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Antiarrhythmic Mechanisms of n-3 PUFA and the Results of the GISSI-Prevenzione Trial

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Abstract. The purpose of this paper is twofold: on the one hand, to confirm the positive results on n-3 PUFA from the overall results Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione trial; on the other, to summarize and describe how the results of an important trial can help generate hypotheses either on mechanisms of action or on differential results in particular subgroups of patients, as well as test the pathophysiological hypotheses that have accompanied in the years the story of the hypothesized mechanisms of action of a drug. GISSI-Prevenzione was conceived as a pragmatic population trial on patients with recent myocardial infarction and it was conducted in the framework of the Italian public health system. In GISSI-Prevenzione, 11,323 patients were enrolled in a clinical trial aimed at testing the effectiveness of n-3 polyunsaturated fatty acids (PUFA) and vitamin E. Patients were invited to follow Mediterranean dietary habits, and were treated with up-to-date preventive pharmacological interventions. Long-term n-3 PUFA at 1 g daily, but not vitamin E at 300 mg daily, was beneficial for death and for combined death, nonfatal myocardial infarction, and stroke. All the benefit, however, was attributable to the decrease in risk for overall (−20%), cardiovascular (−30%), and sudden death (−45%). At variance from the orientation of a scientific scenario largely dominated by the "cholesterol-heart hypothesis", GISSI-Prevenzione results indicate n-3 PUFA (virtually devoid of any cholesterol-lowering effect) as a relevant pharmacological treatment for secondary prevention after myocardial infarction.

Key words: Coronary disease — Prevention and control — n-3 polyunsaturated fatty acids — Sudden death

Introduction

The importance of n-3 polyunsaturated fatty acids (PUFA) in human health was noted 30 years ago by Bang and co-workers [8] who suggested that it was the high n-3 fatty acid content in Eskimos' diets that accounted for the low rate of ischemic heart disease. Some years later, Kromann and Green [41] reported on the low mortality of Greenland Inuit from ischemic heart disease and other chronic diseases. Since then, many clinical studies have been carried out on the role of n-3 PUFA in health and disease and in growth and development; most of the studies have focused, however, on the prevention and management of cardiovascular disease [74, 75].

GISSI-Prevenzione [27, 51] was conceived at the beginning of the last decade as a population-based clinical trial of patients with recent myocardial infarction, aimed at testing the effectiveness of the administration of the following: (i) n-3 PUFA, which are virtually devoid of any cholesterol-lowering effect, and have also been suspected of increasing the susceptibility of low density lipoprotein particles to oxidative phenomena [58]; and (ii) vitamin E, an antioxidant substance, sustained by a wealth of

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*The names of GISSI-Prevenzione Investigators are reported in Lancet 1999; 354; 447–55. (40)

experimental and epidemiologic data [50, 78]. While the results of GISSI-Prevenzione and other relevant trials established the inefficacy of vitamin E in preventing cardiovascular events [22, 81], the positive results of the GISSI-Prevenzione trial on n-3 PUFA were corroborated by those of several studies [1, 15, 31, 32, 42, 60, 61] and prompted the organization of new trials either to confirm its results or to test the efficacy of n3-PUFA in primary prevention as well as in specific populations of patients with diabetes or congestive heart failure [6, 80].

From the wealth of information made available by the first studies that explored and supported the antiatherogenic and antithrombotic effects of n-3 PUFA [74, 75], it now appears that these fatty acids are also able to stabilize myocardial membranes electrically, reducing susceptibility to ventricular dysrhythmias and consequently the risk of sudden death [44, 55].

The purpose of this paper is twofold: on the one hand, to confirm the positive results on n-3 PUFA of the overall results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione trial [27, 45, 48, 49, 51]; on the other, to summarize and describe how the results of an important trial can help generate hypotheses either on mechanisms of action or on differential results in particular subgroups of patients as well as test the pathophysiological hypotheses that have accompanied in the years the story of a drug.

Patients and Methods

Study Design

The study design has been described previously [27, 28, 51, 52]. Patients with recent (3 mo) myocardial infarction were enrolled. Eligible patients had no known contraindications to the dietary supplements, were able to provide informed written consent, and had no unfavorable short-term outlook (e.g., overt congestive heart failure or cancer). Age limits were not defined; the clinicians' decision to include elderly subjects in the study depended merely on the expectation of potential benefits in the light of the patient's clinical condition. A multicenter, open-label design, in which patients were randomly allocated to four treatment groups was adopted. In the absence of evidence for preferred doses of treatments, we decided on daily doses of n-3 PUFA as 1 gelatine capsule containing 850–882 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as ethyl esters, and 300 mg vitamin E, given as one capsule of synthetic α-tocopherol; these doses used existing available formulations to help maintain compliance in patients already receiving many other long-term treatments. Patients were randomly assigned n-3 PUFA alone ($n = 2835$), vitamin E alone ($n = 2830$), n-3 PUFA and vitamin E combined ($n = 2830$), or no supplement (control, $n = 2828$).

Patients were asked to adhere to recommended preventive treatments, i.e., aspirin, β-blockers, and inhibitors of angiotensin-converting enzyme (ACE) (statins were not supported by definitive data on efficacy when the trial was started). The treatment assigned had to be continued until the end of follow-up. As an "open" study, it was recommended that participating cardiologists provide the same care and treatment attitude for all patients, regardless of their allocation. Trial procedures were planned to mimic as closely as possible the routine care after a myocardial infarction. Follow-up visits were scheduled at 6, 12, 18, 30, and 42 mo; these included clinical assessment and the administration of a food-frequency questionnaire. The primary combined efficacy end points were the following: the cumulative rate of all-cause death, nonfatal myocardial infarction, and nonfatal stroke; and the cumulative rate of cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke. We did secondary analyses for each component of the primary end points and for the main causes of death. The validation of the clinical events included in the primary end points was assured by an ad hoc committee of expert cardiologists and neurologists who were not aware of patients' treatment assignment.

Statistical Methods

According to the protocol, data were right-censored at 42 mo, when follow-up information on the vital status of patients was available for the population through clinical visits or census offices. Statistical analyses were carried out by fitting various multivariable Cox regression models adjusted for the confounding effect of relevant prognostic indicators. The efficacy of n-3 PUFA treatment was assessed according to intention-to-treat and adjusted for interaction between treatments to ensure the full assessment of the independent effects of n-3 PUFA. The following confounding variables were included in the multivariable analysis: (A) non-modifiable risk factors: age and sex; (B) complications after myocardial infarction: electrical instability (defined as ≥ 10 premature ventricular beats per hour, or sustained or repetitive arrhythmias during 24-h Holter monitoring), residual ischemia (angina pectoris class I to IV, according to the Canadian Angina Classification or positive exercise testing); (C) cardiovascular risk factors: smoking habits, history of diabetes mellitus and arterial hypertension, total blood cholesterol, HDL cholesterol, and presence of peripheral vascular disease and (D) treatment-related variables: use of antiplatelet agents, ACE inhibitors and beta blockers. We computed χ^2 tests for trend and heterogeneity as appropriate. The linearity of the effect of continuous

Table 1. Baseline characteristics of patients enrolled in GISSI-Prevenzione

Mean time since diagnosis of acute MI*	25.1 (20.9)	
Mean age (± SD)	59.3 ± 10.6 years (16.4% > 70)	
Mean ejection fraction (± SD)	52.6% ± 10.6% (2.5% ≤ 30%)	
Mean LDL-cholesterol (± SD)	137.4 ± 38.0 mg/dl	
Mean HDL-cholesterol (± SD)	41.5 ± 11.5mg/dl	
Mean triglycerides (± SD)	162.0 ± 85.6 mg/dl	
Smokers before acute MI (%)	42.8	
Diabetics (%)	14.9	
Hypertensives (%)	35.6	
Body mass index ≥ 30 kg/m^2 (%)	14.7	
Concomitant medications	Baseline	End of study
Antiplatelet drugs (%)	91.0	82.9
ACE inhibitors (%)	46.9	39.1
Beta-blockers (%)	44.3	38.4
Cholesterol-lowering drugs (%)	4.7	45.5

*50% randomized within 16 days.

variables on the outcome was assessed through fitting classes (e.g., statistical quartiles or quintiles) as a continuous variable and the heterogeneity of the effect of n-3 PUFA by increasing levels of the continuous variable in quintiles by adding n-3 PUFA by variable interaction term. All probability values are 2-sided. All computations were carried out using the SAS statistical package (SAS Institute Inc.).

Results

The characteristics of GISSI-Prevenzione patients were evenly distributed in the experimental groups (Table 1). Briefly, subjects in GISSI Prevenzione appeared to be a relatively low-risk population of acute myocardial infarction (MI) survivors recruited early after the index event, i.e., 50% of subjects were recruited within 16 d after myocardial infarction, mean age (± SD) was 59 ± 1 1 y; 16% were > 70 y old; 15% were women; and 14% had an ejection fraction ≤ 40%. Total blood cholesterol levels were almost normally distributed at recruitment, with a mean of 211 ± 42 mg/100 mL. Arterial hypertension, diabetes mellitus, and claudication were present in 36, 15, and 4% of patients, respectively; 43% of patients smoked before the index events and only one third of them were still smoking thereafter. Patients recruited into the study received life style recommendations, and up to date preventive interventions. At the end of the study, in addition to the drugs tested in the trial, antiplatelet drugs, β-blockers, ACE-inhibitors, and lipid-lowering drugs were prescribed to 83, 38, 39, and 46% of patients, respectively. Finally, 5% of patients had coronary artery bypass graft or angioplasty procedures before recruitment and a total of 24% of patients had been revascularized at the end of the study.

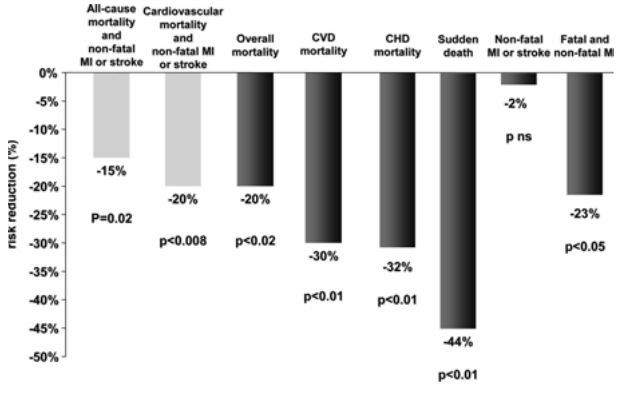

Fig. 1. Use of n-3 PUFA in GISSI-Prevenzione was associated with significant reductions in the risk of the two co-primary endpoint events (*light bars*) as well as of a series of outcome events analyzed in the secondary analyses (*dark bars*).

Main Effects of n-3 PUFA

The full profile of the effects of n-3 PUFA is summarized in Fig. 1 and Table 2. The 15% relative decrease in risk for the combined primary end point of death, nonfatal myocardial infarction, and nonfatal stroke (95% confidence interval (CI), 2–26, $P = 0.022$) and the decrease in risk for the other combined end point of cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke (20%, 6–32, $P = 0.006$) were statistically significant.

Secondary analyses of the individual components of the main study endpoints showed that the decrease in mortality (20% of total deaths, 30% of cardiovascular deaths, and 44% of sudden deaths) obtained with n-3 PUFA accounted for all of the benefit seen in the combined end point. There was no difference across the treatment groups for nonfatal cardiovascular events. Treatment with n-3 PUFA significantly lowered the risk of total coronary heart disease

Table 2. Effect of n-3 PUFA in the primary and secondary analyses of GISSI-Prevenzione

	End-study events in the control group (%)	Relative risk (95% confidence interval)
Primary endpoints		
Death, nonfatal MI, and nonfatal stroke	14.8	0.85 (0.74–0.98)
CV death, nonfatal MI, and nonfatal stroke	11.7	0.80 (0.68–0.94)
Secondary analyses		
All-cause mortality	10.5	0.80 (0.67–0.94)
Cardiovascular mortality	7.2	0.70 (0.56–0.86)
Coronary mortality	5.2	0.68 (0.53–0.88)
Sudden death	3.3	0.56 (0.40–0.79)
CHD death and nonfatal MI	8.9	0.78 (0.65–0.94)
Fatal and non-fatal stroke	1.4	1.24 (0.82–1.87)
Nonfatal MI	4.0	0.95 (0.79–1.14)
Nonfatal stroke	1.1	1.08 (0.75–1.55)

Relative risk calculated by Cox regression analysis adjusted for vitamin E and interaction term; analysis by intention-to-treat; follow-up right-censored at 3.5 years.
Patients with two or more events of different types appear more than once in columns but only once in rows. CV = cardiovascular, CHD = coronary heart disease, MI = myocardial infarction.

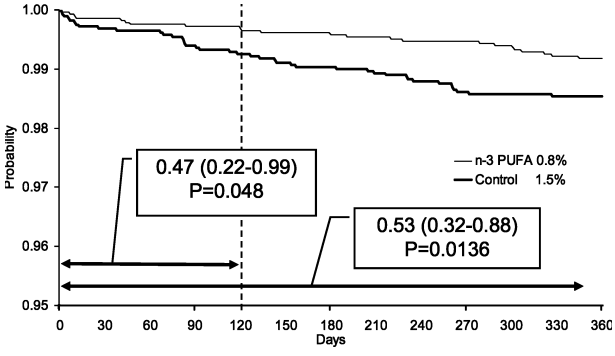

Fig. 2. Early effects of n-3 PUFA on sudden death.

(CHD) events (0.78 (0.65–0.94), $P = 0.008$). No significant change in fatal plus nonfatal stroke was found.

Further Analysis of the GISSI-Prevenzione Database

The striking benefit of n-3 PUFA treatment of the reduction of the risk of sudden death together with the much less evident (and not statistically significant) effect on nonfatal myocardial infarction, prompted us to explore the database to find out evidence sustaining the hypothesis of an antiarrhythmic effect of the experimental drug.

A time-to-event analysis of the precociousness of the appearance of the benefit revealed an early effect of highly purified n-3 PUFA in reducing the risk of sudden death, with a large (53 %) and statistically significant ($P = 0.048$) benefit apparent after only 4 months (Fig. 2) [48]. Survival curves for n-3 PUFA treatment diverged early after randomization, and total mortality was significantly lowered after 3 months of treatment (relative risk (RR) 0.59; 95% confidence interval (CI) 0.36 to 0.97; $P = 0.037$). The reduction in sudden death at 3 months, although not statistically significant ($P = 0.058$) accounted for more than half of the reduction in total mortality at that time (41 % risk reduction for all-cause mortality; $P = 0.037$). By the end of follow-up, the reduction in sudden death was highly significant statistically ($P = 0.0006$) and accounted for 59 % of the total survival benefit of highly purified omega-3 PUFAs versus controls.

Since patients with heart failure are at high risk of sudden death due to arrhythmic events, the GISSI-Prevenzione database facilitated a hypothesis-generating exercise to assess the relationship between left ventricular systolic function and the risk of sudden death in post-MI patients with left ventricular systolic dysfunction [45]. Compared to patients with well-preserved left ventricular function (i.e., ejection fraction $>50\%$), those with left ventricular systolic dysfunction (i.e., ejection fraction $\leq 50\%$) had higher all-cause mortality (6.0% vs. 12.3%) and sudden death (1.4% vs. 3.4%) rates. The administration of n-3 PUFA reduced mortality similarly in patients with (RR 0.76 (0.60–0.96) $P = 0.02$) and without left ventricular systolic dysfunction (RR 0.81 (0.59–1.10) $P = 0.17$) (heterogeneity tests $P = 0.55$). In contrast, the effect on sudden death was markedly asymmetrical: n-3 PUFA produced a marked reduction (RR 0.42 (0.26–0.67) $P = 0.0003$) of risk in patients with left ventricular systolic dysfunction, whereas the effect was less evident (RR 0.89 (0.41–1.69) $P = 0.71$) in patients with ejection fraction EF $> 50\%$ (heterogeneity tests $P = 0.07$). When we performed a more in-depth exploration of the effects of treatment with n-3 PUFA on sudden death in patients with progressively increasing levels of ventricular dysfunction, the effect of n-3 PUFA treatment on sudden death was related to the degree of

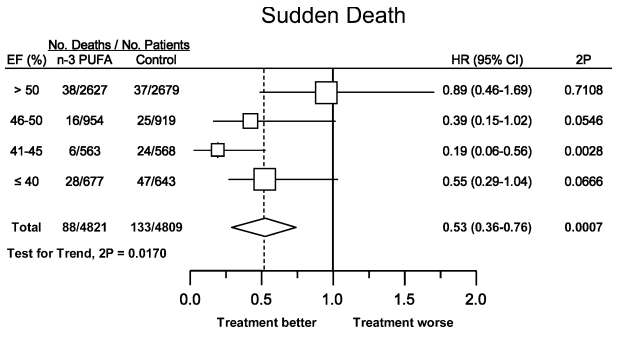

Fig. 3. Effect of n-3 PUFA treatment on total mortality and sudden death in patients with progressive impairment of left ventricular systolic function. Only patients with an echocardiographic measurement of ejection fraction (*EF*) have been included in the present analysis (n = 9,630; 85% of the total cohort). Ejection fraction (*EF*) > 50%: n = 5,306, total mortality 6.01%; *EF* 46% to 50%: n = 1,873, total mortality 8.44%; *EF* 41% to 45%: n = 1,131, total mortality 10.08%; *EF* ≤ 40%: n = 1,320, total mortality 19.62%. White squares indicate hazard ratio in each subgroup, with square size proportional to number of cases and horizontal lines representing 95% confidence intervals. The combined risk ratios and their 95% confidence intervals are indicated by white diamonds.

systolic dysfunction (test for trend P = 0.02) (Fig. 3) with the benefit on sudden death (SD) reduction in patients with ejection fraction ≤ 40% being 4-fold higher than in those with ejection fraction > 50%.

Further analysis of the GISSI-Prevenzione database indicated a somewhat discrepant benefit on sudden death of n-3 PUFA administration in patients treated with and without β-blockers (Fig. 4). At the end of the study, the relative risk reduction of sudden death was higher, though not statistically significant, in patients treated with as compared to those without β-blockers (64% vs. 38%, heterogeneity test P = 0.13136). At one year, however, the benefit of n-3 PUFA in patients taking also β-blockers was even more evident (relative risk reduction 83% vs. 31%) and the results of the test for heterogeneity approached the formal level for statistical significance (P = 0.07908).

Discussion

GISSI-Prevenzione was a secondary prevention, pragmatic, population trial aimed at assessing the effect of promising treatments on top of accepted preventive interventions, such as modification of lifestyle and dietary habits, and pharmacologic therapy, in the framework of the clinical practice of a country-wide network of hospitals within the Italian national public health service. The size of the network (about half of the Italian cardiology departments) and of the recruited population, the pragmatism of the study design aimed at not interfering with clinical practice, and the widespread use of effective preventive interventions assure the transferability of its results to the real patients who are met in a clinical practice for survivors of a myocardial infarction.

When the study was planned [28, 52], n-3 PUFA were supposed to act through a wealth of mechanisms having antithrombotic and antiatherogenic pathways as the mainstream of the benefit (Fig. 5). At that time, the antiarrhythmic mechanisms of n-3 PUFA had been examined only in very few studies on animal models [58]. According to the aforementioned, the two main study endpoints included fatal and nonfatal thrombotic events and sudden death was not a primary outcome measure, though specific information was collected in the case report forms. In the late '80s, in fact, n-3 PUFA raised much expectations for their antiatherogenic effects. The negative results of most of the studies in the mid '90s on the prevention of restenosis after coronary revascularization [25, 33, 39, 43, 53] cooled down the enthusiasm on n-3 PUFA and the weakly positive results of trials on regression of coronary atherosclerosis could not warm it up [72, 83]. During the course of the GISSI-Prevenzione, however, new information became available from epidemiology and laboratory research confirming the protective effects of fatty fish intake on cardiovascular disease and suggesting a possible protective effect of n-3 PUFA on sudden death via antiarrhythmic mechanisms [2, 11, 13, 19, 20, 35–38, 54, 55, 73, 76, 87–89]. The delusion caused by the negative results of trials on prevention of restenosis after coronary angioplasty and the puzzling results of preliminary analyses of relevant databases [7] did not encourage the planning of new clinical trials. As a matter of fact, GISSI-Prevenzione was the only large-scale trial testing formally the efficacy of n-3 PUFA in patients with coronary heart disease. GISSI-Prevenzione positive results sprang up in 1999 in a scenario of unwatchful expectation, but soon after raised enormous interest and a wealth of new positive studies, overall suggesting an antiarrhythmic effect of n-3 PUFA, have been published since then [1, 6, 31, 32, 42, 47, 60, 90]. Not all newly coming evidence on n-3 PUFA was in favor of n-3 PUFA [12, 62, 63, 68]. However, it is not unlikely that either the limited sample size [62, 63, 68] or some limitations and difficulties during the conduction of the study [12] could be the most plausible explanations for such negative results.

The statistically significant reduction of both the two co-primary endpoints, as established in the protocol, and the highly statistically significant results on total, cardiovascular, cardiac, coronary and sudden death allowed to affirm the efficacy of n-3 PUFA therapy [48, 51]. After the results of the GISSI-Prevenzione trial became available, a Consensus Meeting on the overall perspective of its results and on the consistency and articulation of the pieces of experimental and clinical evidence on n-3 PUFA was con-

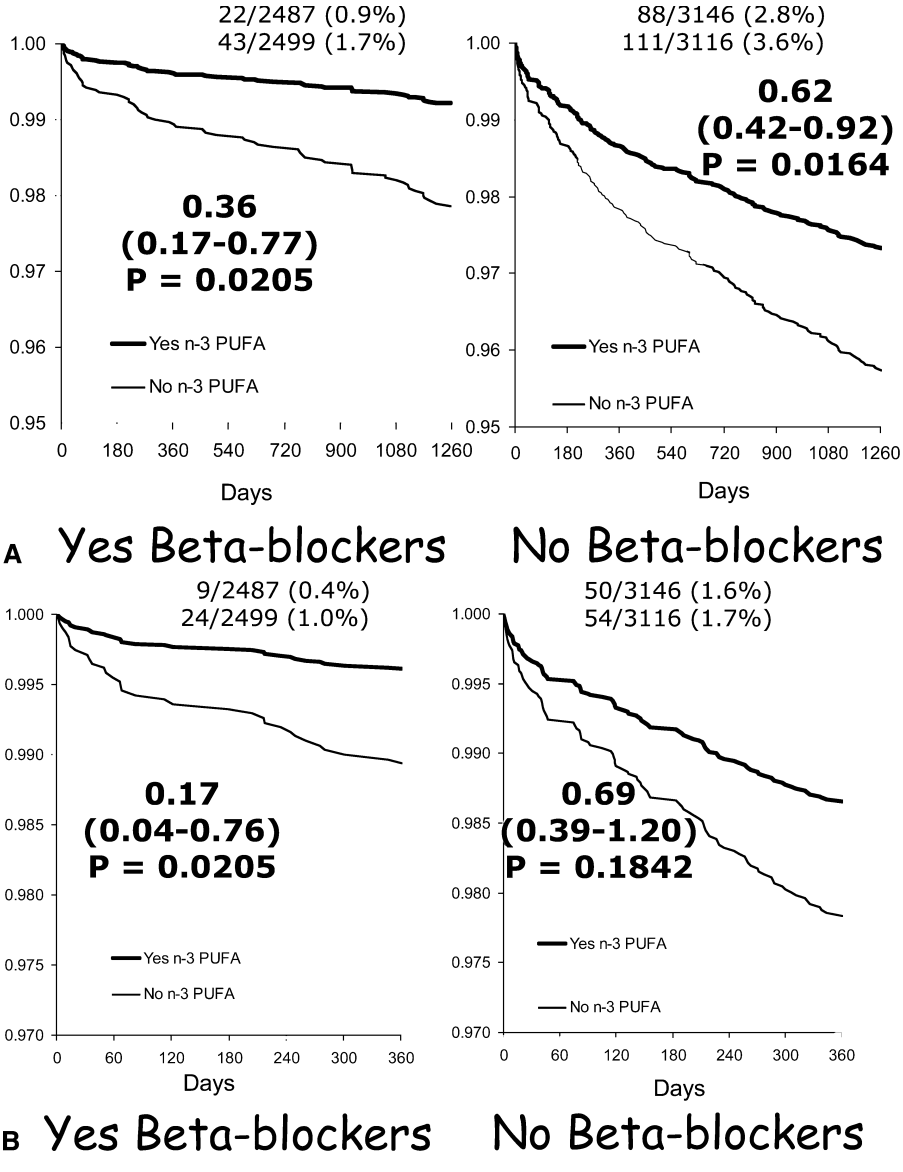

Fig. 4. Effect of n-3 PUFA treatment on sudden death in patients with and without β-blocker treatment. Panel *A*: results at 3.5 years; Panel *B*: results after 1 year.

vened at the Italian National Association of Hospital Cardiologists (ANMCO) centre in Italy and its proceedings have been published as a supplement to the European Heart Journal [49]. GISSI-Prevenzione results have been accepted by various European regulatory authorities to register a new therapeutic indication for n-3 PUFA, i.e., mainly to decrease the risk of death after myocardial infarction. This is a crucial point in the interpretation of GISSI-Prevenzione results. At variance with the expectations declared in the study protocol, n-3 PUFA treatment did not decrease significantly nonfatal myocardial infarction and all the benefit of n-3 PUFA was concentrated in the reduction of fatal events. Such results, though unexpected, strengthened the evidence produced by GISSI-Prevenzione, since death is the hardest, less criticizable, and less likely not to be detected clinical endpoint of a population clinical trial. On the other side, the diagnosis of nonfatal cardiovascular events as well as of cause-specific mortality are often problematic. At any rate, all clinical events have been validated by an ad hoc committee of experts blinded to experimental treatments, therefore any bias related to the validation of nonfatal events and of the causes of death would have been nondifferential in respect to the experimental treatments and would have biased the study results towards the null value.

The impressive results on sudden death prompted us to explore the database to find out evidence in favor of an antiarrhythmic mechanism by examining the effect of n-3 PUFA in situations that were expected to expose GISSI-Prevenzione patients to an increased risk of sudden death due to arrhythmic events. It was hypothesized that if n-3 PUFA had an antiarrhythmic effect then it had to be evident early, in the phase of high "instability" of the first months after myocardial infarction [48] as well as in patients

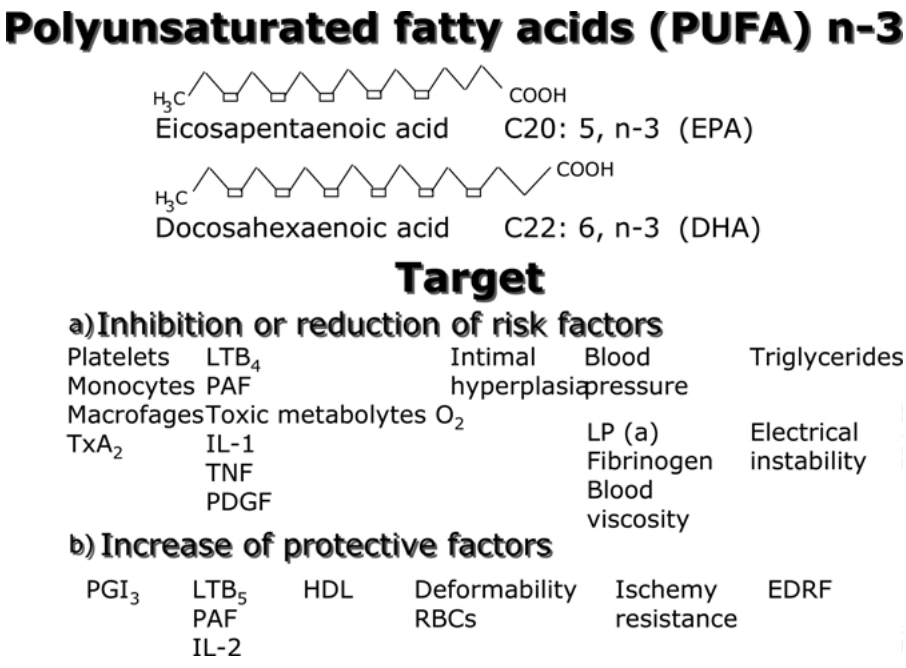

Fig. 5. Mechanisms of action of n-3 PUFA as presented in the original GISSI-Prevenzione protocol.

with left ventricular systolic dysfunction [45]. The results of both the analyses were consistent with the hypothesis of an antiarrhythmic mechanism of action of n-3 PUFA.

The antiarrhythmic and antifibrillatory effects of n-3 PUFAs have been reported in animal studies on marmosets, rats, and dogs, as well as in laboratory experiments on isolated myocytes [11, 35, 54, 55, 58], and were recently comprehensively reviewed [44]. Infusion of an emulsion of n-3 PUFAs just before coronary artery obstruction in an exercising, unanesthetized dog model prevented ischemia-induced sudden cardiac death by preventing ventricular fibrillation. n-3 PUFAs prevented induced fibrillation of cultured neonatal rat cardiomyocytes when various cardiotoxins were tested, and after fibrillation was induced, the arrhythmias were terminated by the PUFAs. According to the results of electrophysiological studies, n-3 PUFAs seem to modulate ion currents (primarily of Na^+ and Ca^{2+}) in the myocyte sarcolemma, shifting the steady-state inactivation potential to more negative values, increasing the depolarizing current necessary to elicit an action potential by 50% and prolong the refractory period by about 3-fold [44]. Xiao et al. [86] hypothesized that during a myocardial infarction a gradient of ischemia occurs from the central core of nonperfused myocardium, where ischemic cells rapidly depolarize and die, whereas myocytes at the junction between remaining normally perfused myocardium and the infarcted zone are only partially depolarized and become hyperexcitable because their resting membrane potentials become more positive, approaching the threshold for the gating of the fast Na^+ channel. Thus, any further small depolarizing stimulus may elicit an action potential, which, if it occurs out-of-phase with the electrical cycle of the heart, may initiate an arrhythmia. In the presence of the n-3 PUFA, however, a voltage-dependent shift of the steady-state inactivation to more hyperpolarized potentials occurs. This effect of the n-3 PUFA on Na^+ channels, together with their effect to inhibit the cardiac L-type Ca^{2+} channels and prevent triggered arrhythmic afterpotential discharges caused by excessive systolic Ca^{2+} fluctuations, is supposed to be the main mechanism for the antiarrhythmic effect of n-3 PUFA [86].

In addition, chronic imbalance of the autonomic nervous system is a prevalent and potent risk factor for adverse cardiovascular events, including mortality. Any factor that leads to inappropriate activation of the sympathetic nervous system can be expected to have an adverse effect on patient outcomes, while any factor that augments vagal tone (e.g., exercise, β-blockers, and n-3 PUFA) tends to improve outcomes. Various studies suggest that n-3 PUFA administration can increase heart rate variability [16–20] and it is well known that low or reduced heart rate variability is associated with poor prognosis and increased risk for sudden death [9, 10, 40, 77]. It has been shown that omega-3 fatty acids improve parameters of autonomic function, including baroreflex sensitivity [84]. Interestingly, Hamazaki et al. showed that the administration of 762 mg of n-3 PUFA per day lowered plasma norepinephrine concentrations in normal volunteers [29]. Although peripheral norepinephrine values measured by Hamazaki et al. do not directly reflect its central activity, it is suggested that peripheral and central norepinephrine activities often change together [67,

79]. Taking into account the importance of sympathetic tone to dangerous arrhythmia, the study of Kamazaki et al. suggests depression of sympathetic tone as one of the mechanisms of the antiarrhythmic action of fish oil.

Omega-3 PUFAs may also be expected to exert arrhythmia-preventing actions in patients with dilated hearts. Experimental observations indicate that enlargement of the ventricles is associated with the activation of ion channels and the emergence of heterogeneity of action potential duration, which may be expected to dispose toward arrhythmia even in the absence of detectable ischemia [44]. The downward spiral of congestive heart failure is mediated by excessive sympathetic tone and activation of the renin-angiotensin system. This causes vasoconstriction, dysrhythmias, apoptosis, and progressive left ventricular dysfunction [57]. Multiple studies have unequivocally documented β-blockers as effective for improving outcomes in patients with congestive heart failure and left ventricular dysfunction. These benefits have been found for carvedilol [65], bisoprolol [21], and metoprolol [56]. β-Blocker therapy not only reduces risk of sudden death but also consistently increases systolic function better than any other therapy [64]. On the other side, the intake of n-3 PUFA can influence directly heart function and improve cardiac responses to ischemia and reperfusion. In an animal model using erythrocyte-perfused isolated working hearts, n-3 PUFA reduced oxygen consumption at any given work output and increased postischemic recovery [66].

According to the aforementioned, the impressive effect of n-3 PUFA on sudden death in coronary heart disease patients taking β-blockers and the statistically significant relationship between degree of left ventricular dysfunction and benefit from n-3 PUFA treatment are highly interesting and, taken together, seem to corroborate the hypothesis of an antiarrhythmic effect of n-3 PUFA.

The results of GISSI-Prevenzione should be also viewed in the light of the original hypothesis of its protocol. GISSI-Prevenzione was designed to assess the combined effects of n-3 PUFAs on death, nonfatal myocardial infarction, and stroke. When the GISSI-Prevenzione trial was planned and started, attention was on much higher doses of n-3 PUFA to aim at antiatherosclerotic and antithrombotic effects via antiplatelet and anti-inflammatory mechanisms, decrease of triglycerides and fibrinogen levels, inhibition of cytokines and gene expression of adhesion molecules, and improvement in arterial compliance and endothelial function [28, 52]. Sudden death was not a primary outcome measure, although its documentation was explicitly foreseen in the collection of end-point events. Further limitations are well known to exist in the definition of sudden deaths: on one hand, the difficulties and differences in the attribution of the cause of death in ambulatory long-term follow-up; on the other, the fact that not all sudden deaths are due to primary ventricular fibrillation initiated by altered membrane ion channel function. Plaque rupture, ischemia, and coronary thrombosis can cause sudden death as a result of pump failure, potassium loss from ischemic cells that initiates injury currents, and inhomogeneities in refractoriness and action potential duration that set up reentrant circuits. According to such hypothesis, the anti-inflammatory effects of n-3 PUFA could be responsible of making atherosclerotic plaques more stable and therefore less prone to rupture and thus decreasing the risk of sudden death due to acute myocardial infarction. On the other hand, the very same mechanism could decrease the probability of arrhythmic events by interrupting the chain of events starting with plaque fissuration, continuing with local thrombosis and ischemia, and leading to electrical instability of myocardial cells. The recent results of Thies et al. suggest that atherosclerotic plaques of carotid arteries readily incorporate n-3 PUFA, inducing changes that can enhance stability of atherosclerotic plaques [82]. In this study, the plaques of patients treated with n-3 PUFA were less likely to have thin fibrous caps, signs of inflammation, and high number of macrophages, compared with plaques in patients in the control and n-6 PUFA-supplemented groups. The anti-inflammatory activity of PUFAs is now well-documented [14, 24, 30, 59] and several clinical studies report PUFA as beneficial in acute and chronic inflammatory diseases [14, 30, 59]. Furthermore, it has been observed that eicosapentaenoic acid can act as a competitive inhibitor of arachidonic acid conversion to prostaglandin E2 and leukotriene B4, while a decreased synthesis of both of these eicosanoids has been observed after inclusion of fish oil in the diet [30, 59]. The inclusion of docosahexaenoic acid in the diet may also result in decreased synthesis of leukotriene B4 [14, 59]. As to the pro-inflammatory cytokines, it has been shown that dietary supplementation with encapsulated fish oil may result in a decreased monocyte synthesis of tumor necrosis factor-alpha and interleukin 1α in humans [24]. Chronic inflammation in the vasculature is now viewed as a cardiovascular risk factor the result of which is a vulnerable plaque, prone to rupture and thrombosis. High-sensitivity C-reactive protein is considered as the most reliable inflammatory marker [85]. In randomized, controlled trials statins have been shown to provide effective therapy for lowering C-reactive protein in conjunction with their cholesterol-lowering effect, though it is unknown whether C-reactive protein lowering per se is an effective cardiovascular therapy [3, 69, 70]. n-3 PUFA have anti-inflammatory effects through a number of mechanisms involving eicosanoids and cytokines [23]. It is worth noting that various studies have shown an

inverse association between n-3 PUFA contents of membranes and levels of C-reactive protein [46, 91]. In GISSI-Prevenzione about one out of two patients was taking a statin at the end of the study. On the other hand, the co-somministration of a statin and n-3 PUFA enhances the conversion of fatty acids to their long-chain polyunsaturated fatty acid derivatives, suggesting increased fatty acid elongase and Δ6- and Δ5-desaturase enzyme activities [34, 71]. Such increased formation of long-chain polyunsaturated fatty acids and their metabolites has been hypothesized to contribute a substantial part of the pleiotropic effects of statins [34].

Another possible interaction between well-known drugs commonly used for cardiovascular prevention and n-3 PUFA (80% to 90% of patients in GISSI-Prevenzione) regards aspirin and their anti-inflammatory effects. It has recently been shown that several endogenous biochemical pathways activated during defense reactions can counter-regulate inflammation [26]. Arita et al. recently identified a new class of aspirin-triggered bioactive lipids, called resolvins, the activity of which may in part explain the beneficial effects of omega-3 fatty acids. Aspirin seems to promote the cyclooxygenase-2 –dependent conversion of n-3 PUFA to a precursor of resolvin El. According to the results of Arita et al., such a precursor is taken by leukocytes and transformed by 5-lipooxygenase to resolvin El that binds to a G protein–coupled receptor called ChemR23 that is expressed on leukocytes, and inhibits the migration of these cells to sites of inflammation [4, 5].

Finally, the discrepant results of the two trials on the ''naturally experimental'' clinical condition represented by patients with implantable cardiac defibrillators (ICD) neither contradict nor confirm the hypothesis of the antiarrhythmic mechanism of n-3 PUFA [6, 68]. It has been documented that in patients with an ICD a low content of n-3 PUFA in serum is associated with a higher incidence of ventricular arrhythmias compared with patients with high serum levels of n-3 PUFA [18]. The small dimensions of the two trials, the existence of some methodological limitations, the possibility that the very fast response of ICD to arrhythmias could have prevented n-3 PUFA to be made ''biologically'' available to exert their antiarrhythmic action (e.g., by making a slow-rate ventricular tachycardia less likely to evolve to ventricular fibrillation), suggest that a clear answer to this question will be provided by further, very specific studies on cardiac arrhythmias and, possibly, by the ongoing large-scale clinical trials in patients at high risk of cardiovascular events [47], with diabetes [6], and with congestive heart failure [80].

GISSI is endorsed by the Associazione Nazionale Medici Cardiologi Ospedalieri (ANMCO), Firenze, Italy and by the Istituto di Ricerche Farmacologiche Mario Negri-Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy. We thank Miss Carla Pera, Miss Barbara Ferri, Mrs. Anna Flamminio, and Mrs. Anna Polidoro for secretarial help. GISSI-Prevenzione was conceived, conducted, analyzed, and published independently by the GISSI group. The study was supported by unrestricted grants from Bristol-Myers Squibb, Pharmacia-Upjohn, Società Prodotti Antibiotici, and Pfizer. Pharmacia-Upjohn and Società Prodotti Antibiotici supplied marketed capsules containing 850–882 mg EPA/DHA ethyl esters. Vitamin E (acetyl d,1-α-tocopherol) was supplied by Bracco.

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