Resuscitation (2008) **78**, 258—264

available at www.sciencedirect.com

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CLINICAL PAPER

Low levels of cellular omega-3 increase the risk of ventricular fibrillation during the acute ischaemic phase of a myocardial infarction☆

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Received 7 December 2007; received in revised form 28 March 2008; accepted 8 April 2008

KEYWORDS
Ventricular fibrillation;
Cardiac arrest;
Myocardial infarction;
Ischaemia;
Fatty acids;
Prevention

Summary
Aim of the study: Animal studies have demonstrated evidence of an anti-arrhythmic effect of marine *n*-3 fatty acids (FAs). In humans the same mechanism may explain the observed reduction in sudden cardiac death (SCD) associated with intake of fish. Whether high levels of *n*-3 FAs could protect against ventricular fibrillation (VF) during the acute ischaemic phase of a myocardial infarction (MI) is, however, not known.
Materials and methods: We measured red blood cell content of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) expressed as a percentage of total FAs (the omega-3 index) at admission in 460 patients hospitalised with an acute coronary syndrome. Out of 265 patients suffering their first MI, 10 (cases) experienced an episode of VF during the initial 6 h of symptom onset. The omega-3 index of these patients was compared to that of 185 first-MI patients (controls) free of VF for at least 30 days post-admission.
Results: The median value of the omega-3 index in the VF cases was 4.88% as compared to 6.08% in the controls ($p = 0.013$). After adjustment for age, sex, ejection fraction, high-sensitivity C-reactive protein, use of beta-blocker, differences of infarct characteristics and previous angina pectoris, a 1% increase of the omega-3 index was associated with a 48% reduction in risk of VF (odds ratio (OR) 0.52, 95% confidence interval (CI) 0.28—0.96; $p = 0.037$).

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at doi:10.1016/j.resuscitation.2008.04.007.

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0300-9572/$ — see front matter
doi:10.1016/j.resuscitation.2008.04.007

Conclusion: Our study supports an anti-arrhythmic effect of *n*-3 FAs through their incorporation into myocardial cell membranes, reducing the risk of VF during ischaemia.

Introduction

Despite significant progress in the understanding and treatment of heart diseases, sudden cardiac death (SCD) still remains a major cause of death in industrialised countries. Incidence rates ranging from 36 to 128 per 100,000 inhabitants per year have been reported,[1] increasing with age and being more frequent in men than in women.[2] 60—80% of sudden deaths are cardiac in origin, primarily due to underlying coronary artery disease (CAD) with acute myocardial ischaemia.[2–4] With the possible exception of high-sensitivity C-reactive protein (hsCRP)[5] and a history of familial sudden death,[6] traditional clinical and biochemical risk factors for atherosclerosis and myocardial infarction (MI) do not seem to explain the risk of SCD. A recent meta-analysis on risk factors for ventricular fibrillation (VF), the most prevalent mechanism of SCD, could only demonstrate ST-elevation and time from onset of symptoms as independent risk factors for VF during the course of an acute MI.[7] Previous studies have also indicated a greater risk associated with occlusion of the left coronary artery as compared to the right coronary artery,[8] and a protective effect of pre-infarction angina pectoris[9,10] and use of beta-blocker.[11] Most of these identified risk factors are, however, un-modifiable characteristics of the actual MI and not useful for risk stratification prior to the event. The overall survival rate of SCD is only 6—23%,[12] and in more than half of the cases, the first symptom of CAD.[3] Accordingly, better methods of identifying individuals at risk, prior to their MI, are critically important for reducing the incidence of this devastating event.

Epidemiological studies and interventional trials have revealed a protective effect from fish on risk of fatal CAD.[13] Increasing fish intake,[14] supplementation with fish-oil capsules,[15] and elevated blood levels of *n*-3 fatty acids (FAs)[16,17] have all been related to a reduced incidence of cardiac death. The proportionately larger decrease in risk of SCD as compared to total cardiac death and non-fatal MI seen in the GISSI-Prevenzione study,[15] has suggested that fish oil might have anti-arrhythmic properties, protecting against serious ventricular arrhythmias during the course of a MI.

Supplementation with fish oil has been found to enrich myocyte membranes with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[18–20] The favourable effects on the electrophysiological properties of myocardial cells (e.g. increased electrical stabilisation[21–23]) are thought to be a result of this altered membrane composition of FAs. According to this hypothesis, the level of EPA and DHA in myocyte membranes could provide information on the risk of SCD. As opposed to other established risk factors, this could also be modified. Direct measurement of FAs in myocardial biopsies is clearly not an option. Both Harris et al.[18] and Owen et al.[19] have, however, shown that red blood cell (RBC) EPA + DHA (expressed as weight percentage of total FAs; hereafter termed the omega-3 index) can serve as a surrogate of cardiac omega-3 FA content.

The aim of our study was to test the hypothesis that patients experiencing VF during the acute ischaemic phase of their MI, had a lower omega-3 index than MI patients free of arrhythmia.

Materials and methods

Study subjects

This study was performed at Stavanger University Hospital, Norway, as part of the Risk factors in Acute Coronary Syndrome (RACS) study, designed to identify early risk markers for development of troponin-T (TnT)-positive coronary events following hospitalisation with chest pain or otherwise suspected acute coronary syndrome (ACS). The only exclusion criteria were previous inclusion in the same study and unwillingness to participate.

Between November 2002 and October 2003, 871 patients over the age of 18 were included, out of which 265 presented with their first MI (defined by a maximal TnT $\geq 0.06\ \mu g\ L^{-1}$). Ten of these patients (cases), nine men and one woman, experienced an episode of VF within 6 h of symptom onset, five prior to hospital admission and five within the first couple of hours after admission. None had received any revascularisation therapy with thrombolytic treatment or primary percutaneous coronary intervention (PCI) before or at the time of their VF. From the remaining 255 patients a final number of 185 controls were identified, consisting of subjects free of VF or sustained ventricular tachycardia (VT) for at least 30 days of follow-up, and matched to cases according to age. Arrhythmias occurring after 30 days were not an issue in this study. At inclusion, a thorough case report form was filled out and ejection fractions (EF) were determined by echocardiography within 3—5 days of admission.

The study was approved by the Regional Board of Research Ethics and the Norwegian Health Authorities and conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983. All patients gave written informed consent, and in a few situations when the patient died just following hospitalisation or stayed unconscious until death, the family was asked for consent on the patient's behalf.

Laboratory methods

Blood samples for measurement of the omega-3 index were harvested at admission together with the hospital's routine samples including TnT, haematological parameters, creatinine, glucose and serum lipids. A repeated measurement of TnT was performed at least once, typically at 8 h after admission. In cases of MI or uncertain diagnosis, further TnT measurements were performed at less fixed intervals. As part of the RACS-study, blood samples were also analysed for brain natriuretic peptide (BNP) (Microparticle Enzyme Immunoassay (MEIA), Abbott AxSYM®) and hsCRP

(Tina-quant® C-reactive protein (latex) high sensitive assay, Roche Diagnostics).

RBCs for analysis of the omega-3 index were prepared from citrated blood after centrifugation. Plasma and buffy coat were discarded and sedimented RBCs washed six times with phosphate buffered saline, followed each time by centrifugation. Samples were stored at −70 °C until extraction of FAs could be performed. At this temperature, the composition of RBC FAs has been demonstrated to remain stable for at least 4 years.[24] After thawing, a 50 μL sample was placed on a filter paper disc (Whatman grade 1, 3.0 cm diameter) that had been treated with butylated hydroxytoluene (50 mg L^{-1}) according to Marangoni et al.[25] Samples were dried at room temperature, placed in sealed plastic bags and shipped in cold packs to Kansas City where they were analysed in the laboratory of Harris. Measurement of RBC FA composition from dried blood spots has been found to reflect the composition of a freshly thawed sample (8.2 ± 3.6% thawed versus 7.8 ± 3.3% dried; $n = 20$, $p = 0.67$).

The blood spot was cut out and placed in a test tube containing 1 mL of boron trifluoride—methanol, and samples were heated for 10 min at 100 °C. After cooling, 2 mL each of water and hexane (Fisher Scientific) were added; the samples were shaken by hand for 30 s, and then centrifuged for 3 min at 3000 rpm at room temperature. The hexane layer was removed and evaporated under nitrogen at 45 °C. FA methyl esters thus generated were then reconstituted in 50 μL hexane and analysed by flame ionisation gas chromatography (GC9A, Shimadazu Corporation, Columbia, MD). FAs identified by comparison with known standards, were reported as weight percentages of total FAs. For each batch, two control RBC samples were included, one high in omega-3 FA and the other low. An external standard containing known amounts of relevant FAs (GLC-673B, Nu-Chek Prep, Elysian, MN) was included in each run to correct for differences in FA response factors. The coefficient of variation for the omega-3 index was 6%. Laboratory personnel performing the analyses were blinded with respect to clinical events.

Statistical analyses

Differences between groups at baseline were tested by the Mann—Whitney Rank-Sum test for quantitative data and by the Fisher's exact test for categorical variables. All measurements are given as median values with the 25th and 75th percentiles (interquartile ranges). Non-parametric testing was also used for the univariate analysis to test for potential differences of the omega-3 index between cases and controls. To adjust for potential predictors of VF besides the omega-3 index, a multivariate analysis using logistic regression was performed. Differences in baseline characteristics and previously identified risk factors for VF during the course of a MI were included in the model: age, sex, hsCRP, use of beta-blocker, infarct location, ST-elevation infarction versus non-ST-elevation infarction, EF day 3—5 post-MI and a history of angina pectoris. We chose not to include maximal TnT and BNP due to the possibility of these parameters being differentially affected by the mechanical manipulation of the heart as well as coronary hypoperfusion during resuscitation.[26,27] The peak TnT values might also be influenced by later reperfusion therapy, such as PCI or thrombolytic therapy.[28] The odds ratio (OR) for VF is presented with 95% confidence intervals (CIs). The statistical analyses were performed with the statistical package SPSS Version 14.0, with p-values derived from the logistic regressions using the Wald chi-square test. All tests were applied two-sided with a significance level of 5%.

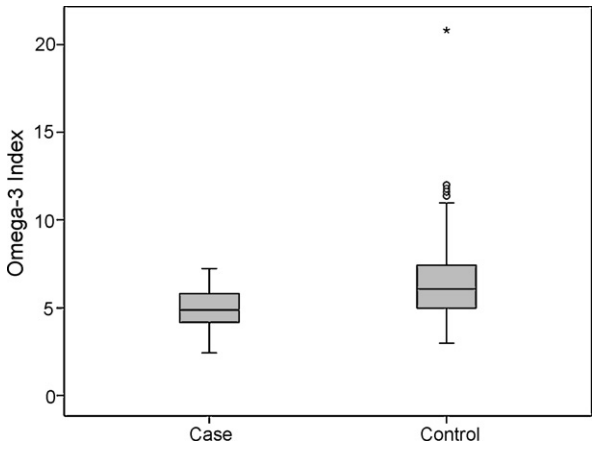

Figure 1 Distribution of the omega-3 index in cases and controls.

Results

Patient characteristics

Only 1 out of 10 case patients had any previous evidence of angina pectoris; for the remaining cases this dramatic event of SCA appeared during their initial presentation of CAD. Except for one patient who died from a non-arrhythmic cardiac cause 7 days after hospitalisation, all cases survived until discharge. One non-sudden cardiac death also appeared among controls during hospitalisation. The only statistically significant differences between the 10 cases and the 185 control patients were in ECG findings at presentation (higher frequency of ST-elevation infarctions in the cases than the controls) and in baseline values of BNP and hsCRP (both lower in cases). A complete list of patient characteristics is presented in Table 1.

Omega-3 index

The median value of the omega-3 index in the VF group was 4.88 (4.10—5.85)% as compared to 6.08 (4.98—7.46)% in the control group (Figure 1). This difference was statistically significant in the univariate analysis, $p = 0.013$. No difference of the omega-3 index could be demonstrated between the five cases suffering a pre-hospital VF (4.83 (4.20—5.87)%) as compared to the five experiencing cardiac arrest after admission (4.92 (3.31—6.35)%). In the logistic regression model, a 1% increase of the omega-3 index was associated with a 44% reduction in odds for VF (OR 0.56, 95% CI 0.34—0.91, $p = 0.021$). This inverse association was altered only slightly after adjustment for age, sex, EF, hsCRP, use of beta-blocker, differences of infarct characteristics and previous angina pectoris (OR 0.52, 95% CI 0.28—0.96, $p = 0.037$).

Table 1 Characteristics of cases and controls

	Cases ($n = 10$)	Controls ($n = 185$)	p-Value
Men	9 (90.0%)	134 (72.4%)	ns
Women	1 (10.0%)	51 (27.6%)	
Age (median, years)	58.0 (50.1—65.5)	64.4 (53.0—72.6)	ns
BMI (median)[a]	26.5 (24.5—27.4)	25.2 (23.3—28.6)	ns
ECG finding at admission[b]			
STEMI	8 (80.0%)	85 (46.4%)	0.051
NSTEMI	2 (20.0%)	98 (53.6%)	
ECG finding at discharge[b]			
Q-infarction	4 (40.0%)	60 (32.8%)	ns
Non-Q-infarction	6 (60.0%)	123 (67.2%)	
Infarct location[c]			
Anterior wall	6 (60.0%)	85 (46.2%)	ns
Inferior wall	3 (30.0%)	64 (34.8%)	
LBBB	1 (10.0%)	1 (0.5%)	
Unidentifiable	0	34 (18.5%)	
Ejection fraction (median, %)[d]	50 (40—60)	55 (50—60)	ns
Known heart disease			
Angina pectoris	1 (10.0%)	56 (30.3%)	ns
Heart failure	0	12 (6.5%)	ns
Previous CABG	0	6 (3.2%)	ns
Previous PTCA	0	8 (4.3%)	ns
Hypertension	3 (30.0%)	61 (33.0%)	ns
Diabetes mellitus	0	21 (11.4%)	ns
Hypercholestrolemia	4 (40.0%)	92 (49.7%)	ns
Smoker	6 (60.0%)	84 (45.4%)	ns
Family history[e]	6 (66.7%)	121 (69.1%)	ns
Creatinine (median, Ref; 45—105 μmol/L)	84.5 (76.5—98.5)	89.0 (75.0—98.0)	ns
Total-cholesterol (median, Ref; 2.9—7.8 mmol/L)	5.7 (4.9—6.2)	5.6 (5.0—6.2)	ns
HDL-cholesterol (median, Ref; 0.8—2.7 mmol/L)	1.3 (1.2—1.4)	1.2 (1.0—1.5)	ns
TG (median, Ref; 0.45—2.60 mmol/L)	1.9 (1.0—2.7)	1.4 (1.0—2.0)	ns
Homocysteine (median, Ref; 5.5—15.0 μmol/L)[f]	10.3 (8.0—12.9)	12.0 (9.7—14.9)	ns
BNP (median, pmol/L)[g]	12.0 (0.0—77.5)	57.5 (29.0—165.5)	0.036
hsCRP (median, mg/L)[b]	2.1 (0.8—4.5)	4.4 (2.0—13.5)	0.046
Medication prior to admission			
Beta-blocker	1 (10.0%)	31 (16.8%)	ns
ACE/AT II-inhibitor	1 (10.0%)	32 (17.3%)	ns
ASA	0	32 (17.3%)	ns
Statin	1 (10.0%)	36 (19.5%)	ns

Median values given with 25th and 75th percentiles (interquartile ranges). ACE, angiotensin converting enzyme; ASA, acetylsalicylic acid; AT II, angiotensin II receptor; BMI, body mass index; BNP, brain natriuretic peptide; CABG, coronary artery bypass grafting; HDL, high density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LBBB, left bundle branch block; NSTEMI, non-ST-elevation myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; STEMI, ST-elevation myocardial infarction; TG, triglycerides; TnT, troponin-T.

[a] One missing value for both cases and controls.
[b] Two missing values for controls.
[c] One missing value for controls.
[d] Seven missing values for controls.
[e] One missing value for cases and ten missing values for controls.
[f] Three missing values for controls.
[g] One missing value for cases and nine missing values for controls.

Discussion

In our study we have demonstrated a lower level of the omega-3 index in patients suffering a VF during the acute ischaemic phase of their MI as compared to MI-patients without such an event. This supports previous observations of a reduced risk of fatal CAD associated with high intake of fish or fish-oil supplements.[13] After adjustment for other

potential predictors of risk, our analyses suggest a 48% (95% CI 4—72%) reduction in risk of VF associated with an increase of 1% of the omega-3 index.

These findings are supported by a case control study on primary cardiac arrest performed by Siscovick et al.[16] demonstrating a 90% risk reduction (OR 0.1, 95% CI 0.1—0.4) associated with RBC EPA + DHA in the upper quartile (mean 6.5%) as compared to the lowest quartile (mean 3.3%). The same inverse relationship has been demonstrated for whole blood long-chain *n*-3 FAs (EPA + DHA + docosapentaenoic acid (DPA)) and risk of SCD in the Physicians' Health Study[17] and for plasma phospholipid DHA + EPA and risk of fatal ischaemic heart disease in the Cardiovascular Health Study.[29] In the latter study, patients over the age of 65 had a 77% lower risk of assumed arrhythmic death for each 1-standard deviation increase in plasma phospholipid DHA + EPA. In all of these studies, case patients were free of clinical heart disease prior to their SCD. By mathematical transformation of whole blood EPA + DPA + DHA (measured in the Physicians' Health Study) to the estimated omega-3 index, Harris and von Schacky[30] have suggested a 90% reduction of risk associated with an omega-3 index in the range of 6.1—10.1% (upper quartile) as compared to an index of 2.4—4.5% (lowest quartile). This corresponds well with the results from Siscovick et al.[16]

None of these studies have, however, documented the mechanism of protection against SCD to be anti-arrhythmic, as the electrical activity of the myocardium at the moment of cardiac arrest was not systematically registered. The incidence of VF as the first documented rhythm at the time of resuscitation seems to be decreasing,[4,31,32] and in some studies has actually been found to be as low as 25—37%.[4,33] The observed reduction in SCD by *n*-3 FAs can therefore only indicate a possible anti-arrhythmic mechanism of protection. One of the strengths of our study is a well documented VF as the eliciting cause of cardiac arrest prior to any reperfusion therapy in patients experiencing their first MI, making it possible to evaluate directly the association between the omega-3 index and the risk of VF in humans.

The first evidence of an anti-arrhythmic potential of *n*-3 FAs came from experimental studies on rats,[34] dogs[35] and monkeys.[36] In a recent review of outcomes in animal models, the authors concluded that there was an apparent beneficial effect of EPA and DHA on ischaemia induced VF and VT across all species.[37] For ventricular arrhythmias induced by reperfusion, the results were inconsistent, and none of the animal models have evaluated the anti-arrhythmic effect in settings not related to ischaemia or reperfusion.

In attempts to determine whether *n*-3 FAs could have the same anti-arrhythmic effects in humans, several studies were conducted using patients with implantable cardioverter defibrillators (ICDs) at high risk of recurrent ventricular arrhythmias.[38—42] Results from these studies have, however, been highly discrepant, with one study actually demonstrating a trend toward increased susceptibility to ventricular arrhythmia after *n*-3 FA-supplementation.[41] One reason for these divergent results might be different mechanisms of arrhythmia in different patients. During an acute ischaemic event, a gradient of depolarisation occurs in the ischaemic myocardium, decreasing the threshold for induction of ventricular arrhythmias. The theoretical mechanism of action of *n*-3 FAs as anti-arrhythmic agents is based on indirect effects on sodium and calcium ion-channels resulting in a membrane hyperpolarisation, increasing the arrhythmic threshold in the ischaemic zone.[21,22] Ventricular arrhythmias based on other mechanisms like myocardial scarring or heart failure, might not be reduced to the same extent by *n*-3 FA treatment.

In our study, patients suffering VF were all in the acute ischaemic phase of their first MI, more or less leaving out the possibility of established myocardial scarring as the substrate for re-entry arrhythmia. None of the patients had a previously established diagnosis of heart failure, supported by a median EF of 50% day 3—5 post-MI. Moreover, their episode of VF appeared prior to revascularisation therapy, strongly suggesting the presence of ischaemia as the cause of arrhythmia. Therefore, our study supports the results of animal studies demonstrating protection from *n*-3 FAs against ischaemia induced VF.

Five out of ten case patients included in our study had suffered VF prior to hospital admission. Blood samples for measurement of the omega-3 index were, however, first taken after hospitalisation. One objection to our conclusion might be the possibility of the *n*-3 FA measurements being affected by the resuscitation itself. Theoretically, adrenaline (epinephrine) injected during the resuscitation as well as the increased stress-response in the body itself, could activate phospholipases in the cell membranes releasing FAs from phospholipids, leading to falsely low levels post-resuscitation. This was, however, not shown to be the case in studies in rats injected with adrenaline.[43] Siscovick et al.[16] cited evidence that the level of EPA + DHA in RBC membranes changed by only 0.33% as a result of the cardiac arrest itself. Even though there were few patients involved, our finding of comparable *n*-3 FA levels in pre-hospital and in-hospital cases supports the hypothesis of low levels of *n*-3 FAs preceding the VF and not being a result of the event.

Moreover, the timing of blood sampling in our study relates the *n*-3 FA level directly to the episode of arrhythmia as opposed to most observational studies where the FA measurements are made years before the event, making it difficult to adjust for intermediary dietary changes. Furthermore, the use of RBC levels instead of plasma and serum measurement will reduce the influence from recent food intake as FAs in RBCs incorporate gradually, reflecting the average exposure to omega-3 in the diet over several weeks.[44,45]

The main limitation of our study is the low number of cases, representing a highly selected population. Only 20—50% of patients with pre-hospital SCD survive until hospital admission.[4,12] Since our blood tests were taken at admission, we have not included patients in whom resuscitation was ended during the pre-hospital phase. Whether this population may differ in their *n*-3 FA level, as compared to those successfully resuscitated, is therefore unknown. It can also be argued that our low number of case patients could limit the power of detecting differences of baseline characteristics as well as the possibility of proper adjustments in the logistic regression analysis. Therefore, our results must not be interpreted as final proof for an anti-arrhythmic effect of *n*-3 FAs, but may serve as a hypothesis generating pilot study. To finally answer both these questions, a larger study of VF-patients with blood samples harvested during pre-hospital resuscitation will be required.

Conclusion

Our study supports a protective effect of *n*-3 FAs against VF during the acute ischaemic phase of a MI. If our results are verified in larger studies, recommendations of increased intake of *n*-3 FAs could reduce the incidence of SCD, with a number of lives being saved every year.

Conflict of interest statement

The analyses of the omega-3 index are performed by William S. Harris who is a consultant to companies with interests in omega-3 fatty acids, including Reliant Pharmaceuticals, Monsanto Company, and OmegaMetrix, LLC. There are otherwise no financial or other relationships associated with the manuscript that might lead to a conflict of interest.

Acknowledgment

The main author was supported with grants from the Regional Health Authorities in Western Norway.

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