Articles

Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial

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Summary

Background There is conflicting evidence on the benefits of foods rich in vitamin E (α-tocopherol), n-3 polyunsaturated fatty acids (PUFA), and their pharmacological substitutes. We investigated the effects of these substances as supplements in patients who had myocardial infarction.

Methods From October, 1993, to September, 1995, 11 324 patients surviving recent (⩽3 months) myocardial infarction were randomly assigned supplements of n-3 PUFA (1 g daily, n=2836), vitamin E (300 mg daily, n=2830), both (n=2830), or none (control, n=2828) for 3·5 years. The primary combined efficacy endpoint was death, non-fatal myocardial infarction, and stroke. Intention-to-treat analyses were done according to a factorial design (two-way) and by treatment group (four-way).

Findings Treatment with n-3 PUFA, but not vitamin E, significantly lowered the risk of the primary endpoint (relative-risk decrease 10% [95% CI 1–18] by two-way analysis, 15% [2–26] by four-way analysis). Benefit was attributable to a decrease in the risk of death (14% [3–24] two-way, 20% [6–33] four-way) and cardiovascular death (17% [3–29] two-way, 30% [13–44] four-way). The effect of the combined treatment was similar to that for n-3 PUFA for the primary endpoint (14% [1–26]) and for fatal events (20% [5–33]).

Interpretation Dietary supplementation with n-3 PUFA led to a clinically important and satistically significant benefit. Vitamin E had no benefit. Its effects on fatal cardiovascular events require further exploration.

Lancet 1999; **354:** 447–55
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Introduction

The protective effects of foods rich in n-3 polyunsaturated fatty acids (PUFA) derived from marine vertebrates, vitamin E (α-tocopherol), and their pharmacological equivalents on cardiovascular risk has been of interest for the past 20 years.[1–4] Since a low rate of coronary heart disease was reported in the Eskimo population exposed to a diet rich in fish oil,[5] several studies have explored and supported antiatherogenic, antithrombotic, and antiarrhythmic effects of n-3 PUFA.[2–4] Although no consensus existed on the underlying mechanism of action, focus was placed on the ability of triglycerides to lower high-dose n-3 PUFA (registration approval was given for this indication), and to modify membrane composition.[2–4] A protective role in the secondary prevention of coronary heart disease was seen for fatty fish in the Diet And Reinfarction Trial (DART).[6]

By contrast, large observational cohort studies[7–10] support the role of vitamin E as an antioxidant against the proatherogenic and prothrombotic effects of LDL oxidation.[11–13] However, controlled trials testing this hypothesis in populations with different background cardiovascular risk produced controversial results. No decrease in cardiovascular events was seen with low-dose (50 mg daily) vitamin E supplementation in smokers;[14] a significant decrease in non-fatal myocardial infarction and an increase in fatal cardiovascular events was reported with a daily regimen of 400–800 mg vitamin E in patients with angiographically proven coronary atherosclerosis.[15]

A possible complementary role for these two dietary components has been purported: vitamin E could improve the role of n-3 PUFA through protection from lipid peroxidation, by acting independently on the same or closely related atherogenic and thrombotic mechanisms, or both.[4,16]

We investigated in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial the independent and combined effects of n-3 PUFA and vitamin E on morbidity and mortality after myocardial infarction.[17]

Patients and methods

Patients

We enrolled patients with recent (⩽3 months) myocardial infarction. Eligible patients had no contraindications to the dietary supplements (ie, known allergy to n-3 PUFA or α-tocopherol, or known congenital defects of coagulation), were able to provide informed written consent, and had no unfavourable short-term outlook (eg, overt congestive heart failure, cancers, &c). We did not define age limits.

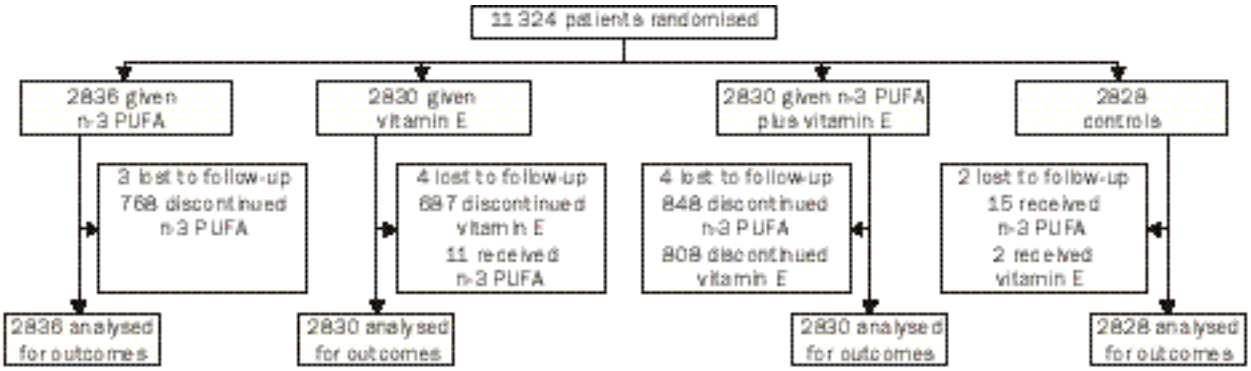

Figure 1: **Trial profile**

Study design

We used a multicentre, open-label design, in which patients were randomly allocated to four treatment groups. In the absence of evidence for preferred doses of treatments, we decided on the daily doses of n-3 PUFA as 1 gelatin capsule containing 850–882 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as ethyl esters in the average ratio of EPA/DHA 1:2, and 300 mg vitamin E, given as one capsule of synthetic α-tocopherol; these doses used existing available formulations to help compliance in patients already receving many other long-term treatments. We asked patients to adhere to recommended preventive treatments—aspirin, β-blockers, and inhibitors of angiotensin-converting enzyme (statins were not supported by definitive data on efficacy when the trial was started).

	n-3 PUFA (n=2836)	Vitamin E (n=2830)	n-3 PUFA plus vitamin E (n=2830)	Control (n=2828)	All (n=11 324)
Male/female	2403 (84·7%)/433 (15·3%)	2398 (84·7%)/432 (15·3%)	2451 (86·6%)/379 (13·3%)	2407 (85·1%)/421 (14·9%)	9659 (85·3%)/1665 (14·7%)
Age (years)					
≤50	592 (20·8%)	560 (19·8%)	596 (21·0%)	577 (20·4%)	2325 (20·5%)
51–60	827 (29·1%)	849 (30·0%)	875 (31·0%)	844 (31·0%)	3395 (30·0%)
61–70	943 (33·2%)	946 (33·4%)	930 (32·8%)	937 (33·1%)	3756 (33·1%)
71–80	415 (14·6%)	424 (15·0%)	370 (13·0%)	418 (14·7%)	1627 (14·3%)
>80	59 (2·0%)	51 (1·8%)	59 (2·0%)	52 (1·8%)	221 (1·9%)
Time from AMI to randomisation (days)					
<10	752 (26·5%)	727 (25·7%)	731 (25·8%)	754 (26·7%)	2964 (26·2%)
10–15	641 (22·6%)	661 (23·4%)	665 (23·5%)	637 (22·5%)	2604 (23·0%)
16–30	613 (21·6%)	644 (22·8%)	675 (23·9%)	645 (22·8%)	2577 (22·8%)
≥31	830 (29·3%)	798 (28·2%)	759 (26·8%)	792 (28·0%)	3179 (28·1%)
Secondary diagnoses					
Arterial hypertension	1019 (36·0%)	1007 (35·6%)	1033 (36·5%)	967 (34·2%)	4026 (35·6%)
Diabetes mellitus	405 (14·2%)	426 (15·0%)	426 (15·0%)	426 (15·0%)	1683 (14·8%)
Non-smokers	632 (22·4%)	636 (22·6%)	618 (22·0%)	613 (21·9%)	2499 (22·2%)
Ex-smokers	996 (35·4%)	1016 (36·1%)	972 (34·5%)	953 (34·0%)	3937 (35·0%)
Smokers	1189 (42·2%)	1161 (41·3%)	1223 (43·5%)	1234 (44·0%)	4807 (42·4%)
Body-mass index ≥30 kg/m²	419 (14·7%)	403 (14·2%)	432 (15·2%)	390 (13·8%)	1644 (14·5%)
Previous myocardial infarction	326 (11·6%)	333 (11·8%)	365 (13·0%)	333 (11·9%)	1357 (12·0%)
Claudication	127 (4·5%)	125 (4·4%)	122 (4·3%)	127 (4·5%)	501 (4·4%)
Angina grade (CCVS)					
No angina	1688 (59·5%)	1667 (58·9%)	1679 (59·3%)	1696 (60·0%)	6730 (59·4%)
No limitation (I)	897 (31·6%)	923 (32·6%)	881 (31·1%)	1895 (31·7%)	3596 (31·8%)
Slight limitation (II)	126 (4·4%)	125 (4·4%)	136 (4·1%)	122 (4·3%)	509 (4·5%)
Severe limitation (III)/at rest (IV)	29 (2·1%)	50 (1·8%)	54 (1·9%)	46 (1·6%)	209 (1·8%)
Dyspnoea grade (NYHA)					
No dyspnoea	968 (34·4%)	941 (33·5%)	955 (34·0%)	966 (34·6%)	3830 (34·1%)
No limitation (I)	1561 (55·0%)	1600 (56·5%)	1554 (54·9%)	1537 (54·3%)	6252 (55·2%)
Dyspnoea on normal/mild exertion (II–III)	287 (10·1%)	264 (9·3%)	294 (10·4%)	291 (10·3%)	1136 (10·0%)
Ejection fraction					
≤0·30	56 (2·3%)	69 (2·9%)	59 (2·5%)_	65 (2·7%)	249 (2·6%)
0·31–0·40	283 (11·7%)	245 (10·2%)	279 (11·6%)	264 (11·0%)	1071 (11·1%)
>0·40	2089 (86·0%)	2092 (87·0%)	2059 (85·9%)	2079 (86·3%)	8319 (86·3%)
Premature ventricular beats >10/h	259 (13·1%)	252 (12·6%)	278 (14·1%)	279 (14·1%)	1068 (13·5%)
Previous sustained ventricular tachycardia	17 (0·9%)	25 (1·3%)	18 (0·9%)	13 (0·7%)	73 (0·9%)
Ventricular arrhythmias	373 (18·8%)	376 (18·7%)	400 (20·2%)	385 (19·4%)	1534 (19·3%)
Positive exercise-stress test	550 (29·8%)	511 (27·8%)	542 (29·0%)	534 (29·0%)	2137 (28·9%)
Mean (SD) characteristics					
Age	59·4 (10·7%)	59·5 (10·5%)	59·1 (10·5%)	59·4 (10·5%)	59·4 (10·6%)
Days since diagnosis of AMI	25·4 (21·0%)	25·0 (20·7%)	24·7 (20·7%)	25·2 (21·1%)	25·1 (20·1%)
Body-mass index (kg/m²)	26·5 (3·9%)	26·5 (3·6%)	26·6 (3·6%)	26·4 (3·5%)	26·5 (3·7%)
Ejection fraction	52·6 (10·6%)	52·9 (10·5%)	52·4 (10·5%)	52·5 (10·8%)	52·6 (10·6%)
Lipids (mg/dL)					
Total blood cholesterol	210·2 (42·1%)	211·1 (42·4%)	210·6 (41·5%)	211·6 (42·3%)	210·9 (42·1%)
LDL cholesterol	137·3 (39·1%)	138·0 (38·1%)	138·2 (38·1%)	138·5 (37·6%)	137·4 (38·0%)
HDL cholesterol	41·5 (11·3%)	41·3 (11·2%)	41·6 (11·5%)	41·7 (12·0%)	41·5 (11·5%)
Triglycerides	162·6 (81·7%)	163·3 (85·3%)	160·3 (80·3%)	161·9 (94·5%)	162·1 (85·6%)

AMI=acute myocardial infarction; CCVS=Canadian Cardiovascular Society; NYHA=New York Heart Association. In some sections numbers do not add up because of missing values.

Table 1: **Baseline characteristics of randomised patients**

	n-3 PUFA (n=2836)	Vitamin E (n=2830)	n-3 PUFA plus vitamin E (n=2830)	Control (n=2828)	All (n=11 324)
Dietary habits					
Fish (≥1 serving/week)					
Baseline	2050 (72·9%)	2053 (73·1%)	2057 (73·3%)	2053 (73·4%)	8213 (73·2%)
6 months	2170 (85·9%)	2184 (87·7%)	2137 (86·2%)	2125 (85·5%)	8616 (86·3%)
42 months	1676 (87·7%)	1622 (87·5%)	1651 (88·1%)	1578 (87·2%)	6527 (87·6%)
Fruit (≥1 serving/day)					
Baseline	2243 (79·9%)	2269 (80·8%)	2239 (79·8%)	2259 (80·9%)	9010 (80·3%)
6 months	2185 (86·7%)	2169 (87·4%)	2181 (88·4%)	2145 (86·7%)	8680 (87·3%)
42 months	1670 (87·9%)	1625 (88·0%)	1635 (87·5%)	1590 (88·5%)	6520 (88·0%)
Fresh vegetables (≥1 serving/day)					
Baseline	1121 (39·8%)	1088 (38·7%)	1145 (40·8%)	1107 (39·6%)	4461 (39·7%)
6 months	1341 (53·0%)	1299 (52·1%)	1333 (53·8%)	1331 (53·4%)	5304 (53·1%)
42 months	1055 (55·1%)	1010 (54·4%)	1026 (54·6%)	988 (54·4%)	4079 (54·6%)
Olive oil (regularly)					
Baseline	2092 (74·3%)	2085 (74·3%)	2016 (71·8%)	2066 (73·9%)	8259 (73·6%)
6 months	1998 (79·1%)	1993 (80·2%)	1955 (79·0%)	1990 (80·0%)	7936 (79·6%)
42 months	1566 (82·2%)	1542 (83·4%)	1542 (82·5%)	1486 (82·0%)	6136 (82·5%)
Pharmacological therapy					
Antiplatelet drugs					
Baseline	2601 (92·2%)	2565 (91·2%)	2582 (91·8%)	2562 (91·5%)	10 310 (91·7%)
6 months	2308 (88·2%)	2262 (87·4%)	2261 (87·5%)	2267 (88·3%)	9098 (87·8%)
42 months	1707 (83·4%)	1649 (82·5%)	1685 (83·2%)	1627 (82·1%)	6668 (82·8%)
Angiotensin-converting-enzyme inhibitors					
Baseline	1298 (46·0%)	1287 (45·7%)	1352 (48·1%)	1343 (48·0%)	5280 (46·9%)
6 months	1033 (39·5%)	1074 (41·5%)	1045 (40·4%)	1083 (42·2%)	4235 (40·9%)
42 months	788 (38·5%)	774 (38·7%)	826 (40·8%)	754 (38·0%)	3142 (39·0%)
β-blockers					
Baseline	1237 (43·9%)	1261 (44·8%)	1250 (44·4%)	1238 (44·2%)	4986 (44·3%)
6 months	1092 (41·7%)	1085 (41·9%)	1052 (40·7%)	1043 (40·6%)	4272 (41·2%)
42 months	807 (39·4%)	790 (39·5%)	764 (37·7%)	738 (37·2%)	3099 (38·5%)
Cholesterol-lowering drugs					
Baseline	124 (4·4%)	130 (4·6%)	135 (4·8%)	145 (5·1%)	534 (4·7%)
6 months	782 (28·6%)	780 (28·8%)	757 (27·9%)	786 (29·1%)	3105 (28·6%)
42 months	1003 (46·0%)	962 (44·8%)	1013 (46·7%)	941 (44·4%)	3919 (45·5%)
Revascularisation procedures*					
CABG or PTCA					
Baseline	135 (4·8%)	142 (5·0%)	157 (5·6%)	126 (4·5%)	560 (5·0%)
6 months	433 (15·3%)	439 (15·5%)	481 (17·0%)	429 (15·2%)	1782 (15·7%)
42 months	689 (24·3%)	651 (23·0%)	707 (25·0%)	670 (23·7%)	2717 (24·0%)

CABG=coronary artery bypass; PTCA=percutaneous transluminal coronary angioplasty.
In some sections numbers do not add up because of missing values. Patients alive at baseline=11 324, 6 months=11 092, and 42 months=9289.
*Number and percentage of patients revascularised during study are cumulative.

Table 2: **Dietary habits and main therapeutic interventions at baseline and during study**

Patients were randomly assigned n-3 PUFA alone (n=2836), vitamin E alone (n=2830), n-3 PUFA and vitamin E combined (n=2830), or no supplement (control, n=2828). Treatment was administered by investigators or, in some instances, by hospital pharmacists.

Randomisation was done over the telephone and by computer network. Treatments were automatically assigned from a program based on the biased-coin algorithm, which allowed stratification by hospital.[18] Randomisation data were kept at the coordinating centre.

We planned the procedures of the trial to mimic as far as possible the routine of care after myocardial infarction. We scheduled follow-up visits at 6 months, 12 months, 18 months, 30 months, and 42 months that included clinical assessment and the administration of a food-frequency questionnaire. We measured compliance by refilling drug supplies every 3 months. Blood samples were taken for measurement of lipids at baseline and at follow-up visits for a companion study run by the research group of the Italian Society of Clinical Biochemistry (SIBioC) that was investigating the quality control and the monitoring of main biochemical markers.[19]

The primary combined efficacy endpoints were: the cumulative rate of all-cause death, non-fatal myocardial infarction, and non-fatal stroke; and the cumulative rate of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke. We did secondary analyses for each component of the primary endpoints, and for the main causes of death.

Myocardial infarction was taken to be present if the investigator had identified this complication on a standard form or if a death certificate or hospital records showed a fatal myocardial infarction. Non-fatal acute myocardial infarction was defined as at least two of the following: chest pain of typical intensity and duration; ST segment elevation or depression of 1 mm or more in any limb lead of the electrocardiogram, of 2 mm or more in any precordial lead, or both; or at least a doubling in necrosis enzymes. Diagnosis of non-fatal stroke required unequivocal signs or symptoms of remaining neurological deficit, with sudden onset and a duration of more than 24 h. Diagnosis of fatal stroke also used these criteria. Alternatively, we used the diagnosis documented in hospital records or on death certificates. The validation of the clinical events included in the primary endpoints was assured by an ad-hoc committee of expert cardiologists and neurologists blinded to patients' treatment assignment.

The study was conceived, managed, and analysed by the coordinating centre, under the responsibility of the steering committee. We obtained the approval of existing ethics committees before the start of the trial. All patients gave informed written consent. The external safety and efficacy monitoring committee did one interim analysis, masked to treatment assignment.

Statistical methods

We estimated that the cumulative rate of death, non-fatal myocardial, and stroke in the control group over the planned 3·5 years of the study would be 20%. The sample size of the trial was calculated to compare the rate of the main endpoint in each of the three study-drug groups to that of the control group (3000 patients per group, relative-risk decrease 20%) and to test the hypothesis that the combined treatment would decrease by a further 20% the rate of the main endpoint compared with n-3 PUFA alone or vitamin E alone. According to the protocol,

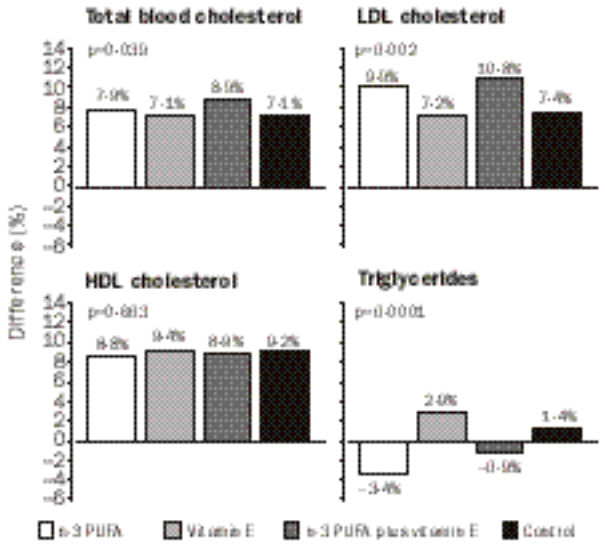

Figure 2: **Percentage differences in blood lipid concentrations at 6 months**
Bars show percentage change from baseline to 6 months.

follow-up data were right-censored at 42 months, when follow-up information on the vital status of patients, through clinical visits or census, was available for 99·9% of the population. Analysis was done by intention to treat and according to the two strategies defined in the protocol: first, a factorial design with two-way analysis of efficacy of n-3 PUFA supplements compared with no n-3 PUFA and efficacy of vitamin E supplements compared with no vitamin E; second, a four-way analysis of efficacy of n-3 PUFA supplements, vitamin E supplements, and the combined treatment compared with control, as well as the efficacy of the combined treatment compared with individual interventions.[20,21]

We analysed data by Kaplan-Meier survival curves and the log-rank test. Treatment efficacy was assessed by baseline values of the risk-stratification variables fitting various Cox's regression models adjusted for the confounding effect of relevant prognostic indicators. The assumption of proportionality in the hazard functions for the experimental groups was assessed visually.[22] In addition, we fitted a logistic function to the data, which gave the same results as the fitting of the Cox's proportional hazards model. Criteria for hierarchical use of events as endpoints have been reported elsewhere.[23] Briefly, we first looked at information on vital status and, if the patient was alive at the end of the study, we assessed whether a non-fatal event had occurred. We used the Kruskal-Wallis test for continuous variables. All p values are two-sided.

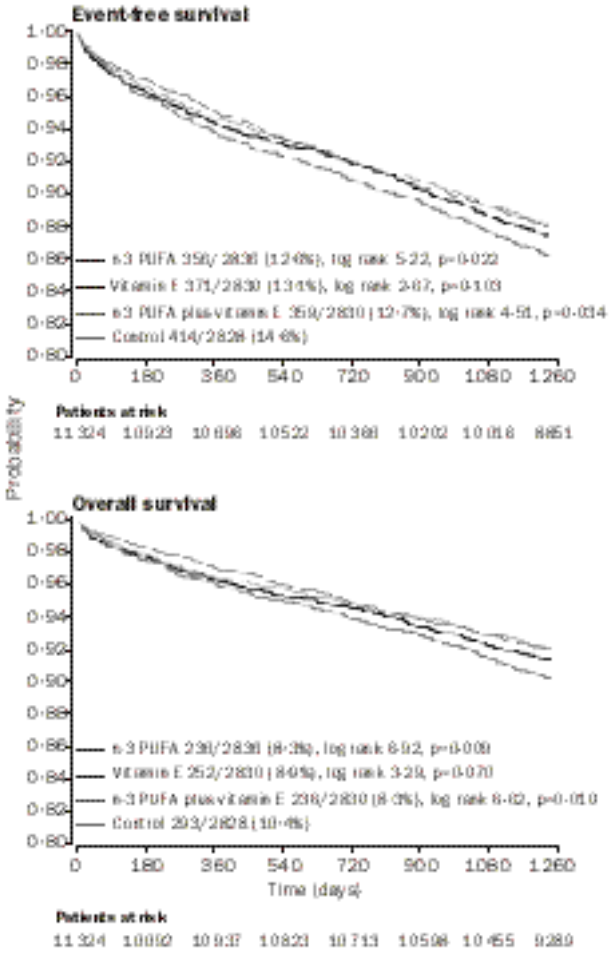

Figure 3: **Event-free survival and overall survival**

To explore interaction, we fitted multivariate models including the two experimental treatments and the interaction term. If significant, the latter indicates effect modification when the two treatments are given together.

Results

Between October, 1993, and September, 1995, 11 324 patients were recruited (figure 1) by 172 participating centres (130 cardiological departments and 42 rehabilitation centres) across Italy. Information on vital status at the end of the study was 99·9% complete for a

	All (n=11 324)	Two-way analysis			Four-way analysis		
		n-3 PUFA (n=5666)	Control (n=5668)	Relative risk (95% CI)	n-3 PUFA (n=2836)	Control (n=2828)	Relative risk (95% CI)
Main endpoints							
Death, non-fatal MI, and non-fatal stroke	1500 (13·3%)	715 (12·6%)	785 (13·9%)	0·90 (0·82–0·99)	356 (12·3%)	414 (14·6%)	0·85 (0·74–0·98)
Cardiovascular death, non-fatal MI, and non-fatal stroke	1155 (10·2%)	547 (9·7%)	608 (10·8%)	0·89 (0·80–1·01)	262 (9·2%)	322 (11·4%)	0·80 (0·68–0·95)
Secondary analyses							
All fatal events	1017 (9·0%)	472 (8·3%)	545 (9·6%)	0·86 (0·76–0·97)	236 (8·3%)	293 (10·4%)	0·80 (0·67–0·94)
Cardiovascular deaths	639 (5·6%)	291 (5·1%)	348 (6·2%)	0·83 (0·71–0·97)	136 (4·8%)	193 (6·8%)	0·70 (0·56–0·87)
Cardiac death	520 (4·6%)	228 (4·0%)	292 (5·2%)	0·78 (0·65–0·92)	108 (3·8%)	165 (5·8%)	0·65 (0·51–0·82)
Coronary death	479 (4·2%)	214 (3·8%)	265 (4·7%)	0·80 (0·67–0·96)	100 (3·5%)	151 (5·3%)	0·65 (0·51–0·84)
Sudden death	286 (2·5%)	122 (2·2%)	164 (2·9%)	0·74 (0·58–0·93)	55 (1·9%)	99 (3·5%)	0·55 (0·40–0·76)
Other deaths	378 (3·3%)	181 (3·2%)	197 (3·5%)	0·91 (0·74–1·11)	100 (3·5%)	100 (3·5%)	0·99 (0·75–1·30)
Non-fatal cardiovascular events	578 (5·1%)	287 (5·1%)	291 (5·1%)	0·98 (0·83–1·15)	140 (4·9%)	144 (5·1%)	0·96 (0·76–1·21)
Other analyses							
CHD death and non-fatal MI	909 (8·0%)	424 (7·5%)	485 (8·6%)	0·87 (0·76–0·99)	196 (6·9%)	259 (9·2%)	0·75 (0·62–0·90)
Fatal and non-fatal stroke	178 (1·6%)	98 (1·7%)	80 (1·4%)	1·21 (0·91–1·63)	54 (1·9%)	41 (1·5%)	1·30 (0·87–1·96)

MI=myocardial infarction; CHD=coronary heart disease.
Patients with two or more events of different types appear more than once in columns but only once in rows.

Table 3: **Overall efficacy profile of n-3 PUFA treatment**

	All (n=11 324)	Two-way analysis			Four-way analysis		
		Vitamin E (n=5666)	Control (n=5668)	Relative risk (95% CI)	Vitamin E (n=2830)	Control (n=2828)	Relative risk (95% CI)
Combined endpoints							
Death, non-fatal MI, and non-fatal stroke	1500 (13·3%)	730 (12·9%)	770 (13·6%)	0·95 (0·86–1·05)	371 (13·1%)	414 (14·6%)	0·89 (0·77–1·03)
Cardiovascular death, non-fatal MI, and non-fatal stroke	1155 (10·2%)	571 (10·1%)	584 (10·3%)	0·98 (0·87–1·10)	286 (10·1%)	322 (11·4%)	0·88 (0·75–1·04)
Secondary analyses							
All fatal events	1017 (9·0%)	488 (8·6%)	529 (9·3%)	0·92 (0·82–1·04)	252 (8·9%)	293 (10·4%)	0·86 (0·72–1·02)
Cardiovascular deaths	639 (5·6%)	310 (5·5%)	329 (5·8%)	0·94 (0·81–1·10)	155 (5·5%)	193 (6·8%)	0·80 (0·65–0·99)
Cardiac death	520 (4·6%)	247 (4·4%)	273 (4·8%)	0·91 (0·76–1·08)	127 (4·5%)	165 (5·8%)	0·77 (0·61–0·97)
Coronary death	479 (4·2%)	228 (4·0%)	251 (4·4%)	0·91 (0·76–1·09)	114 (4·0%)	151 (5·3%)	0·75 (0·59–0·96)
Sudden death	286 (2·5%)	132 (2·3%)	154 (2·7%)	0·86 (0·68–1·08)	65 (2·3%)	99 (3·5%)	0·65 (0·48–0·89)
Other deaths	378 (3·3%)	178 (3·1%)	200 (3·5%)	0·89 (0·73–1·09)	97 (3·4%)	100 (3·5%)	0·96 (0·73–1·28)
Non-fatal cardiovascular events	578 (5·1%)	294 (5·2%)	284 (5·0%)	1·04 (0·88–1·22)	147 (5·2%)	144 (5·1%)	1·02 (0·81–1·28)
Other analyses							
CHD death and non-fatal MI	909 (8·0%)	454 (8·0%)	455 (8·0%)	1·00 (0·88–1·14)	226 (8·0%)	259 (9·2%)	0·87 (0·73–1·04)
Fatal and non-fatal stroke	178 (1·6%)	83 (1·5%)	95 (1·7%)	0·87 (0·65–1·17)	39 (1·4%)	41 (1·5%)	0·95 (0·61–1·47)

MI=myocardial infarction; CHD=coronary heart disease.
Patients with two or more events of different types appear more than once in columns but only once in rows.

Table 4: **Overall efficacy profile of vitamin E treatment**

total person-time of 38 053 years. Median time from the index myocardial infarction to randomisation was 16 days. Baseline demographic and clinical characteristics were well balanced across the groups (table 1) and define a relatively low-risk population, with 16% of patients aged 70 years or older, 14% with an echo-documented ejection fraction of 40% or less, and 29% with positive exercise-stress tests. Dietary habits, recommended secondary-prevention treatments, and revascularisation procedures at baseline and during the study were also well balanced across all groups (table 2).

Compared with baseline values, there were no clinically important changes for cholesterol (total, HDL, and LDL), glycaemia, and fibrinogen in any of the treatment groups at the first visit (figure 2). The difference in blood lipids, however, was more slight than any other value during the study (data not shown). Compared with controls, the small decrease in triglyceride concentrations was significant in patients receiving n-3 PUFA.

The full profile of the effects of n-3 PUFA is summarised in table 3. In the two-way factorial analysis, the 10% relative decrease in risk for the combined primary endpoint of death, non-fatal myocardial infarction, and non-fatal stroke was significant (95% CI 1–18, p=0·048), but the decrease in risk for the other combined endpoint of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke was not significant (11% [1–20], p=0·053).

The four-way analysis provides a clearer profile of the effects of n-3 PUFA (figure 3), with a relative decrease in risk for the combined endpoint of 15% (2–26, p=0·023) and for cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke of 20% (5–32, p=0·008).

Analyses of the individual components of the main endpoint showed that the decrease in mortality (20% for total deaths, 30% for cardiovascular deaths, and 45% for sudden deaths) which was obtained with n-3 PUFA accounted for all the benefit seen in the combined endpoint. There was no difference across the treatment groups for non-fatal cardiovascular events. The results of the tests for interaction were not significant when the two combined endpoints and overall mortality were analysed. The significance values reached when a similar analysis was applied to the individual components of the endpoints (p=0·0242 for cardiovascular mortality; p=0·0226 for coronary mortality; p=0·024 for fatal plus non-fatal coronary events; and p=0·010 for sudden death) better approximate the true unconfounded effect of n-3 PUFA and show that the results of the four-way analysis are not influenced by an effect modification due to the combination of the two treatments.

Patients receiving vitamin E and controls did not differ significantly when data were analysed according to the factorial design (table 4). The lack of evidence of effect is similar for the combined endpoint and for its individual components. The results were similar for the combined endpoints and overall mortality analysed by treatment group. An indication of a possible beneficial effect of vitamin E is provided, however, in the secondary analyses of the individual components of cardiovascular death of the combined endpoints, for which the increasing benefit (from 20% for all cardiovascular deaths to 35% for sudden death) is similar to the picture for n-3 PUFA. The absence of a difference in the rate of non-fatal cardiovascular events between vitamin E and the control group is also similar to the findings related to n-3 PUFA.

The results for combined treatment compared with controls are shown in table 5. The effects seen on the primary combined endpoint and on total mortality were consistent with those obtained with n-3 PUFA alone. No increased benefit was apparent when the rate of the combined endpoint of death, non-fatal myocardial infarction, and non-fatal stroke that was seen in patients receiving n-3 PUFA plus vitamin E was compared with

	n-3 PUFA plus vitamin E (n=5666)	Control (n=2828)	Relative risk (95% CI)
Main endpoints			
Death, non-fatal MI, and non-fatal stroke	359 (12·7%)	414 (14·6%)	0·86 (0·74–0·99)
Cardiovascular death, non-fatal MI, and non-fatal stroke	285 (10·1%)	322 (11·4%)	0·88 (0·75–1·03)
Secondary analyses			
All fatal events	236 (8·3%)	293 (10·4%)	0·80 (0·67–0·95)
Cardiovascular deaths	155 (5·5%)	193 (6·8%)	0·80 (0·65–0·99)
Cardiac death	120 (4·2%)	165 (5·8%)	0·72 (0·57–0·91)
Coronary death	114 (4·0%)	151 (5·3%)	0·75 (0·59–0·96)
Sudden death	67 (2·4%)	99 (3·5%)	0·67 (0·49–0·92)
Other deaths	81 (2·9%)	100 (3·5%)	0·80 (0·60–1·08)
Non-fatal cardiovascular events	147 (5·0%)	144 (5·1%)	1·01 (0·80–1·27)
Other analyses			
CHD death and non-fatal MI	228 (8·1%)	259 (9·2%)	0·87 (0·73–1·04)
Fatal and non-fatal stroke	44 (1·6%)	41 (1·5%)	1·06 (0·70–1·63)

MI=myocardial infarction; CHD=coronary heart disease.
Patients with two or more events of different types appear more than once in columns but only once in rows.

Table 5: **Overall efficacy profile of n-3 PUFA plus vitamin E treatment**

the group receiving n-3 PUFA alone (1·01 [0·87–1·17) or with patients treated with vitamin E alone (0·96 [0·83–1·12]).

At 1 year and at the end of the study, 11·6% and 28·5% of patients receiving n-3 PUFA and 7·3% and 26·2% of those receiving vitamin E, respectively, had permanently stopped taking the study drug. Conversely, during the whole course of the study, only two patients not assigned vitamin E and 26 patients not assigned n-3 PUFA were receiving these drugs. Side-effects were reported as a reason for discontinuing therapy for 3·8% of patients in the n-3 PUFA groups, and in 2·1% of those in the vitamin E groups. Overall, gastrointestinal disturbances and nausea were the most frequently reported side-effects (4·9% and 1·4% of n-3 PUFA recipients, and 2·9% and 0·4% of vitamin E recipients, respectively).

Cancer occurred in 61 (2·2%) patients in the control group, in 77 (2·7%) in the n-3 PUFA group, in 73 (2·6%) in the vitamin E group, and in 65 (2·3%) in the combined treatment group. There were 33 (1·2%) non-fatal cases of cancer in the control group, 41 (1·5%) in the n-3 PUFA group, 35 (1·2%) in the vitamin E group, and 26 (0·9%) in the combined treatment group.

Discussion

Treatment with n-3 PUFA significantly decreased, over 3·5 years, the rate of death, non-fatal myocardial infarction, and stroke. No effect was seen for vitamin E. When data were analysed by four-way analysis, the size of the beneficial effect of n-3 PUFA became more evident and more clearly significant; the absence of a significant effect was confirmed for vitamin E.

The degree of the effects on rates of death deserves to be specifically highlighted and is suggestive of hypotheses that could have more general implications for secondary-prevention trials in patients who have had myocardial infarction, as well as for pathophysiological interpretation of trial results. The results obtained with n-3 PUFA are consistent with those of the DART trial.[6] They found a 29% decrease over 2 years in overall mortality in men who ate fatty fish twice a week, with no decrease in the rate of non-fatal myocardial infarction. This pattern of effects was reproduced in two large-scale observational studies, the Health Professionals Study[24] and the US Physicians' Health Study.[25] Significant associations between fish intake and lower risk of coronary heart disease were shown in the Zutphen study,[26] the 30-year follow-up of the Western Electric study,[27] the observational cohort of the Multiple Risk Factor Intervention Trial,[28] and the Honolulu Heart Program.[29] The significant results of the Lyon Diet Heart study[30] and of the Indian trial by Singh and colleagues,[31] strongly suggest a protective effect of n-3 PUFA. Because of the high frequency of stroke of non-defined cause, there were only 11 haemorrhagic strokes and, therefore, distribution in the experimental groups could not be clearly inferred.

The pathophysiological basis of the clinical and epidemiological suggestions in favour of a more direct cardiac effect of n-3 PUFA has been explored in a wealth of experimental, animal,[32–35] human,[36–39] and in-vitro[40–45] studies, which together support a role for n-3 PUFA on arrhythmogenesis. The lack of evidence of benefit on atherosclerotic-thrombotic events, despite the well-documented activity of n-3 PUFA on eicosanoid metabolism, inflammation, tissue factor, β-oxidation, endothelial dysfunction, cytokine growth-factors, and gene expression of adhesion molecules, is difficult to explain.[2–4] In our trial, an explanation could partly be the intensive preventive interventions that were documented for the whole duration of the study (table 2).[46]

By contrast with n-3 PUFA, the results for vitamin E did not support the strong epidemiological evidence available at the beginning of the trial and to date,[1,7–13] although the significant decrease of cardiovascular deaths in the four-way analysis cannot be easily dismissed. The information available before the GISSI-Prevenzione trial was contradictory. The suggestion of a striking decrease in non-fatal myocardial infarction, and of a non-significant excess of total and cardiovascular deaths originated from a trial that had severe weaknesses in the methods.[47,48] The data on the absence of any significant effect of low doses (50 mg daily) of vitamin E on cardiovascular death, and on the non-significant (positive and negative) modifications of non-fatal cardiovascular events were obtained in a population that could not be compared with that in our study.[14,49]

Discrepant findings between expectations of benefit based on epidemiological observations and results of clinical trials, however, are not especially surprising.[50,51] The biological background of the suggested mechanisms of action of vitamin E[4,10–13,52] should be considered in the general framework of the biological effects of all the other treatments already prescribed to myocardial-infarction patients, as well as the effects of those attributable to the protection of the Mediterranean eating habits of the GISSI population.[53,54] In addition, it is possible that a longer duration of intervention is needed to allow the action of biological mechanisms of benefit, which might be different from those of n-3 PUFA, and to shift significantly the overall risk profile and, as a consequence, the incidence of fatal events. However, similar considerations would apply also to n-3 PUFA, for which the same experimental context produced consistently positive and significant results.

To better qualify the results of our trial, a few comments are appropriate with respect to: the doses of experimental treatments; the open design of the study; the overall clinical importance; and the implications of the size of the effects seen with n-3 PUFA.

The regimen we used for n-3 PUFA corresponds to a diet that contains a large amount of fatty fish, to be maintained every day (eg, 100 g of fatty fish/day), although most of the data available on the mechanisms of this product had been obtained with much higher, purely "pharmacological" doses of n-3 PUFA (≥3–4 g/day). The choice in favour of a regimen more acceptable for long-term treatment seems also to fit well with the favourable clinical and epidemiological "dietary" results, and with emerging suggestions about other mechanisms of action of n-3 PUFA not directly related to a rapid and substantial modification of the saturation ratio of cell membranes.

The dose of vitamin E that we used was in the lower range of those chosen in other continuing clinical trials (only the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study[14] trial used a lower dose of 50 mg/day). It is important to take into account, however, that a dose of 300 mg per day is already in excess of any achievable dose through dietary intake (eg, corresponding to 200 table-spoons of olive oil daily) and is more than ten times higher than current recommended dietary allowances for optimum health in adults. Notably, however, the results of

observational studies suggest no increasing benefit for intakes of vitamin E higher than 100 mg daily.[7,8] In addition, evidence exists that short-term treatment with doses lower than that used in our trial for long-term treatment could prevent LDL oxidation.[55–59] The equivalence between prevention of LDL oxidation and clinical efficacy, however, may be oversimplistic.[52] It is more likely that the gradient of the beneficial effects seen in the most striking results of epidemiological studies reflected the overall behavioural attitudes of the people regularly taking high doses of antioxidant substances over many years. Therefore, the dose of vitamin E that is most effective and safe, as well as the minimum duration of treatment that is required to produce the postulated protective effects of vitamin E are still unknown.[60] Results of continuing large randomised trials with other doses of vitamin E supplements will better elucidate the efficacy profile of this antioxidant substance in lowering cardiovascular risk in patients with myocardial infarction and in other patients, possibly in different clinical settings.[10]

The main risk of any open-label design for a mortality trial could be seen in the possibility of biased behaviour by prescribing doctors and of patients adopting different dietary habits. Our data, however, provide good evidence that dietary habits, secondary prevention with recommended treatments, and revascularisation procedures were well balanced across the four groups throughout the study (table 2). Conversely, the pragmatic strategy used for monitoring was expected to lead to the risk of incomplete compliance, which would have mimicked what is likely to happen in general long-term secondary preventive care in a population whose relatively low-risk profile is already intensively covered with other preventive interventions. The strict adherence to the intention-to-treat principle assures that the effects seen correspond closely to what is achievable in clinical practice.

The size of effect of n-3 PUFA treatment on the primary endpoint of total death, non-fatal myocardial infarction, and non-fatal stroke could be quantified as corresponding to a 10% relative decrease in risk in the two-way analysis and to a 15% relative decrease in risk in the four-way analysis. Although significant, these results are clearly lower than the 20% relative decrease of risk expected in our original planning. An efficacy result that is smaller than expected is quite common in trials in which patients receive more intensive background treatments than populations taken as reference at the time of trial design. Therefore, the rate of events in the control group that was 25% less than expected was not surprising. Although the four-way analysis, which avoids the possible interference of the interaction of effects between treatments, should be seen preferentially as the one showing the "true" results, it is important to take into account that the more relevant effects were seen on the harder component of the primary combined endpoint (20% relative decrease overall and 30% relative decrease of cardiovascular mortality). The effect of multiple comparisons of the various components of the endpoint checked with appropriate statistical approaches did not modify importantly the significance values of the four-way analysis for fatal events.

In this population of patients who had myocardial infarction and Mediterranean dietary habits, and who were well treated with up to date preventive pharmacological interventions, long-term n-3 PUFA 1 g daily, but not vitamin E 300 mg daily, was beneficial for death and for combined death, non-fatal myocardial infarction, and stroke. All the benefit, however, was attributable to the decrease in risk for overall and cardiovascular death.

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Acknowledgments

GISSI is endorsed by Associazione Nazionale Medici Cardiologi Ospedalieri (ANMCO), Firenze, Italy and by Istituto di Ricerche Farmacologiche Mario Negri-Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy.

This study was supported by 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, l-α-tocopherol) was supplied by Bracco. We thank Miss Barbara Ferri for secretarial help.

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