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A New Predictor of Risk for Sudden Cardiac Death

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ABSTRACT

Background. Long-chain fatty acids, particularly omega-3 fatty acids found primarily in fish oil, are beneficial in different physiological conditions in the human body. High intake of omega-3 fatty acids has been found to have a strong inverse relationship to sudden cardiac death. An index showing the relationship between different fatty acids in the blood could be an important risk indicator for sudden cardiac death.

Methods. Whole-blood samples from the fingertip were collected and fatty acids were measured by a new simple method using direct transmethylation. Two groups were compared: subjects who had been taking fish oil daily during the last 6 years, and subjects who had not been taking fish oil.

Results. Six different fatty acid indices were calculated. Five of them take both DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) into consideration, and the sixth includes only EPA and not DHA in the calculation. This latter index, the ratio between EPA and arachidonic acid, gave the best result, with the largest difference between the two groups. This index varied between 5 and 118 among the different individuals; 70% of the fish oil consumers having an index ≥50. Based on the present knowledge about the relationship between blood levels of omega-3 fatty acids and sudden cardiac death obtained from studies of 14,000 individuals, a subject with an index below 50 should be advised to increase his/her intake of omega-3 fatty acids.

Conclusion. The EPA/arachidonic acid index may be an important new predictor of risk for sudden cardiac death, and reflects the individual requirement for consumption of omega-3 fatty acids.

INTRODUCTION

Low omega-3 blood levels have been found to be a more important risk factor for sudden cardiac death than high blood levels of cholesterol or C-reactive protein (CRP) (1-3.) Subjects with high blood levels of omega-3 fatty acids have been reported to

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have a 90% lower risk for sudden cardiac death than subjects with low blood levels (1). Recently a meta-analysis of 97 randomized controlled trials comprising 276,000 individuals showed that intake of omega-3 fatty acids is more effective in reducing cardiac death than statins, fibrates, resins, or niacin (4). Intake of omega-3 fatty acids decreased total mortality by 23%, whereas statins decreased this rate by 13%. The corresponding reductions of cardiac mortality were 32% and 22%, respectively.

The more favourable effects of omega-3 fatty acids may be due to their antiarrhythmic and antithrombotic actions. Thus, in addition to its beneficial effects on blood lipids (5-8) stable fish oil also decreases ventricular fibrillation (9), the most common cause of sudden cardiac death, and inhibits arterial thrombus formation (10). Blood levels of cholesterol and CRP can be measured by simple tests which are easy to evaluate. No such method for determination of omega-3 fatty acids has hitherto been available.

Recently a new method for measuring fatty acids in human blood, using just a few blood drops from the fingertip, was reported (11). In comparison with conventional methods, this new technique is much simpler and less time consuming. Another group (3) applying a conventional method for measuring fatty acids in red blood cells, recently used a new index, the ratio between EPA+DHA and all fatty acids in the sample, as a measure of the omega-3 fatty acid status. Our aim was to combine modifications of these two techniques to get a simple, useful indicator for sudden cardiac death. We used one drop or a few drops of blood from the fingertip in high fish oil and non-fish oil consumers and developed a new index which reflects the omega-3 fatty acid status in the body.

MATERIALS AND METHODS

Subjects

One group consisted of 36 subjects, 12 males and 24 females, aged between 49 and 75 years (mean age 63 years), who had been taking natural stable fish oil (FO) (Eskimo-3, Cardinova, Sweden), 5 ml daily, during the last 6 years because of increased blood lipids. The other group consisted of 18 subjects, 11 males and 7 females, aged between 42 and 76 years (mean age 59 years), who had not been taking fish oil supplements (NFO). Before blood sampling the subjects had fasted for 10 hours.

Methods

Blood sampling

Blood from the fingertip, 25 µl-100 µl (1-4 drops) was absorbed onto a special filter paper (Schleicher Schuell, 591), which did not interfere with the fatty acids and showed no peaks in the chromatogram. This paper was analyzed directly or stored at 4oC in envelopes with airtight closure.

Transmethylation

The filter paper was transferred to a glass vial with 1 ml of 3N methanol/HCl (Supelco) and kept in a dry bath at 90°C for 60 minutes. In the transmethylation process there is a transfer of methyl groups from methanol to fatty acids.

Extraction

After the vial had been returned to room temperature, 2 ml of distilled water and 2 ml of saturated KCl solution were added, and the contents of the vial were mixed by a Whirlmixer. Fatty acids were extracted twice using 2 ml n-hexane. The collected volume of the n-hexane phase was reduced totally by N_2 gas at 25°C. The fatty acids were then dissolved in 50 µl n-hexane.

Separation

Five microliters of the solution was injected in a gas chromatograph (Hewlett Packard 5890) equipped with a 50 m PE-FFAP column (Perkin Elmer), split-injector, FID detector and a datasystem. Temperature programming went from 170°C to 250°C with an increase 5°C/minute. Peaks were identified by comparisons with an external standard containing 20 fatty acids typical for fish oil fatty acids.

RESULTS

Blood samples from all 54 subjects were analyzed. The mean percentages of the different fatty acids in the blood samples in Groups NFO and FO are shown in Table 1.

FA	NFO	FO
14:0	**1.12**± 0.59	**1.63**± 0.92
16:0	**25.65**± 1.61	**24.73**±2.28
16:1 (n-7)	**1.96**± 0.64	**1.71**± 0.63
18:0	**11.11**± 1.28	**13.29**± 3.58
18:1 (n-9)	**19.84**± 2.15	**17.68**± 2.00
18:1 (n-7)	**1.88**± 0.22	**1.76**± 0.44
18:2 (n-6)	**21.09**± 3.07	**17.42**± 3.47
18:3 (n-3)	**0.70**±0.25	**0.65**± 0.19
20:0	**0.38**± 0.18	**0.83**± 0.62
20:1 (n-3)	**0.17**± 0.10	**0.14**± 0.05
20:3 (n-6)	**1.35**± 0.26	**0.98**± 0.31
20:4 (n-6)	**6.93**± 1.19	**6.13**± 1.64
EPA (n-3)	**1.19**± 0.49	**3.48**± 1.42
22:1 (n-11)	**1.11**± 0.36	**1.70**± 0.74
22:4 (n-6)	**0.55**± 0.15	**0.42**± 0.31
24:0	**2.79**± 0.50	**3.56**± 0.91
DHA (n-3)	**2.62**± 0.59	**4.81**± 0.93

Table 1. Mean (and standard deviation) percentages of different fatty acids in blood samples from non-fish oil (NFO) and fish oil (FO) consumers.

Several fatty acid indices were calculated. Index 1 is EPA+DHA expressed as percentage of total fatty acids; Index 2 is the ratio between EPA+DHA and linoleic acid (18:2, n-6) + arachidonic acid (%); Index 3 is the ratio between EPA+DHA and only linoleic acid (%); Index 4 is calculated like Index 1, but here only the main peaks are included (EPA+DHA)/(16:0+16:1+18:0+18:1+18:2+18:3+20:4+EPA+ DHA); Index 5 is the ratio between EPA+DHA and arachidonic acid (%); Index 6 is the ratio between EPA and arachidonic acid (%).

	NFO	FO	Ratio (FO/NFO)
Index 1	**3.82**± 0.99***	**8.29**± 2.02	2.17
Index 2	**13.90**± 4.37***	**36.47**±11.22	2.62
Index 3	**18.62**± 6.10***	**49.47**± 16.32	2.66
Index 4	**4.10** ± 1.05***	**9.08**± 2.12	2.21
Index 5	**56.78**± 19.00***	**142.70**± 42.98	2.51
Index 6	**17.84**± 8.31***	**60.66** ± 27.67	3.40

***$p \leq 0{,}001$

Table 2. Mean (and standard deviation) of different fatty acid indices in NFO (non- fish oil) and FO (fish oil) consumers.

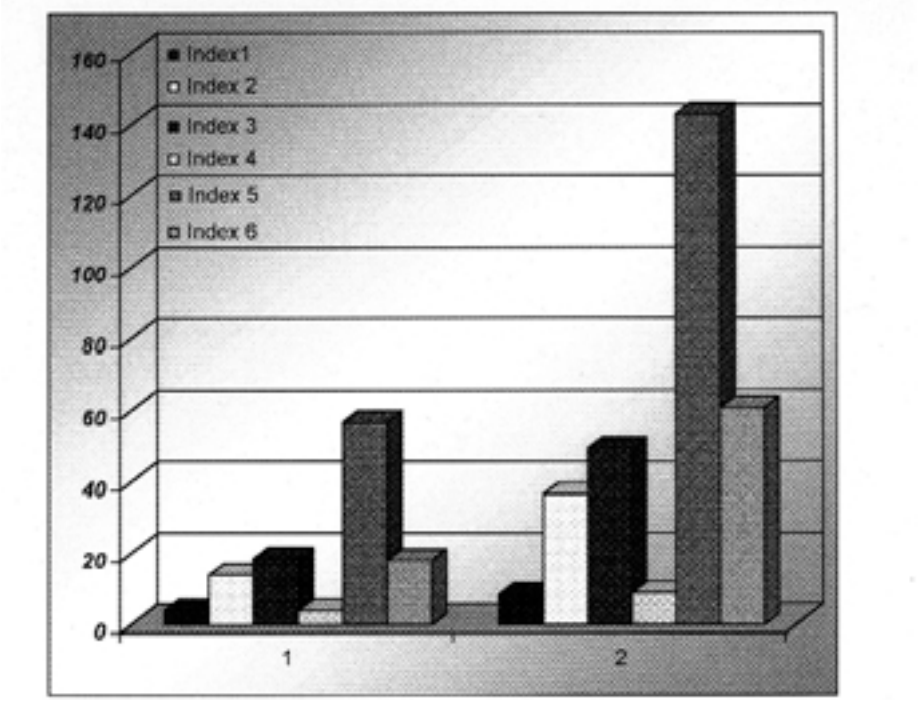

Figure 1. Different fatty acid indices in the non-fish oil (1) and the fish oil (2) group

The mean values of these indices and the ratio between group FO and group NFO for each index are shown in Table 2, and the mean indexes are also illustrated in Figure 1. As seen in Table 2 Index 1 showed the smallest ratio for FO/NFO, whereas Index 6 showed the largest ratio.

DISCUSSION

One aim of this study was to set up a new simple method for collection and analysis of whole blood from the fingertip using direct transmethylation. The conventional methods have a disadvantage associated with the collection of blood samples from the antecubital vein, which requires the aid of specialized personnel. There are also risks with transport of blood samples, for example the tubes can get broken or the blood samples can be destroyed by hemolysis. The analysis of fatty acids in the conventional methods is a time consuming process, since several centrifugations are required for separation of plasma from red blood cells. In addition, for identifying the fatty acids in plasma, thin layer chromatography is needed.

The method with direct transmethylation is very simple. The blood sampling does not require special personnel. Patients can collect blood samples at home with a fingerstick lancet and send the samples by mail. The blood samples can be analyzed without centrifugation, and time consuming thin layer chromatography is not needed. The results obtained with the present method reflect reality to a greater extent than plasma samples, since whole blood samples contain a large number of red blood cells and the fatty acids are incorporated into cell membranes. Plasma fatty acids reflect recent dietary intake, while red blood cells are not influenced by fasting or the recent fed state (3), as the half-life of EPA and DHA is 4-6 times longer in red blood cells than in serum (12).

This new method has thus been shown to be a simple procedure, noninvasive, and time and cost saving. The most important modification we have introduced is a reduction of the injected volume from the 80 µl used by Marangoni and coworkers (11) to 5 µl, which is a pronounced advantage. Another modification is that we use a PE-FFAP column Perkin Elmer and split-injector instead of an Omegawax 320 Supelco column with PTV injector as used by Marangoni and coworkers (11). The column used by us is more easily available and less expensive but nevertheless appropriate, giving accurate peaks for all fatty acids of interest.

Harris and von Schacky (3) used a fatty acid index which they called the Omega-3 index, reflecting the level of EPA and DHA in red blood cells in relation to all fatty acids. An Omega-3 index of 8 or more was associated with the greatest cardioprotection, whereas an index of 4 or less was associated with the least cardioprotection. A fatty acid index identical to the Omega-3 index was calculated in our study and was called Index 1. Group FO, who had been taking 5 ml fish oil (1.2 g

EPA+DHA) daily, had a mean index 1 of 8.3, and group NFO, who had not been taking fish oil, had a mean index of 3.8. This result agrees well with that of the Harris study (3). Translation of Omega-3 index to cardioprotection was based on the results from several human studies (1, 13-17).

Albert *et al* (1) reported that the subjects with the lowest risk of sudden cardiac death were those whose blood levels of long-chain omega-3 fatty acids were in the third and fourth quartiles, who had an average blood level of long-chain omega-3 fatty acids of 5.63 and 6.87, respectively. Similar results were obtained by Siscovick et al (14). In the study by Harris and von Schacky (3) based on the studies mentioned above, it was found that an Omega-3 index of about 8 was associated with a 70% reduction in the risk of fatal ischemic disease. This shows that protection against heart diseases can be achieved by intake of the dose of fish oil that we used (1.2 g EPA+DHA daily), which corresponds to an index ≥8.

Another aim of this study was to find an index which is even better than that used by Harris and von Schacky (3). We therefore calculated other indices (Index 2-Index 6), which are more sensitive to intake of fish oil, easy to present and understand, and based on the difference between our FO and NFO groups. Index 2 - Index 5 take both EPA and DHA into consideration, whereas Index 6 uses only EPA and arachidonic acid in the calculation. Exclusion of DHA from the calculation is an advantage, since DHA, in contrast to EPA and arachidonic acid, is not a substrate for eicosanoids. All these indices differed significantly between groups FO and NFO ($p \leq 0.001$). The best index should be the one which shows the highest ratio between FO and NFO.

Index 6 gave the highest ratio between these groups (3.40), indicating that it is a better index than Index 1, which gave a ratio of 2.17. To make it possible for us to estimate the association between Index 6 and cardioprotection, we utilized findings in previously mentioned studies in 14,000 subjects (1, 13-17). According to those studies, a value of 50 or higher for Index 6 should give the best cardioprotection and was reached by 70% of the subjects in group FO. This index corresponds to an EPA/arachidonic acid ratio of 1:2.

The remaining 30% of group FO had a value ≤50. Why some subjects who were supposed to have had a fish oil intake of 5 ml daily for 6 years had a lower index than 50 needs to be discussed. One possibility is that compliance was not optimal and that they in fact had consumed a smaller amount of fish oil than they had reported. Another possibility is that their ability to metabolize the omega-3 fat was abnormal, resulting in lower blood levels than expected (12). The availability of a simple method for measuring the fatty acid levels in the blood is especially important in the latter group.

One result of this study is that an Index 6 of ≤40 represents the highest risk, a value between 40 and 50 an intermediate risk and a value ≥50 the lowest risk for sudden

cardiac death. It was recommended that subjects in the fish oil group who had an index ≤50 should increase their intake of fish oil by about 2.5 ml (0.6 mg EPA+DHA) daily to reach the adequate value. The mean Index 6 in the non-fish oil group was 18. Non of the subjects in this group reached an index 6 of 50, the three highest values being 45, 41, and 40. This indicates that in the Swedish population supplementation with fish oil may be is necessary to reach a low risk level for sudden cardiac death. Individuals in this category are recommended a daily dose of 5 ml of fish oil (1.2 g EPA+DHA). The requirement of fish oil intake is, however, individual and the Index 6 ratio should be tested until a value of 50 is reached.

An advantage of this risk factor is that it is easily modifiable and can quickly be increased by consuming food rich in omega-3 fatty acids, such as fatty fish. Fatty fish, however, contains environmental contaminants such as mercury, PCBs and dioxins. This is one of many reasons why fish oils have been developed as replacement for fish. Natural stable fish oil contains a balanced mixture of different fatty acids from the fish, and also antioxidants (5), and intake of such an oil in the present investigation was associated with a high Index 6 in most patients.
Index 6 (EPA/arachidonic acid) may be an important predictor of risk for sudden cardiac death, and it reflects the individual requirement of omega-3 fatty acids.The conclusion that the ratio between EPA and arachidonic acid is a better marker for cardiovascular disease than the tissue content of EPA or arachidonic acid is supported by the observed correlation between ethnic differences in fatty acid concentrations of platelet phospholipids and frequency of cardiovascular disorders. Thus, cardiovascular mortality rates among Greenland Eskimos, Japanese, and populations in Europe and the United States have been reported to be 7%, 12%, and 45%, respectively (18). Whereas in these countries the proportions of EPA in platelets were 8.0%, 1.6%, and 0.5%, and those of arachidonic acid in platelets 8.3%, 21%, and 26%, the arachidonic acid/EPA ratios 1, 12, and 50, corresponding to an index 6 of 100, 8 and 2, respectively, showed the best correlation to cardiovascular disorders (18).

The recommendation given by the American Heart Association for reducing the risk of death from CAD in secondary prevention is 1g of EPA+DHA daily, and in primary prevention 500 mg daily. However, the recommendations of the American Heart Association and FDA are based on averages and may not reflect the individual requirement for omega-3 fatty acids. The needs depend on age, medical condition and personal health habits. A good way to know if the diet is giving enough cardio-protection through omega-3 fatty acid intake could be to take a test to determine the EPA/arachidonic acid index.

In conclusion, this fatty acid index may be an important new predictor of the risk of sudden cardiac death, and reflects the individual requirement for consumption of omega-3 fatty acids.

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