Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease

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Summary

In a prospective, randomised single-blinded secondary prevention trial we compared the effect of a Mediterranean alpha-linolenic acid-rich diet to the usual post-infarct prudent diet.

After a first myocardial infarction, patients were randomly assigned to the experimental (n=302) or control group (n=303). Patients were seen again 8 weeks after randomisation, and each year for 5 years. The experimental group consumed significantly less lipids, saturated fat, cholesterol, and linoleic acid but more oleic and alpha-linolenic acids confirmed by measurements in plasma. Serum lipids, blood pressure, and body mass index remained similar in the 2 groups. In the experimental group, plasma levels of albumin, vitamin E, and vitamin C were increased, and granulocyte count decreased. After a mean follow up of 27 months, there were 16 cardiac deaths in the control and 3 in the experimental group; 17 non-fatal myocardial infarction in the control and 5 in the experimental groups: a risk ratio for these two main endpoints combined of 0·27 (95% CI 0·12–0·59, p=0·001) after adjustment for prognostic variables. Overall mortality was 20 in the control, 8 in the experimental group, an adjusted risk ratio of 0·30 (95% CI 0·11–0·82, p=0·02).

An alpha-linolenic acid-rich Mediterranean diet seems to be more efficient than presently used diets in the secondary prevention of coronary events and death.

Lancet 1994; **343:** 1454–59

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Introduction

Previous trials of diet modification to prevent recurrence after a first myocardial infarction did not show the results that could have been expected from epidemiological studies.[1] Among three main studies,[2-4] only the Oslo trial[2] reported a significant reduction of 23% in coronary events but no improvement in survival. In these trials[2,4] in order to lower serum cholesterol, the intake of saturated fat was decreased and that of polyunsaturated fat increased. Even in primary prevention with a diet rich in polyunsaturated (P) and low in saturated (S) fat (P/S, 1·6), the rates of coronary events and deaths were not decreased.[5] In another trial—the diet and reinfarction trial (DART)—it was only when intake of n-3 fatty acids (fish or oil) was increased that mortality was reduced.[6]

We have reported that a dietary polyunsaturated to saturated fat ratio more than 1, even if associated with a decrease in plasma cholesterol, enhanced platelet aggregation to adenosine diphosphate.[7] High platelet aggregation is associated with myocardial infarction[8] and closely predicts coronary events.[9] In addition, a high concentration of linoleic acid could be associated with increased lipid peroxidation[10] and platelet-induced aggregation.[7]

In the present trial of diet to reduce cardiac mortality and morbidity after myocardial infarction, we adapted a diet associated with a low mortality rate from coronary heart disease and all causes in the Seven Country Study.[11] The Cretan Mediterranean diet includes a high intake of alpha-linolenic acid (the precursor of n-3 long chain fatty acids), known for its beneficial effect on platelet reactivity.[12] Being rich in vegetables and fruits, the diet also supplied a high intake of antioxidants.

Methods

Design

The Lyon Diet Heart Study is a prospective, randomised, single-blinded, multi-clinic (6 Services within Lyon Cardiovascular Hospital), secondary prevention trial aimed at reducing the risk of cardiovascular deaths by diet modification and recurrent myocardial infarction in survivors of a first myocardial infarction. The protocol was approved by the Ethical Committee of the Institut National de la Santé et de la Recherche Médicare (INSERM). An independent Scientific Committee met every two years to advise the investigators on all aspects of the trial. The randomisation list was kept confidentially at INSERM and consulted by phone for each patient enrolled. Assignment of patients were not known by the attending physicians. Mortality and morbidity outcomes were validated and classified by an independent Committee that worked only on the blinded data from hospital files concerning outcomes which involved hospital admission.

Patients

Patients of both sexes, less than 70 years old, who survived a myocardial infarction within 6 months of enrolment were eligible. Exclusion criteria included heart failure (stage III and IV of the New York Heart Association functional class), hypertension

(systolic > 180 mm Hg, diastolic > 110 mm Hg), and inability to complete an exercise test due to recurrent angina, ventricular arrhythmias, or atrioventricular block. Among patients who had coronary angioplasty or bypass, only those who were clinically stable were entered. Patients were also excluded if they had any other conditions thought to limit survival or ability to participate in a long-term trial.

Inclusion of patients was according to a modification of Zelen's design.[3] During hospital stay, patients were asked to participate in a cohort study with a follow-up of 5 years and to sign a first informed consent. To avoid between-group contamination, with the approval of the Ethical and Scientific Committees, patients were not fully informed of the design of the study, especially of the comparison between two diets. To be included in the study, they had to come to the outpatient clinic, 2 weeks after discharge, and be randomised. Patients assigned to the experimental group had to sign a second informed consent in which they agreed to modify their diets.

Sample size and recruitment

Sample size was calculated assuming an annual recurrence rate of 8% in the control group, 4% in the experimental group, and 4% annual loss to follow-up in both groups. For a power of 90%, with a two-sided test and a 5% type 1 error, 250 patients (125 patients per group per year) would be required in each group with a follow-up of 5 years after 2 years of recruitment.

However, after one year of recruitment and follow-up, both the accrual rate and recurrence rate were lower than expected. Assuming an annual recurrence rate of 5·3% in the control and 2·65% in the experimental groups, the number of subjects to be recruited was 300 per group over 4 years with a minimum follow-up of 2 years. Consequently, enrolment which began in March, 1988, was ended in March, 1992. An intermediate analysis was proposed by the Scientific Committee in March, 1993, after a minimum follow-up of one year, assuming a type 1 error of 3%, to ensure a total 5% error at final analysis, in 1994. In the case of a statistically significant result at the intermediate analysis, the trial would be stopped. This publication results from that decision being made. The trial was stopped after the intermediate analysis.

Diet intervention

To avoid between-group contamination, control patients received no dietary advice apart from that of hospital dieticians or attending physicians. Patients in the experimental group were advised by the research cardiologist and dietician, during a one-hour-long session, to adopt a Mediterranean-type diet: more bread, more root vegetables and green vegetables, more fish, less meat (beef, lamb, and pork to be replaced with poultry), no day without fruit, and butter and cream to be replaced with margarine supplied by the study.

Because the patients would not accept olive oil—traditional to the Mediterranean diet—as the only fat, a rapeseed (canola) oil-based margarine (Astra-Calvé, Paris, France) was supplied free for the whole family to all experimental subjects. This margarine had a composition comparable to olive oil with 15% saturated fatty acids, 48% oleic acid but 5·4% 18:1 trans. However, it was slightly higher in linoleic (16·4 *vs* 8·6%) and more so in alpha-linolenic acid (4·8 *vs* 0·6%), a fatty acid markedly higher (3 fold) in the plasma of the Cretan cohort in the Seven Country study compared to that of Zutphen (Netherlands).[14]

The oils recommended for salads and food preparation were rapeseed and olive oils exclusively. Moderate alcohol consumption in the form of wine was allowed at meals. At each subsequent visit of the experimental patients, a dietary survey and further counselling were done by the research dietician. Diet evaluations comprised a 24-hour recall and a frequency questionnaire.

Initial examination and follow-up

Acute-phase data on myocardial infarction were recorded from hospital files. After the randomisation visit where baseline characteristics were recorded, patients of both groups were scheduled to be seen 8 weeks later and then annually.

Weeks	Control n = 303		Experimental n = 302	
	Number	Rate %	Number	Rate %
0	8	2	13	4
8	3	1	9	3
52	13	4	10	3
104	6	2	6	2
156	0	0	1	0 04

Number and rate at each step of follow up.

Table 1: **Withdrawal from follow-up**

At all visits, a systematic interview was conducted by the research cardiologist concerning new hospital admissions, drug treatment changes, invasive and non-invasive cardiac investigations, exercise tolerance, angina, and arrhythmia. Blood pressure was measured by an automatic sphygmomanometer. Blood samples for routine biochemical and haematological determinations and for platelet aggregation studies were done as previously described.[15] Plasma total lipid fatty acids were measured by gas-liquid chromatography[16] but with a capillary column. Plasma vitamin C was measured by colorimetry,[17] and vitamins A and E by high-performance liquid chromatography.[18]

For the first 4 years, dietary habits were evaluated only in the experimental group so as not to influence the behaviour of controls. Nevertheless, the diet of 192 consecutive controls was evaluated once, starting in June, 1992.

Clinical endpoints

Primary endpoints were death from cardiovascular causes and non-fatal acute myocardial infarction. Subsidiary endpoints included non-cardiac deaths (evaluated in patients withdrawn from follow up by contacts with the family or birth-place city halls) and the following conditions, providing they required hospital admission: unstable angina, postinfarct recurrent stable angina, heart failure (stage III and IV of the New York Heart Association), stroke, pulmonary embolism, peripheral embolism, and venous

	Control n = 303	Experimental n = 302
Age (years)	53 5 (10)	53 5 (10)
Sex ratio, M/F (%)	92 1/7 9	89 4/10 6
Current smokers (%)	4 9	7 6
Infarction history		
Primary ventricular fibrillation (%)	4 3	4 0
Thrombolytic therapy (%)	45 9	49 7
Highest serum creatine kinase (IU/L)	1972 (1581)	2067 (1789)
Non-Q wave infarction (%)	18 9	20 8
Infarct location (%)		
Anterior	33 3	42 4
Lateral	5 2	5·7
Inferoposterior	61 5	51 9
Positive exercise test (%)	17 6	19·3
Coronary angiography (%)	38 7	44 7
Coronary angioplasty (%)	15 6	14 7
Medication at randomisation (%)		
Anticoagulant agents	26 4	29·4
Antiplatelet agents	64 8	62 6
Beta-blocking agents	63 4	60 2
Calcium-channel blockers	21 7	20 4
Angiotensin-converting-enzyme inhibitors	6 1	9 3
Haematocrit (%)	41 9 (3 4)	41 3 (3 9)
Haemoglobin (g/L)	139 (11)	141 (51)
Leucocyte count (10^9/L)	6 61 (1 7)	6 62 (1 8)
Granulocyte count (10^9/L)	3 93 (1 3)	3 96 (1 3)
Lymphocyte count (10^9/L)	1 87 (0 6)	1 85 (0 7)
Platelet aggregation (%)		
Thrombin-induced	13 9 (8 7)	14 1 (8 1)
ADP-induced		
First wave	34 4 (8 6)	34 5 (8 7)
Secondary wave	21 4 (16 3)	19 9 (14 1)

(Percent or mean [SD]) values obtained at the first visit at the outpatient clinic, week 0.

Table 2: **Baseline characteristics of patients**

	Experimental group—(weeks)				At 1 to 4 years follow up*		
	0 n=210	8 n=228	52 n=243	104 n=145	Control n=192	Experimental n=219	p
Total calories	2062 (43)	1964 (37)	1944 (32)	1991 (43)	2140 (45)	1928 (32)	<0 001
Proteins (g)	17 2 (0 3)	17 1 (0 2)	17 0 (0 3)	17 2 (0 3)	16 5 (0 3)	17 2 (0 3)	0 12
Comprising—% calories							
Total lipids	30 7 (0 6)	28 6 (0 5)	29 2 (0 5)	30·0 (0 6)	32 7 (0 7)	30 5 (0 5)	0 008
Saturated fats	10·5 (0 3)	7 1 (0 2)	7 7 (0 2)	7 9 (0 3)	11 7 (0 4)	8·3 (0 2)	<0 001
18:1 (n-9)	9 0 (0·2)	12 2 (0 2)	12 6 (0 3)	12 9 (0 3)	10 3 (0 3)	12 9 (0 3)	<0 001
18:2 (n-6)	5.8 (0·2)	3 6 (0 1)	3 5 (0 1)	3 6 (0 1)	5 3 (0 2)	3 6 (0 1)	<0 001
18:3 (n-3)	0 24 (0 01)	0 76 (0 03)	0 77 (0 03)	0 80 (0 04)	0 27 (0 02)	0 81 (0 03)	<0 001
Alcohol	5 1 (0 4)	5 0 (0 4)	5 5 (0 4)	4 9 (0 5)	6 4 (0·5)	5 5 (0 4)	0 19
P/S ratio	0 78 (0 04)	0·77 (0 03)	0 70 (0 03)	0·73 (0 03)	0 69 (0 07)	0 65 (0·06)	0·59
Ascorbic acid (mg)	120 5 (4 6)	129 1 (5 4)	118 6 (4 1)	125 0 (5 5)	101 7 (4 7)	115 8 (4 2)	0 02
Alpha-tocopherol (mg)	13 9 (0·5)	11 9 (0 3)	11 7 (0 3)	11 6 (0 4)	12 9 (0 5)	11 6 (0 3)	0 02
Cholesterol (mg)	333 (16)	252 (14)	219 (11)	237 (14)	318 (15)	217 (11)	<0 001

*Evaluation of controls done only June 1992 to March 1993 so as not to influence diètary habits of the control group earlier in the study, compared to the experimental groups evaluated during the same period. Mean (SE). P/S = polyunsaturated/saturated fat.

Table 3: **Nutrient daily intake in the experimental group at different periods, and in control and experimental groups at the same period of follow-up**

thrombophlebitis. Recurrent myocardial infarction was defined as prolonged chest pain with characteristic electrocardiographic changes and/or increases in creatine kinase to more than two times the upper limit of normal, and/or the presence of positive results of specific myocardial isoform. Cardiovascular death included death occurring during the course of a suspected or confirmed myocardial infarction, sudden death, unwitnessed death, and death occurring during the course of episodes of heart failure. Patients who had cardiac transplant or cardiomyoplasty were classified by the Validation and Classification Committee as cardiac death as in the Pfeffer et al captopril trial.[19] Coronary bypass surgery, angioplasty, and their complications were considered as secondary endpoints.

All endpoints were ascertained at the annual visit. Detailed information on the clinical event and its evolution was obtained from the family and medical and hospital records. Data were reviewed blindly and classified by the Validation and Classification Committee according to predefined criteria.

Fatty acids	Control n=139	Experimental n=141	p
18:0 (stearic)	7 07 (0 07)	6 55 (0 06)	<0 001
Cis 18:1 (n-9) (oleic)	19 54 (0 29)	21 81 (0 32)	<0 001
18:1 Trans	0 22 (0 02)	0 25 (0 02)	0 42
18:2 (n-6) (linoleic)	29 25 (0 48)	27 27 (0 42)	0 002
18:3 (n-3) (linolenic)	0·37 (0 02)	0 62 (0 03)	<0 001
20:4 (n-6) (arachidonic)	6 88 (0 13)	6 38 (0 13)	0 005
20:5 (n-3) (eicosapentaenoic)	0 76 (0 05)	1 03 (0 06)	<0 001

Table 4: **Selected plasma fatty acids after 52 weeks follow-up (mean [SEM])**

Foods	Control n=192	Experimental n=219	p
Bread	145 (7)	167 (6)	0 01
Cereals	99 4 (11)	94 0 (10)	0 22
Legumes	9 9 (3 0)	19 9 (4 3)	0 07
Vegetables	288 (12)	316 (10)	0 07
Fruits	203 (12)	251 (12)	0 007
Delicatessen	13 4 (2 4)	6 4 (1 5)	0 01
Meat	60 4 (5 5)	40 8 (5 0)	0 009
Poultry	52 8 (6 0)	57 8 (5 0)	0 42
Cheese	35 0 (2 6)	32 2 (2 0)	0 25
Butter and cream	16 6 (1 6)	2 8 (0 6)	<0 001
Margarine	5 1 (0 6)	19 0 (1 0)	<0 001
Oil	16 5 (0 9)	15 7 (0 8)	0 65
Fish	39 5 (5 7)	46 5 (5 6)	0 16

Results in g/day. Mean (SEM). Delicatessen = ham, sausage, and offal.

Table 5: **Intake of the main foodstuffs after 1 to 4-years follow-up in the 2 groups**

Controls for bias

Since the study was single blinded, bias could result from a beneficial placebo effect in the group with dietary counselling. A questionnaire designed and analysed by independent psychosociologists (manuscript in preparation) indicates that there was no significant difference between the 2 groups in their perceptions of clinic visits—judgments on the staff; consultation discussions on drug use, suffering, job, family life, anxiety, and physical problems—except for dietary habits.

Analysis

Analyses were done on the intention-to-treat principle. Differences between groups were evaluated by χ^2 tests for categorical variables and unpaired student's t-tests for continuous variables (two-sided tests). In life-table analyses, the date of randomisation was used as the starting point and the date of any event as the endpoint. In combined analyses, the time of the first event was used. Kaplan Meier estimates of the probabilities of survival and Rothman 95% confidence limits were computed to make the survival curves which were compared by log rank test. Cox proportional hazards model was used to estimate the risk ratio of events before and after controlling for prognostic variables.

Results

Patient accrual, eligibility, and follow-up

Enrolment was from March, 1988 to March, 1992. 679 patients were contacted in hospital; 71 refused to participate and 3 were considered ineligible. Shortly after randomisation, 21 (8 in the controls and 13 in the experimental group refused follow-up) (table 1). Refusals were included only in the total mortality analysis. Consequently, the cardiovascular morbidity and detailed mortality analyses were on 584 randomised patients; 289 in the experimental and 295 in the control group. Among these 584 patients, the mean rate of withdrawal from follow-up (did not attend two consecutive appointments) was similar in the experimental (8·0%) and control (7·0%) groups, as was the length of time until withdrawal (mean [standard deviation]) 8 (9) *vs* 7 (9) months. 11 patients in the control and 10 in the experimental group missed only one visit and were not withdrawn. 2 in the experimental group reported margarine-related side-effects: one with colitis and one with diarrhoea.

Survivors free from myocardial infarction were followed for 27·1 (15) months in the control and 26·9 (15) months in the experimental group. At the time of this report the vital status of 3 controls is unknown.

	Week 0		Week 8		Week 52		Week 104	
	Control n=295	Experimental n=289	Control n=280	Experimental n=278	Control n=241	Experimental n=239	Control n=168	Experimental n=171
Weight (kg)	73 7 (0 6)	74 2 (0 7)	74 4 (0 6)	74 4 (0 7)	75 3 (0 7)	75 2 (0 8)	76 0 (0 9)	75 6 (1 1)
Body mass (kg/m²)	25 8 (0 2)	25 8 (0 2)	26 0 (0 2)	25 9 (0 2)	26 5 (0 2)	26 0 (0·2)	26 2 (0 3)	26·1 (0 3)
Systolic BP (mmHg)	120 (1)	119 (1)	122 (1)	120 (1)	125 (1)	124 (1)	129 (1)	126 (1)
Diastolic BP (mmHg)	74 (1)	74 (1)	76 (1)	75 (1)	78 (1)	77 (1)	79 (1)	78 (1)
Cholesterol (mmol/L)	6 47 (0 07)	6 50 (0 08)	6 41 (0·07)	6 30 (0 08)	6 11 (0 07)	6 16 (0 07)	6 16 (0 10)	6 17 (0 09)
Triglycerides (mmol/L)	2 00 (0 07)	2 15 (0 09)	1 96 (0 07)	2 14 (1 00)	1 85 (0 09)	1 99 (0 09)	1 92 (0 13)	1 85 (0 12)
HDL-cholesterol (mmol/L)	1 17 (0 01)	1 16 (0 02)	1 22 (0 02)	1 18 (0 02)	1 28 (0 02)	1 23 (0 02)	1 32 (0 03)	1·28 (0·03)
LDL-cholesterol (mmol/L)	4 54 (0 06)	4 52 (0 07)	4 31 (0 11)	4 32 (0 07)	4 16 (0·07)	4 20 (0 06)	4 11 (0 09)	4 18 (0 08)
Apoprotein B (g/L)	1 49 (0 02)	1 52 (0 02)	1 45 (0 02)	1 47 (0 02)	1 37 (0 02)	1·39 (0 02)	1 37 (0 03)	1 39 (0 03)
Apoproterin A1 (g/L)	1 24 (0 01)	1 24 (0·01)	1 31 (0 02)	1 26 (0 02)	1 37 (0 02)	1 34 (0 02)	1 46 (0 02)	1 34 (0 02)
Lipopreotein a (g/L)	0 30 (0 02)	0 28 (0 02)	0·34 (0 04)	0 29 (0 02)	0 29 (0 03)	0 30 (0 02)	0 27 (0 03)	0 31 (0 03)
Ascorbic acid (mg/L)†	.				3.5 (0 3)	4 3 (0 3)*		
α-tocopherol (μmol/L)†	.		.		26 2 (0 8)	28 9 (0 9)		
Retinol (μmol/L)†	.				2 7 (0 1)	2 9 (0 1)		.

(Mean ± SEM). *p<0 05 vs; †for vitamins only, n=128 (control) and 122 (experimental); BP=blood pressure. †Determined in 128 in control and 122 in experimental groups.

Table 6: **Main cardiovascular risk factors**

	Control (n=303) person-years 594		Experimental (n=302) person-years 606		Cox proportional-hazards model†		
	No events	Rate	No events	Rate	Risk ratio	95% confidence interval	p
Cardiovascular deaths	16 (10)	2 69	3 (0)	0 50	0 24	0 07–0 85	0 02
Non-fatal myocardial infarction	17	2 86	5	0 82	.	.	
Total major primary endpoints	33	5 55	8	1 32	0 27	0 12–0·59	0 001
Non cardiovascular deaths	4	0 67	5	0 82			
Overall mortality	20	3 37	8	1 32	0 30	0.11–0 82	0 02

*Major primary endpoints are mutually exclusive. †In the analyses, the time of first event was used. Adjusted for age, sex, smoking, serum cholesterol, systolic blood pressure, and infarct location. Rates shown are per 100 patient-years of follow-up. Numbers in parentheses correspond to sudden death (8 vs 0), transplantation (1) and cardiomyoplasty (1).

Table 7: **Endpoints in the two groups over 27 months mean follow-up**

Baseline characteristics

Patients were mostly male (table 2). There was no difference in sex ratio, body mass index, blood pressure, or other features between randomised patients. Characteristics of patients who withdrew were also similar to those of the whole group—there is no major selection bias in this study.

Dietary intervention

At randomisation, the diet of the experimental group (table 3) was assumed to be that of controls, ie, close to the prudent diet of the American Heart Association (total lipids, 31% energy; saturated fats, 10·5%; polyunsaturated/saturated ratio, 0·78). Eight weeks later, the experimental group had decreased their intake of saturated fat, cholesterol, and linoleic acid while increasing that of oleic and alpha-linolenic acid.

Differences in dietary habits between groups after 1 to 4 years (table 3) were similar to those between week 0 and weeks 8 to 104 in the experimental group, confirmed by the fatty-acid analysis of plasma lipids (table 4). After 52 weeks, there were higher concentrations of oleic, alpha-linolenic, and eicosapentaenoic acids and reduced concentrations of stearic, linoleic, and arachidonic acids in the experimental group (table 4). The increase in eicosapentaenoic acid was probably related to alpha linolenic acid since intake of fish was not significantly increased (table 5). In terms of foodstuffs (table 5), the experimental group had a significantly higher intake of bread, fruit, and margarine; and a lower intake of butter, cream, meat, and delicatessen such as ham, sausage, and offal.

Cardiovascular disease risk factors

At 8, 52, and 104 weeks cholesterol, triglycerides, lipoproteins, apoproteins and lipoprotein a, weight, and blood pressure were similar in both groups (table 6). Nevertheless, the trend with time was a decrease in total and low density lipoprotein (LDL) cholesterol and an increase of high density lipoprotein (HDL) cholesterol and apoprotein A_1. The number of smokers was slightly higher in the experimental group (19 *vs* 15%).

Antioxidant vitamins, determined only in the last 120 patients in each group at 52 weeks (table 6) were

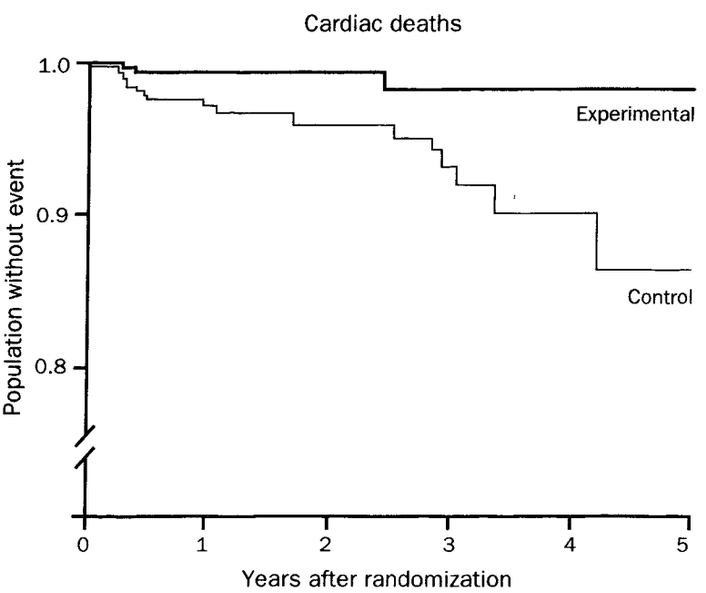

Figure 1: **Survival curves for cardiac death, including 1 heart transplant and 1 cardiomyoplasty**

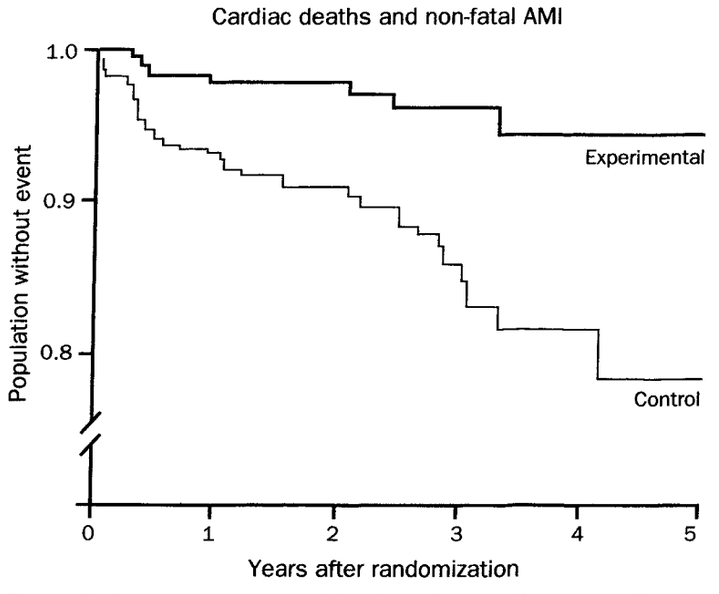

Figure 2: **Survival curves combined cardiac death and non-fatal acute myocardial infarction (AMI)**
Log rank test, using only the time of the first event.

significantly higher in the experimental group. At 8 weeks, the concentration of albumin in plasma was higher (47·5 [0·3] g/L *vs* 46·7 [0·3], $p < 0·05$) and the granulocyte count lower (3·58 [0·09] $\times 10^9$/L *vs* 3·80 [0·10], $p < 0·05$) in the experimental group. Probably due to aspirin being given to the majority of patients, no difference between the groups in platelet aggregation was observed.

Mortality

There were 20 deaths (8 sudden deaths) in the control and 8 (0 sudden death) in the experimental group (table 7), 1 cardiac transplant and 1 cardiomyoplasty in controls being classified as cardiac deaths. The probability of survival by the product-limit method (Kaplan Meier) was 0·82 in the control and 0·955 in the experimental group ($p = 0·02$), confirmed by Cox model after adjustment for prognostic variables (age, sex, smoking, cholesterol, blood pressure, and infarct location) the risk ratio being 0·30 ($p = 0·02$) (table 7).

16 deaths in the control and 3 in the experimental group were due to cardiac causes. The probability of absence of cardiac death was 0·865 in the control and 0·98 in the experimental group.

The risk ratio of cardiac death was 0·19 (95% CI 0·06–0·65, $p < 0·002$), a reduction of 81%. After adjustment for prognostic variables, the risk ratio was 0·24 (0·07–0·85, $p = 0·02$), a reduction of 76% (table 7) and (97% CI 0·15–0·91) when taking into account the early stopping rule. Survival curves are shown in figure 1 (log rank test, $p < 0·003$).

Primary endpoints

There were 33 events in the control and 8 in the experimental group (table 7). The probability of absence of these events was 0·787 and 0·947 respectively (log rank test, $p < 0·001$, figure 2). With the proportional-hazards model, the risk ratio was 0·24 (0·11–0·55, $p < 0·001$), 0·27 (0·12–0·59, $p = 0·001$), after adjustment (table 7) (97% CI 0·11–0·65).

Discussion

In this study, the survivors of a first myocardial infarction, assigned to a Mediterranean alpha-linolenic acid-rich diet, had a markedly reduced rate of recurrence, other cardiac events and overall mortality.

Except for the DART trial[6], with an increased intake of fish, previous dietary secondary prevention trials were unsuccessful.[2-4] Even in primary prevention, the only trial successful in preventing coronary death was the Hjermann trial.[20] Owing to a small polyunsaturated to saturated fat ratio of 0·7:1·0,[1] serum cholesterol was reduced only by 13% compared to 3% in the control group, a mean net difference of 10% in that trial. The recommendations were to decrease saturated fat without increasing polyunsaturated fat and to eat more vegetables, fish, and fruit in addition to smoking reduction. In the present trial, a reduction in coronary events and cardiac deaths of close to 70% was achieved without a reduction of serum cholesterol, triglycerides, or an increase in HDL compared to controls. Compared with the DART trial[6] the protective effect we observed was associated with dietary supply of the n-3 long-chain fatty acids precursor (alpha-linolenic acid) instead of eicosapentaenoic acid and extended to non-fatal myocardial infarction.

The margarine used contained approximately 5% alpha-linolenic acid. Recent studies indicate that the subjects of the Crete cohort from the Seven Country Study had 3-fold higher concentrations of alpha-linolenic acid compared to the Zutphen (Netherlands) cohort and 21% lower linoleic acid.[14] In the present study, the concentration of alpha-linolenic acid was increased by 68% in the experimental group, and that of linoleic acid reduced by 7%. Another interesting comparison is with the Japanese population of Kohama Island[26] who have the lowest incidence of cardiovascular diseases in Japan, probably in the World. In Kohama, the concentration of oleic acid was 21·5% *vs* 21·8% in the experimental group in Lyon, linoleic acid, 26·9% *vs* 27·3%; alpha-linolenic 1·1 *vs* 1·0%; eicasapentaenoic acid 1·4 *vs* 1·1%; arachidonic acid, 6·5 *vs* 6·4%—a very similar profile.

The two populations with the lowest coronary heart disease mortality in the world have a high intake of alpha-linolenic acid; the Japanese in the form of canola and soybean oils, the Cretans, possibly through the consumption of purslane[22] and walnuts. Whether it is alpha-linolenic acid that plays a protective part cannot be determined by this study; it is one of the most striking differences between the experimental and control groups and already present at 8 weeks. Also, whether alpha-linolenic acid acts by competing with arachidonic acid for prostaglandin E_2 synthesis[23] or as the precursor of eicosapentaenoic acid is not known; this last hypothesis, however, concurrs with the results of Burr,[6] showing that a small increase in intake of fish decreased mortality within a few months, possibly through the prevention of ventricular fibrillation during acute myocardial ischaemia.[29]

The increase in intake of oleic acid, less susceptible to peroxidation,[10] and in natural antioxidants, probably also play a protective part. At one year, plasma vitamins E and C were higher in the experimental groups, although only the intake of vitamin C was increased. This could be due to the known protective effect of vitamin C on vitamin E, or to reduced peroxidation due to additional natural antioxidants from fruits, vegetables, legumes, wine, and to a lower intake of linoleic acid, easily oxidized in low density lipoproteins.[10] The results of the present study seem consistent with recent observations of a lower risk of coronary heart disease with a high intake of vitamin E[25] and higher concentrations of β-carotene in adipose tissue.[26] Whether or not red wine was

protective as has been suggested[27], could not be evaluated here since there were very few events in the experimental group, and the controls consumed approximately the same amount of wine.

That protection started quickly is shown by the survival curves (figure 1 and 2). In contrast to cholesterol-lowering drugs such as cholestyramine[28] requiring 3 to 4 years to show a protective effect, the rapid protection induced by diet agrees with the DART, and Hjermann[20] trials (a similar 50% reduction after 1 or 7 years), and a recent study[29] showing that a low-fat vegetarian diet containing nuts (some rich in alpha-linolenic acid) reduced the rate of coronary events within six weeks.

The rapid protective effect and similarity of serum lipids in our 2 groups suggest that the protective effect of the experimental diet could be through thrombogenesis since the incidence of myocardial infarction was markedly reduced. The fact that no sudden death occurred in the experimental group against 8 in the control group, suggests a possible additional antiarrhthmic effect, consistent with observations in man,[6,24] and animals[30] indicating that n-3 fatty acids, especially alpha-linolenic acid, markedly reduced the incidence of lethal arrythmias.

The heads of Department or Services who participated in this study in addition to Prof J Delaye and Prof P Touboul were: **Cardiology** (Prof J Beaune MD, Prof J P Delahaye MD, Prof A Froment MD, Prof J Normand MD), **Hematology** (Prof M Dechavanne MD), **and Biochemistry** (Prof P Louisot MD), **Hôpital Cardiovasculaire, Lyon, France.**

We thank Dr P Ducimetière (INSERM, Unit 258, Paris), Dr F Epstein (Institute for Social and Preventive Medicine, Zurich, Switzerland), Dr A Nordoy (Department of Clinical Medicine, Tromso, Norway), Dr W Rutishauser (Geneva University Hospital, Geneva, Switzerland) who were members of the Scientific Committee: Dr F Paillard (Cardiologist, University Hospital, Rennes, France) and Dr A Righetti (Cardiologist, Geneva University Hospital, Geneva, Switzerland), the members of the Validation Committee, and to Mr F Jacquard for computer programs. The study was supported by grants from INSERM (Réseau Clinique), Ministry of Research (Aliments 2000 and 2002), CNAMTS, CETIOM, and ONIDOL, Astra-Calvé BSN, and the Fondation pour la Recherche Médicale.

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