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OMEGA-3 FATTY ACIDS VS. CARDIAC DISEASE – THE CONTRIBUTION OF THE OMEGA-3 INDEX

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Abstract – Although statistically and clinically significant, reductions of clinical events seen in large scale intervention studies with omega-3 fatty acids in the cardiovascular field were smaller than would have been predicted from the results of epidemiologic studies. In epidemiologic studies, assessment of intake of fish or eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) predicted clinical events less well than assessing blood levels of EPA and DHA, e.g. by the Omega-3 Index. The Omega-3 Index is the percentage of EPA+DHA in red cell lipids, determined with a highly standardized methodology. Using the perspective of the Omega-3 Index facilitates reconciling discrepancies in results from intervention studies and from epidemiologic studies. Moreover, a low Omega-3 Index can be considered a risk factor for sudden cardiac death and for non-fatal cardiovascular events, whereas a high Omega-3 Index can be used as a therapeutic target. Currently ongoing and future scientific work on the basis of the Omega-3 Index will further define its value.

Key words: Eicosapentaneoic acid, docosahexaenoic acid, Omega-3 Index, sudden cardiac death, congestive heart failure, coronary artery disease

INTRODUCTION

A vast body of literature exists on the use of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in intervention studies in the cardiovascular field (e.g. reviewed in 10, 15, 19, 37). Typically, like in GISSI-Prevenzione or GISSI-HF (4,17), a fixed dose of EPA+DHA (1 g / day) was investigated like an experimental drug, comparing its effect to a control or placebo group, on the basis of an otherwise guideline oriented treatment in both groups. The study participants were defined by inclusion and exclusion criteria that encompassed disease features, but usually no assessment of intake or endogenous levels of EPA+DHA (4,17). As reviewed in more detail elsewhere in this issue, this approach has been successful: Total mortality was reduced (-21%), especially driven by a reduction in occurrence of sudden cardiac death (SCD, -45%) in patients after a first myocardial infarction in GISSI-P, while a reduction in non-fatal myocardial infarctions was not seen (17). In Patients with congestive heart failure, GISSI-HF demonstrated a reduction in total mortality (-9%) plus hospitalizations (-8%)(4). A special case is JELIS, a very large randomized intervention study conducted in Japan in a population with cardiovascular risk factors (40). Total incidences of sudden cardiac death and of fatal myocardial infarctions were low, and not reduced by 1.8 g EPA / day. The overall positive study result was driven by a reduction in non-fatal events by 19 % (40).

Thus, although statistically and clinically significant, reductions of clinical events were

Abbreviations: **CHD,** coronary heart disease; **DHA,** docosahexaenoic acid; **EPA,** eicosapentaenoic acid; **GISSI-P,** Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico, -P: prevenzione, -;**HF,** heart failure; **JELIS,** Japan Eicosapentaenoic Lipid Intervention Study; **PUFA,** polyunsaturated fatty acids; **SCD,** sudden cardiac death.

(much) smaller than would have been predicted from the results of epidemiologic studies discussed below. Here, reasons for these discrepancies are discussed, with particular reference to the Omega-3 Index, a novel biomarker.

EPIDEMIOLOGY

Ingestion of fish and omega-3 fatty acids

In most epidemiologic studies, intake of fish was associated with reduced total or cardiovascular mortality (reviewed e.g. in 19, 37). A typical example is the Nurses health study, where risk for fatal CHD decreased roughly according to the amount of fish ingested (data in quintiles): From a relative risk for fatal CHD of 1.0 (lowest quintile of fish ingested), to 0.81 (0.57-1.15), 0.66 (0.47-0.92), 0.72 (0.49-1.08), to 0.55 (0.33-0.90), with a p for trend <0.01 (95%CI given, multivariate analysis I, ref. 11). A similar picture emerged, when the amount of EPA+DHA ingested was calculated from the dietary questionnaires and factored into the data: From a relative risk for fatal CHD of 1.0 (lowest quintile of omega-3 fatty acid containing fish ingested) to 0.63 (0.45-0.88), with a p for trend <0.001 (95%CI given, ref.11). Thus, a 37 % lower relative risk for mortality from coronary heart disease was seen in the highest quintile of intake of omega-3 fatty acid containing fish, as compared to the lowest quintile.

Similarly, non-fatal myocardial infarctions were less frequent in persons ingesting fish in epidemiologic studies, although, in general, the data was found to be less impressive (reviewed e.g. in 19, 37): In the Nurses Health Study, risk decreased roughly with frequency of fish meals: From a relative risk for non-fatal CHD of 1.0 of 1.0 (lowest quintile of fish ingested), to 0.78 (0.60-1.00), 0.74 (0.58-0.94), 0.68 (two-four times / week, 0.51-0.90), to 0.73 (0.51-1.04), with a p for trend <0.03 (data in quintiles, 95%CI given, multivariate analysis I, ref. 11). When the amount of EPA+DHA intake was estimated, and factored into the data, risk for non-fatal CHD decreased from 1.0 (lowest quintile of omega-3 fatty acid containing fish ingested) to 0.92 (0.75-1.13), to 0.83 (0.67-1.02), to 0.75 (0.60-0.94) to 0.69 (0.55-0.88), p for trend <0.01 (data in quintiles, 95%CI given, multivariate analysis I, ref. 11). Thus, a 31 % lower relative risk for non-fatal myocardial infarctions was seen in the highest quintile of intake of omega-3 fatty acid containing fish, as compared to the lowest quintile

Less data are available for congestive heart failure: In a study in Japan, an inverse association of fish and omega-3 PUFA intakes with relative risks of mortality from heart failure (multivariable hazard ratio [95% confidence interval] for highest versus lowest quintiles = 0.76 [0.53 to 1.09] for fish and 0.58 [0.36 to 0.93] for EPA+DHA intake was seen (39). A retrospective analysis of the GISSI-P trial found a 24 relative % lower mortality in participants with congestive heart failure in the fish oil group, as compared to such participants in the control group (16).

BLOOD LEVELS OF OMEGA-3 FATTY ACIDS

In two case-control studies on sudden cardiac death, levels of omega-3 fatty acids were measured: In Seattle, WA, USA, an inverse relation between red cell omega-3 fatty acid concentrations and risk for sudden cardiac death was seen: 6.5 % was associated with a relative risk of 1.0, whereas 3.3 % was associated with a relative risk of 0.1 (95% CI 0.14 – 0.37, p not given), – a tenfold difference (26). A tenfold difference was also seen by analyzing whole blood samples from the Physicians Health study (1). When comparing different populations, an even larger difference in incidence of SCD can be found: In the general population of Western countries like Germany or the US, where an Omega-3 Index among 4 % is frequent, the incidence of SCD is among 150 / 100 000 person years, whereas in the general population in Japan, where red cell levels of EPA and DHA are among 10 %, the incidence of SCD is 7.8 / 100 000 person years – a 20 fold difference (12, 40). Combining the numbers mentioned results in fig. 1, which is partly hypothetical, since analytical methods varied.

The incidence of myocardial infarctions in the general population of Western countries like Germany is tenfold higher than in the general population in Japan (31). Environmental factors and differences in cholesterol levels, rather than genetic differences, are thought to explain this tenfold difference (31). A lower burden of atherosclerosis correlated inversely with serum levels of EPA and DHA (25, 30). A case-control study from Kansas found that an Omega-3 Index >8% was associated with 1/3 of the risk for acute

coronary syndrome, as compared to an Omega-3 Index<4% (RR 0.31, 95%CI 0.14-0.67, p for trend <0.0001), with a multivariable-adjusted odds for case status of 0.77 (95% CI 0.70-0.85) for a one unit increase in the Omega-3 Index (2). Similar data, in this case based on measurements of EPA and DHA in plasma, have been published independently (29).

Nothing is known on a relation between blood levels of omega-3 fatty acids and development of congestive heart failure or atrial fibrillation and their complications.

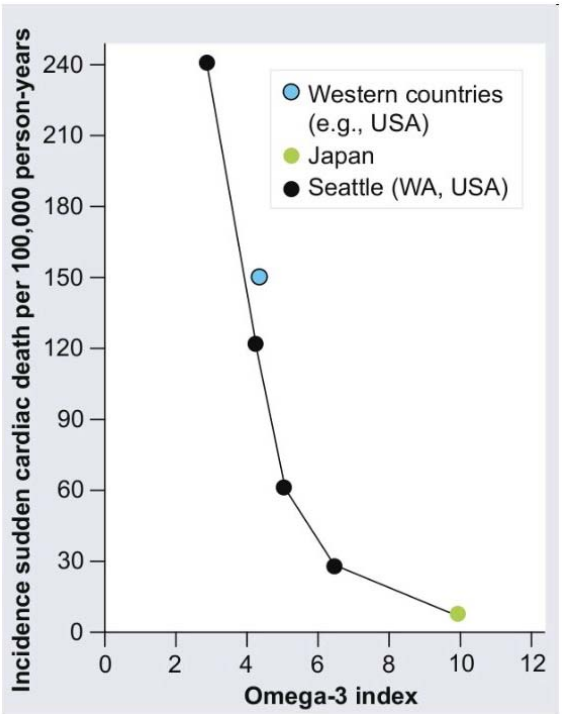

Figure 1. Relation between incidence of sudden cardiac death and Omega-3 Index. The relation is partly hypothetical, because different analytical methods were used. Reproduced with permission from Biomarkers Med

Taken together, in randomized intervention studies, EPA+DHA reduced total mortality less efficiently than predicted from epidemiologic studies assessing fish or fish-related omega-3 fatty acid consumption. One of the reasons that can be invoked to explain these differences is non-compliance with study intervention: A per protocol analysis of GISSI-HF showed a 14 % reduction in total mortality in per protocol analysis, as compared to a 9% reduction in the intention to treat analysis (4). A more difficult issue is the question of dosing. However, 1 g / day has repeatedly been demonstrated to resemble two fish meals per week, which approximates the intake, at which a minimum of risk was seen in the Nurses Health Study mentioned (8,11). A much larger difference in risk can be observed from the perspective of blood levels: approx. 10 - 20 fold for SCD, 7 fold for myocardial infarctions in general, 3 fold for non-fatal myocardial infarctions. So far, the results found are rather consistent. Epidemiologic studies, however, may be subject to publication bias. In conclusion, assessment of intake of fish or supplementation with 1 g EPA+DHA / day predicted clinical events considerably less well than assessing blood levels of EPA and DHA e.g. by the Omega-3 Index.

THE OMEGA-3 INDEX

The Omega-3 Index was defined in 2004 as the percentage of EPA+DHA in red cell lipids, using a highly standardized analytical methodology (8). This methodology has been installed in three laboratories in the world (US, Germany, Korea), and successfully subjected to an interlaboratory comparison (9). A high analytical reproducibility with a low coefficient of variation (4 – 7 %) was found, a prerequisite to acknowledge the low biological variability of the Omega-3 Index (5). The low biological variability may be due to the fact that the half-life of RBC EPA+DHA is 4 – 6 times longer than that of serum EPA+DHA (5). Also, RBC fatty acid composition is less influenced by day-to-day variations and by dyslipidemias than are plasma fatty acids, which contributes to the fact that the Omega-3 Index is unaffected by fasting or fed state (5). In contrast to other assessments of levels of omega-3 fatty acids, like in plasma, the Omega-3 Index correlates with cardiac ventricular or atrial tissue levels during steady intake of EPA and DHA, as well as after an increase in intake (7, 18). Taken together, the Omega-3 Index can be considered a parameter that reflects a persons´ status in EPA and DHA, rather than short term intake, which may be better reflected by measurements in plasma (33, 34, 36). A pre-analytical advantage is that samples are stable for 7 days at room temperature, and can be shipped by regular mail, if taken into EDTA-coated tubes (5). Taken together, from a methodological point of view, determining the Omega-3 Index has advantages over determining levels of EPA+DHA in other fatty acid compartments.

Intake of EPA and DHA is only one determinant of the Omega-3 Index: for every 4 g of EPA and DHA ingested per month, the

Omega-3 Index increased by 0.24 % (23). Other determinants found in Kansas City were age (+ 0.50 % / decade), diabetes (- 1.13 %, if present), body mass index (- 0.30 % / 3 units) (23). In Japan, gender and physical activity were also found to influence RBC EPA+DHA (13). Only a part of the population converts some alpha-linolenic acid to EPA, whereas another part is unable to perform this metabolic step (20). Less is know on genetic influences of catabolism of EPA and DHA. Therefore, a genetic influence on the Omega-3 Index seems likely. Moreover, drugs impeding lipid absorption are likely to also impede absorption of omega-3 fatty acids. Other factors, yet to be defined may also play a role. Taken together, intake of EPA+DHA contributes to, but does not predict, the Omega-3 Index. This may explain some of the difference between the epidemiologic studies focusing on fish consumption and the epidemiologic studies focusing on levels of omega-3 fatty acids in blood, especially on the Omega-3 Index.

The Omega-3 Index has been measured in a number of populations, e.g. in study participants with subclinical atherosclerosis (defined in ref 3, fig. 2). In this population, as in all other populations studied so far, a normal, Gaussian distribution has been found, as illustrated by fig. 3. Means, however, differ: In a general population in Kansas City, a mean of 4.9±2.1 % was measured, whereas in Japan, red cell EPA+DHA were measured using a different methodology at 8.5 % (SD not reported)(13,23, also fig. 1, 2). More measurements are currently being performed in other populations.

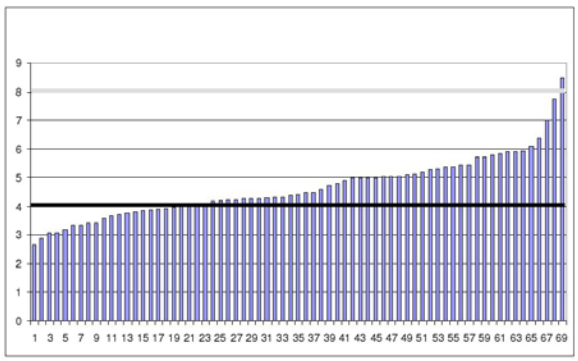

Figure 2. Omega-3 Index in 69 persons with sub-clinical atherosclerosis, as defined in ref. 3. Mean(±SD) = 4.65±1.09 %. Each bar represents one person.

LARGE SCALE INTERVENTION STUDIES AND THE OMEGA-3 INDEX

The large scale intervention studies reported so far used omega-3 fatty acids like an experimental drug. However, in contrast to levels of an experimental drug, levels of omega-3 fatty acids can be assessed in every human being. Therefore, rather than comparing the presence with the absence of an experimental drug, intervention studies with omega-3 fatty acids intend to compare an increase to no increase of pre-existing levels of omega-3 fatty acids – clearly a less potent approach.

None of the large-scale intervention studies factored an assessment of a pre-study mean Omega-3 Index or any other assessment of mean levels of EPA and DHA into the study design (e.g. 4, 15, 17). As mentioned, cardiovascular risk factors were similar in participants of GISSI-P and JELIS (17, 40). Incidence of SCD was 828 / 100 000 person years in the GISSI-P control group, and 615 / 100 000 person years in the intervention group, whereas it was 40 / 100 000 person years in JELIS (17, 40). The incidence of non-fatal myocardial infarctions in GISSI-P was 1151 / 100 000 person years in GISSI-P, not reduced by 1 g / day EPA+DHA, whereas it was 178 / 100 000 person years in the control group of JELIS, with a non-significant 25 % reduction to 133 / person years by 1.8 g EPA / day (17, 40). Thus, differences in incidence of events were much larger between GISSI-P and JELIS than between the respective intervention and control groups. We previously estimated the baseline Omega-3 Index in GISSI-P among 4.5%, whereas in JELIS, it probably was around 10 % (8, 13, 40). Therefore, in order to estimate event rates in clinical trials, and thus size and duration of the trial, it seems prudent to factor in important characteristics of the study population, like a pre-study Omega-3 Index. Conversely, in the authors´ personal opinion, results of intervention studies can better be understood and interpreted in light of pre-study Omega-3 Index or other levels of EPA+DHA.

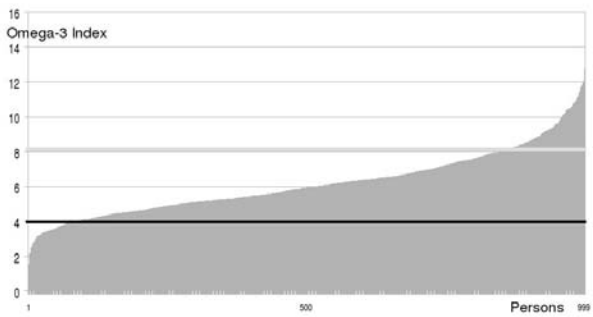

Figure 3. Omega-3 Index in 1000 unselected persons in Germany. Mean(±SD) = 6.14±1.83 %

None of the large-scale intervention studies reported so far on recruited study participants depending on an individual pre-study Omega-3 Index or any other assessment of levels or intake of EPA and DHA. It can safely be assumed that in such study populations, the Omega-3 Index had a normal, Gaussian, distribution, like in Figs. 2, 3, 4. Therefore, in any given study population, some participants had a relatively low Omega-3 Index, the majority was around the mean, and some participants had a relatively high Omega-3 Index. Participants with a high Omega-3 Index pre-study were not likely to experience a large effect of the study intervention, if randomized to the intervention group, and were not likely to develop clinical events, if randomized to the control group. Conversely, not all participants with a low Omega-3 Index pre-study achieved a sufficiently high Omega-3 Index to protect them from clinical events, if randomized to the intervention group; if randomized to the control group, however, they were likely to experience clinical events. Thus, only for these reasons, a substantial portion of the study population was unlikely to benefit from being in the intervention group / not benefit from being in the control group, and thus to contribute to the study results – resulting in a dilution of the effect of the intervention with EPA+DHA.

Non-compliance with study medication also has a negative impact on the size of the effect seen in an intervention study. In any randomized controlled study, non-compliance can occur in the intervention group, i.e. study participants refrain from ingesting the study medication. In contrast to studies with experimental drugs that are not available to the study participant elsewhere, participants of studies with omega-3 fatty acids can increase their intake by ingesting omega-3 fatty acids from other sources; i.e. non-compliance can also occur in the placebo group. This is likely, since ethics demand that prospective study participants are informed that the study medication might be of value for them. In JELIS, but not in the GISSI trials, changes in blood levels of EPA+DHA were documented (JELIS 2, 4, 17, 22). Compliance, however, was not assessed this way. Therefore, the magnitude of this problem remains unknown. Non-compliance in the control and the intervention groups also results in a dilution of the effect of the intervention with omega-3 fatty acids – a larger dilution than in studies with an experimental drug.

As discussed above, dietary intake is only one determinant of the Omega-3 Index. Therefore, inter-individually, a heterogeneous response to a uniform dosage is to be expected, and illustrated in Fig. 4 This phenomenon is also likely to be seen in large-scale intervention studies, although comparable measurements have not yet been performed. Heterogeneity of response to the study intervention also serves to dilute the effect of the intervention.

Taken together, a number of reasons and mechanisms jointly minimized effects of EPA+DHA discernible by randomized intervention studies published so far. These reasons and mechanisms are less effective in studies with experimental drugs. However, this discussion also indicates that an Omega-3 Index – based approach is likely to be more efficient, and maybe also more successful in demonstrating effects of omega-3 fatty acids.

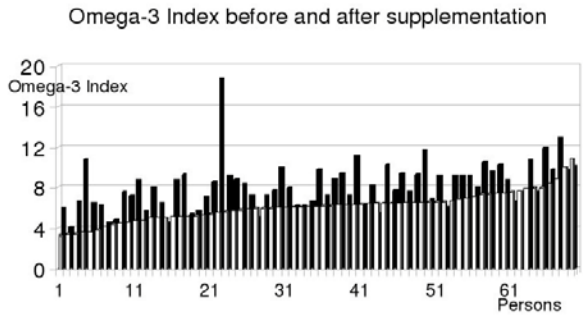

Figure 4. Response to supplementation with 1 g EPA + DHA / day in 69 persons. Mean (±SD) before supplementation (white bars) = 6.17±1.43 %. Mean (±SD) after supplementation of 1 g EPA+DHA for approx. 3 months (black bars) = 8.13±2.30 %. Each person represented by a white and a black bar, adjacent to each other. Please note the heterogeneity of response to supplementation.

STRENGTHS AND LIMITATIONS

Currently, determining the Omega-3 Index with the method that has been thoroughly evaluated and is subject to rigorous quality control is available in only a limited number of laboratories in the world. However, samples – in a tube or on specially prepared paper – can be transported with ease (5). A number of epidemiologic research projects, in patients with coronary artery disease or at risk for it (e.g. in collaboration with the Framingham group), in patients with congestive heart failure, in carriers of implanted cardioverters / defibrillators and other diseases and conditions outside cardiology are currently in progress, as are a number of

intervention studies (unpublished). Thus, the amount of evidence accumulating based on this specific analytical method is increasing rapidly. Therefore, other laboratories have begun to join into using the method to determine the Omega-3 Index. It is envisioned that more will join in the future.

Systematic comparative studies comparing the Omega-3 Index to other methods of assessing a persons´ status in omega-3 fatty acids, including the response to a change in diet, have yet to be conducted. Other compartments also reflect a change in intake of omega-3 fatty acids: plasma free fatty acids within hours, plasma phospholipids fatty acids within days, as do platelet phospholipids fatty acids (33, 34, 36). Some fatty acid compartments, like platelet phosphatidylinositol, do not incorporate EPA or DHA, while others, like platelet phosphatidylcholine do so preferentially (35). Therefore, it cannot be excluded that another fatty acid compartment exists that better reflects risk and change in risk than the omega-3 index. Also, the Omega-3 Index calls for determination in erythrocytes, obtained by centrifugation of EDTA-blood – a fact that may limit the widespread application of this determination. Determining the fatty acid composition of whole blood can be converted into the Omega-3 Index using a conversion factor under constant dietary conditions; kinetics after a change in diet, however, still need to be comparatively characterized. When analysing the fluid obtained by a fingerprick for fatty acid composition, reproducible results can only be obtained when using strictly standardized conditions (fasting, specific lancet apparatus, etc., unpublished). The results on EPA and DHA can be transformed into the Omega-3 Index by use of a conversion factor with some certainty (unpublished).

As yet, intervention studies based on the Omega-3 Index, i.e. using a pre-specified Omega-3 Index for participant selection and / or as a target criterion have not been published. Some small studies are, however, being conducted at present to the authors´ knowledge (unpublished). Clearly, there is a need for Omega-3 Index-based randomized intervention studies with clinical endpoints. Entry criteria and target levels to be achieved for the Omega-3 Index could be based on data derived from epidemiologic studies Japanese vs. Western populations, as discussed here. Estimating the treatment effect, necessary for case estimations, for these studies could be based on current epidemiologic evidence. This should lead to much smaller study sizes in comparison to published studies. This way, research on omega-3 fatty acids could become less costly and more efficient.

Presently, guidelines advocate the use of omega-3 fatty acids in cardiovascular prevention, treatment after myocardial infarction and secondary prevention, generally in a dose of 1 g / day (27, 32). This reflects the evidence provided by studies such as GISSI-P (17). These studies, however, can also be considered as providing proof-of-principle evidence in favour of an effect of omega-3 fatty acids in the clinical situations mentioned. Similarly, large intervention studies established the use of statins in cardiovascular risk populations, without aiming at given target (e.g. 21, 24). Nevertheless, target levels were established by guideline committees, and were subsequently challenged by further studies, like TNT (14, 27). Several studies in progress try to relate the occurrence of clinical events over time to levels of the Omega-3 Index. Results of these studies may add to the scientific basis for target levels of the Omega-3 Index.

When the Omega-3 Index was proposed as a risk factor for sudden cardiac death in 2004, high risk levels were suggested to be $\leq$ 4%, intermediate risk levels >4 and < 8 %, and low risk levels > 8% (8). These suggestions were largely derived from circumstantial evidence, but are in line with fig. 1. From fig. 1 it can also be extrapolated that an Omega-3 Index > 11% will offer little, if any further protection from sudden cardiac death. Ongoing research aims at prospectively validating fig. 1. Therefore, the levels suggested remain to be scrutinized by ongoing research. Further research, some of it ongoing, will define target levels for other cardiac and non-cardiac diseases and conditions.

There is a debate, whether a need for new biomarkers exits (28, 38). The Omega-Index fulfils the criteria put forth for a biomarker (28, 38). In contrast to most biomarkers, however, the results of intervention studies mentioned above indicate causality, at least in principle. Unpublished data indicate that in patients with coronary artery disease, with similar characteristics to participants of GISSI-P, a high Omega-3 Index predicts a survival benefit in comparison to a low Omega-3 Index. Although necessary from a strict scientific point of view, a placebo-controlled randomized intervention

study with the aim of proving that increasing the Omega-3 Index improves survival in coronary artery patients might therefore become difficult to justify from an ethical point of view. A randomized study comparing the present approach, as derived from GISSI-P, to an Omega-3 Index-based approach seems more prudent.

CONCLUSION

Large scale randomized intervention trials demonstrated that 1 g / day EPA and DHA reduced mortality and morbidity in patients with coronary artery disease or congestive heart failure. The beneficial effect seen was smaller than was to be expected based on data derived from epidemiologic studies. When using the perspective of the Omega-3 Index, the percentage of EPA and DHA in red cells, these differences can partly be explained. A low Omega-3 Index (<4%) can be considered a risk factor for SCD and cardiovascular events. Conversely, a high Omega-3 Index (>8, < 11%) can be used as a therapeutic target. Most methodologic issues of the Omega-3 Index have been resolved. Therefore, in the future, a more widespread use of the Omega-3 Index is anticipated in clinical studies, as well as in patient care. A more widespread use of the Omega-3 Index is likely to render clinical research more efficient, and contribute to a reduction in mortality and morbidity by a more intelligent use of EPA and DHA.

Other articles in this theme issue include references (41-52).

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