyte fatty acids and prostate cancer risk: a comparison of methods, Prostaglandins Leukot. Essent. Fatty Acids. 83 (2010) 161–169.
[106] L.M. Newcomer, I.B. King, K.G. Wicklund, J.L. Stanford., The association of fatty acids with prostate cancer risk, Prostate 47 (2001) 262–268.
[107] M. Okuno, K. Hamazaki, T. Ogura, H. Kitade, T. Matsuura T, R. Yoshida, et al., Abnormalities in fatty acids in plasma, erythrocytes and adipose tissue in Japanese patients with colorectal cancer, In Vivo 27 (2013) 203–210.
[108] M.H. Bloch, A. Qawasmi., Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis, J. Am. Acad. Child Adolesc. Psychiatry 50 (2011) 991–1000.
[109] P. Montgomery, J.R. Burton, R.P. Sewell RP, T.F. Spreckelsen, A.J. Richardson, Low blood long chain omega-3 fatty acids in UK children are associated with poor cognitive performance and ehaviour: a cross-sectional analysis from the DOLAB study, PloS One 24 (8) (2013) e66697.
[110] M.E. Sublette, S.P. Ellis, A.L. Geant, J.J. Mann, Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression, J. Clin. Psychiatry, 72, 20111577–1584.
[111] M.H. Bloch, J. Hannestad., Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis, Mol. Psychiatry1. 17 (2012) 1272–1282.
[112] W. Stonehouse, C.A. Conlon, J. Podd, S.R. Hill, A.M. Minihane, C. Haskell, D. Kennedy., DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial, Am. J. Clin. Nutr. 97 (2013) 1134–1143.
[113] A.V. Witte, L. Kerti, H.M. Hermannstädter, J.B. Fiebach, S.J. Schreiber, J. P. Schuchardt, et al., Long-Chain Omega-3 Fatty Acids Improve Brain Function and Structure in Older Adults, Cereb. Cortex. (2013) (Epub ahead of print).