Effect of an increased intake of α-linolenic acid and group nutritional education on cardiovascular risk factors: the Mediterranean Alpha-linolenic Enriched Groningen Dietary Intervention (MARGARIN) study[1–3]

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ABSTRACT

Background: The effect of long-term increased intakes of α-linolenic acid (ALA; 18:3n−3) on cardiovascular risk factors is unknown.

Objectives: Our objectives were to assess the effect of increased ALA intakes on cardiovascular risk factors and the estimated risk of ischemic heart disease (IHD) at 2 y and the effect of nutritional education on dietary habits.

Design: Subjects with multiple cardiovascular risk factors (124 men and 158 women) were randomly assigned in a double-blind fashion to consume a margarine rich in either ALA [46% linoleic acid (LA; 18:2n−6) and 15% ALA; *n* = 114] or LA (58% LA and 0.3% ALA; *n* = 168). An intervention group (*n* = 110; 50% ALA) obtained group nutritional education, and a control group (*n* = 172; 34% ALA) received a posted leaflet containing the standard Dutch dietary guidelines.

Results: Average ALA intakes were 6.3 and 1.0 g/d in the ALA and LA groups, respectively. After 2 y, the ALA group had a higher ratio of total to HDL cholesterol (+0.34; 95% CI: 0.12, 0.56), lower HDL cholesterol (−0.05 mmol/L; −0.10, 0), higher serum triacylglycerol (+0.24 mmol/L; 0.02, 0.46), and lower plasma fibrinogen (−0.18 g/L; −0.31, −0.04; after 1 y) than did the LA group (adjusted for baseline values, sex, and lipid-lowering drugs). No significant difference existed in 10-y estimated IHD risk. After 2 y, the intervention group had lower saturated fat intakes and higher fish intakes than did the control group.

Conclusions: Increased ALA intakes decrease the estimated IHD risk to an extent similar to that found with increased LA intakes. Group nutritional education can effectively increase fish intake. *Am J Clin Nutr* 2002;75:221–7.

KEY WORDS α-Linolenic acid, linoleic acid, prevention, ischemic heart disease, nutritional education, cardiovascular risk factors, Netherlands, MARGARIN study

INTRODUCTION

Hypercholesterolemia, hypertension, and overweight are risk factors for ischemic heart disease (IHD) that are modifiable by dietary habits. The standard dietary advice for hypercholesterolemic persons is a decrease in intakes of total fat and replacement of saturated fatty acids with polyunsaturated fatty acids, predominantly linoleic acid (LA; 18:2n−6), or carbohydrates (1). However, an increased intake of LA may increase platelet aggregation (2), which is associated with an elevated risk of IHD (3). It can be questioned whether partial replacement of LA with other polyunsaturated fatty acids may be more useful.

In 1994, the Lyon Diet Heart Study (LDHS) showed that a Mediterranean diet rich in α-linolenic acid (ALA; 18:3n−3) prevented the recurrence of cardiovascular events more than did the usual postinfarct prudent diet in the secondary prevention of IHD (4, 5). The traditional Mediterranean diet contains more legumes, fruit, fish, ALA, and oleic acid and contains less meat and LA (6). This type of diet was associated with a low IHD mortality rate in Crete in the 1960s (7). Cretans had a 3-fold higher concentration of plasma ALA and a 21% lower concentration of LA than did the Zutphen cohort (Netherlands) (8). In the Nurses' Health Study the baseline intake of ALA was inversely associated with a risk of fatal IHD (9).

The cardioprotective mechanisms of ALA may include the prevention of ventricular fibrillation (10) or a decreased platelet response to aggregation (11). Furthermore, ALA is the metabolic precursor of the long-chain n−3 fatty acid eicosapentaenoic acid (20:5n−3). Consumption of fish that is rich in eicosapentaenoic acid or supplementation with long-chain n−3 fatty acids decreases IHD mortality independently of changes in serum total cholesterol (12, 13). Hence, the cardioprotective effects of ALA are not due primarily to an influence on the traditional risk factors. However, for consumption of ALA to be advised in the primary prevention of IHD, the effects of ALA should be similar to those of a standard

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TABLE 1
Study design and number of participants[1]

	NEC group		NEI group	
Educational subgroup	LA (n = 105)[2]	ALA (n = 58)	LA (n = 52)	ALA (n = 51)
Group education about a Mediterranean-type diet	−	−	+	+
Posted leaflet	+	+	−	−

[1] LA, linoleic acid–rich margarine; ALA, α-linolenic acid–rich margarine; NEC, nutritional education control; NEI, nutritional education intervention.
[2] The NEC + LA subgroup had a weighing factor of 2 during the randomization procedure.

LA-rich diet. The effects of long-term increased intakes of ALA on IHD risk factors are not well known (14). In subjects with multiple cardiovascular risk factors, we investigated the effects of an LA-rich margarine and an ALA-rich margarine on 10-y estimated IHD risk, serum lipids, blood pressure, plasma fibrinogen, and the von Willebrand factor after 2 y. Furthermore, we investigated the effect of group nutritional education on dietary habits.

SUBJECTS AND METHODS

Experimental design

The MARGARIN (Mediterranean Alpha-linolenic enRiched Groningen dietARy INtervention) study is a prospective prevention project for persons with moderate hypercholesterolemia and ≥2 other cardiovascular risk factors (4). The study design was inspired by the LDHS, in which the intervention group consumed an ALA-rich margarine and received nutritional education. Contrary to the LDHS, the MARGARIN study investigates separately the effects of increased ALA intake and of nutritional education. Subjects in the intervention municipality Winschoten [nutritional education intervention (NEI) group; n = 110] received nutritional education about a Mediterranean-type diet, and subjects in the control region consisting of neighboring municipalities [nutritional education control (NEC) group; n = 172] received a posted leaflet containing the standard Dutch dietary guidelines. Because we wanted to minimize the crossover of information, this part of the study was not randomized on an individual level. The study area is culturally and socioeconomically homogeneous. Participants in the NEI and NEC groups were randomly assigned to consume either an ALA-rich margarine (NEI+ALA and NEC+ALA subgroups) or an LA-rich margarine (NEI+LA and NEC+LA subgroups) in accordance with a predefined schedule in a double-blind fashion (**Table 1**). The schedule was generated by computer with the use of a random number generator. An independent Trial Coordination Center allocated the margarines and organized the masked distribution during the study. The 2 margarines were identical with respect to taste and packaging. The amount of margarine prescribed was based on the participants' usual consumption of added fat, and the margarine was supplied by household. The baseline examination was conducted in November 1997, and follow-up examinations were conducted after 16, 52, and 104 wk.

Subjects

Eligible subjects were 30–70 y old, had a serum total cholesterol concentration between 6 and 8 mmol/L (mean of 2 separate measurements), and had ≥2 of the following cardiovascular risk factors: high blood pressure (diastolic pressure ≥95 mm Hg, systolic pressure ≥160 mm Hg, or both) or use of antihypertensive medication, body mass index (in kg/m^2) ≥27, smoking, history of cardiovascular disease or a family history of early onset of cardiovascular disease. The exclusion criteria were diabetes mellitus, hypothyroidism, and use of acetylsalicylic acid, anticoagulants, or cholesterol-lowering drugs.

The recruitment procedures were described previously (15). In short, all inhabitants in the study region who were >30 y old were invited for a blood pressure screening program, the registration systems of pharmacies were screened for patients taking antihypertensive medications, and a local radio program invited potential participants to contact the research team.

The medical ethical committee of the Groningen University Hospital approved the study protocol. Written, informed consent was obtained from 282 persons, after which the margarines were randomly allocated. Before the baseline examination, 16 persons dropped out (n = 7 in the NEI group and 9 in the NEC group), and 1 person missed the study entirely. After 2 y, 93% of the NEI group and 91% of the NEC group were still involved in the study.

Nutritional education program

In February and March 1998, subjects in the NEI group were invited by the research dietitian to attend three 2-h nutritional education meetings, which were held in groups of 10 subjects. The detailed content of the education program was described previously (16). The nutritional guidelines called for daily consumption of 5–7 slices of bread, 400 g vegetables, 2 pieces of fruit, 1–2 alcoholic beverages (only for those who regularly consumed alcohol), 2–3 lean dairy products, 2 servings of fish/wk at dinner, less red meat and more poultry, less fatty cheese and eggs, and more ALA-rich foods (9). Participants were encouraged to use the project margarines for baking and on bread. In February 1998, the NEC group received a posted leaflet containing the standard Dutch dietary guidelines, which differed from the Mediterranean guidelines in their advice on the intake of vegetables (200 g/d); the leaflet also contained no specific guidelines on the consumption of fish, alcohol, or ALA-rich foods.

Composition of the margarines

The ALA-rich margarine contained 80% fat, of which 21% was from saturated fatty acids (SFAs), 18% from monounsaturated fatty acids, 46% from LA, and 15% from ALA. The LA-rich margarine also contained 80% fat, of which 23% was from SFAs, 18% from monounsaturated fatty acids, 58% from LA, and 0.3% from ALA. Both margarines contained 0.66 mg vitamin E/g, 9 μg vitamin A/g, and 0.023 μg vitamin D/g.

Measurements

The 10-y estimated risk of developing IHD was calculated with the use of the Framingham risk function. The following variables were included: age (at all measurements the age at baseline was included to adjust for increased age during the study period), sex, serum total and HDL cholesterol, systolic blood pressure, current smoking, and left ventricular hypertrophy (17). A standardized questionnaire was used to take an inventory of the mortality and morbid-

TABLE 2
Baseline characteristics of the participants[1]

Variable	NEC group		NEI group		P^2
	LA (n = 105)	ALA (n = 58)	LA (n = 52)	ALA (n = 51)	
Age (y)	53.3 ± 9.7[3]	54.1 ± 9.2	55.2 ± 9.9	54.8 ± 10.0	NS
Men (%)	49[a]	48[a,b]	39[b,c]	35[c]	<0.01
Family history of CVD (%)[4]	44	40	47	37	NS
Current smoker (%)	51	51	46	47	NS
Use of antihypertensive medication (%)	42[a]	44[a,b]	52[b,c]	63[c]	<0.01
Estimated 10-y IHD risk (%)[5]					
Men	20.4 ± 10.2	19.9 ± 9.7	19.9 ± 8.4	19.4 ± 9.3	NS
Women	10.7 ± 6.0	11.8 ± 7.3	11.6 ± 6.6	13.2 ± 7.9	NS

[1] LA, linoleic acid–rich margarine; ALA, α-linolenic acid–rich margarine; CVD, cardiovascular disease; IHD, ischemic heart disease; NEC, nutritional education control; NEI, nutritional education intervention. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (one-way ANOVA with multiple comparisons with Bonferroni adjustment or chi-square test).
[2] Reflects the difference between the NEC and NEI groups (chi-square test).
[3] $\bar{x}$ ± SD.
[4] First-degree relative with a diagnosis of cardiovascular disease before 60 y of age.
[5] Calculation based on systolic blood pressure (17).

ity outcomes for the participants. Using information from patient records, 2 independent physicians, a cardiologist and a general practitioner, validated and classified the results in a blinded fashion.

A standardized questionnaire was used to establish smoking habits and the presence of a family history of cardiovascular disease. The subjects' body weight was measured while they were not wearing shoes or heavy clothing. The subjects' blood pressure was measured with the use of Dinamap (Critikon Inc, Tampa, FL) after the subjects had rested and while they were in a sitting position. The subjects' dietary intake was assessed by a self-administered, semiquantitative food-frequency questionnaire (15). The intakes of energy, macronutrients, and dietary fiber were validated by using a 3-d 24-h recall method in a pilot study within the study population (n = 43). Pearson's correlation coefficients were as follows ($P < 0.01$): energy intake, 0.76; total fat as a percentage of energy, 0.66; SFAs, 0.51; monounsaturated fatty acids, 0.52; polyunsaturated fatty acids, 0.70; alcohol, 0.72; and fiber (in g/d), 0.74.

Analytic methods

In fasting samples, serum concentrations of total and HDL cholesterol and triacylglycerol were measured with the use of enzymatic methods on a Vitros 950 (Ortho-Clinical Diagnostics, Rochester, NY); blanks were not run. HDL cholesterol was isolated by precipitation of LDL and VLDL with dextran sulfate and magnesium chloride (CV: 2.8–3.5%; interassay CV: 3.3–3.6%). Serum LDL cholesterol was calculated by using the Friedewald formula and excluding persons with serum triacylglycerol concentrations >5.0 mmol/L. The fatty acid composition of the cholesteryl ester (CE) was determined as described previously (18). Plasma fibrinogen was measured against a World Health Organization standard with the use of a Clauss assay on an STA coagulation analyzer. For a sample containing 2.8 g/L, the CV was 3.6%. Von Willebrand factor antigen was determined by enzyme-linked immunosorbent assay techniques with the use of commercially available antibodies (DAKO A/S, Glostrup, Denmark) and calibrated with pooled normal plasma (n = 65 healthy volunteers aged 19–55 y) containing 100% von Willebrand factor antigen by definition (intraassay CV: 3.0–4.5%; interassay CV: 3.0–6.5%).

Sample size

The main objective of the study was to investigate the effect of increased ALA intake, and the power analysis was based on the plasma ALA concentration. With a control group of 100 persons and intervention groups of 50 persons each, a difference of 0.10 mol% would be significant (5% two-sided type 1 error). The LDHS showed a difference of 0.25 mol% (4). If our study were equally effective, it would have a power >90%.

Statistics

Comparisons between the subgroups at baseline were analyzed for significant differences ($P < 0.05$) by using one-way analysis of variance and multiple comparisons with Bonferroni correction for normally distributed variables and the Mann-Whitney U test for variables not normally distributed, and the chi-square test was used for categorical variables. Differences in changes of dietary intake between the NEI and NEC groups were investigated separately for men and women by using a one-factor analysis of variance with the presence or absence of education as the main effect and the baseline intake as covariate. Analysis of covariance was used to analyze the differences between the treatments in changes of the fatty acid composition of the CE and in changes in values of IHD risk factors. We used two-factor analysis of variance in which margarine type and the presence or abscence of nutritional education were the 2 factors (main effects), and sex, baseline value, and use of lipid-lowering drugs (only in the case of estimated risk of IHD and serum lipids as the dependent variables) were the covariates. In addition, the interaction between the margarine type and the presence or absence of nutritional education was investigated. The differences between the individual means were compared with the use of Bonferroni adjustments. We used the statistical package SPSS 8.0 (SPSS Inc, Chicago) to conduct analyses on an intention-to-treat basis.

RESULTS

Baseline characteristics

At baseline, 265 persons with a mean (±SD) age of 55 ± 10 y were investigated. The mean (±SD) 10-y estimated IHD risk was 20.0 ± 9.5% for men and 11.7 ± 6.9% for women. As shown in **Table 2**, the NEI group consisted of fewer men and more persons who took antihypertensive medication than did the NEC group. No significant differences were observed in the other IHD risk factors.

TABLE 3
Changes in dietary intakes of nutrients and foods during 104 wk of follow-up, stratified by sex and adjusted for baseline intake[1]

	Men			Women		
		Changes[3]			Changes[3]	
	Baseline (n = 117)[2]	NEC group (n = 60)	NEI group (n = 31)	Baseline (n = 144)[2]	NEC group (n = 70)	NEI group (n = 50)
Energy (MJ/d)	12.0 ± 3.5	−0.4 ± 0.3	−1.2 ± 0.4	8.8 ± 2.5	−0.3 ± 0.2	−0.1 ± 0.2
Total fat (% of energy)	38.8 ± 5.9	2.0 ± 0.6	−0.6 ± 0.9[4]	37.4 ± 7.5	0.6 ± 0.6	−0.9 ± 0.7
SFA (% of energy)	14.3 ± 3.2	−0.5 ± 0.3	−1.8 ± 0.4[4]	14.1 ± 3.3	−1.2 ± 0.2	−1.8 ± 0.3
MUFA (% of energy)	13.0 ± 2.5	−0.4 ± 0.3	−1.2 ± 0.4	12.3 ± 2.8	−0.7 ± 0.2	−1.4 ± 0.3
PUFA (% of energy)	8.9 ± 2.4	3.6 ± 0.4	3.0 ± 0.5	8.4 ± 3.0	3.2 ± 0.3	2.9 ± 0.4
ALA (% of energy)						
ALA group	0.5 ± 0.2	2.0 ± 0.2	1.8 ± 0.2	0.5 ± 0.2	1.6 ± 0.2	1.8 ± 0.2
LA group	0.5 ± 0.2	−0.1 ± 0	−0.1 ± 0	0.5 ± 0.2	−0.1 ± 0	−0.1 ± 0
LA (% of energy)						
ALA group	7.9 ± 2.0	2.7 ± 0.5	1.9 ± 0.6	7.7 ± 3.0	1.8 ± 0.5	1.4 ± 0.5
LA group	7.9 ± 2.7	3.2 ± 0.4	2.5 ± 0.7	7.3 ± 3.0	3.1 ± 0.4	2.5 ± 0.5
Dietary fiber (g/d)	30.0 ± 8.6	−1.9 ± 0.9	−0.8 ± 1.3	24.8 ± 6.7	−0.4 ± 0.6	1.2 ± 0.8
Bread (g/d)	190 ± 87	−11 ± 8	5 ± 11	132 ± 44	−6 ± 4	6 ± 5
Vegetables (g/d)	150 ± 78	−10 ± 7	−9 ± 10	136 ± 52	8 ± 6	15 ± 7
Fruit (g/d)	246 ± 178	−3 ± 21	15 ± 30	284 ± 171	−5 ± 18	37 ± 21
Meat (g/d)	103 ± 42	−3 ± 6	−2 ± 9	88 ± 47	−9 ± 3	−19 ± 4
Fish (g/d)	27 ± 38	0 ± 3	8 ± 5	19 ± 21	0 ± 3	20 ± 4[4]
Margarine (g/d)	26 ± 26	32 ± 4	21 ± 5	19 ± 20	17 ± 2	16 ± 2
Total added fat (g/d)	62 ± 28	0 ± 5	−10 ± 7	43 ± 24	−2 ± 3	−5 ± 3

[1] NEC, nutritional education control; NEI, nutritional education intervention; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; ALA, α-linolenic acid.
[2] $\bar{x}$ ± SD.
[3] $\bar{x}$ ± SEM.
[4] Significantly different from the NEC group, $P < 0.05$ (one-factor ANOVA adjusted for baseline).

Dietary intake and fatty acid composition of the CE

At baseline, the men in the NEC group had a higher mean (±SD) intake of fish (32 ± 43 g/d) than did the men in the NEI group (15 ± 15 g/d; $P < 0.05$). No significant differences were observed in the intakes of the other nutrients and foods. During the study, all groups decreased their SFA intake and increased their LA intake. In the NEI group, the men increased their consumption of fish and the women increased their intake of fish and vegetables and decreased their intake of meat (**Table 3**). After adjustment for sex and baseline intake, the NEI group had a lower intake of total fat (net difference: −2.0% of energy; 95% CI: −3.4, −0.6) and SFAs (net difference: −0.9% of energy; 95% CI: −1.4, −0.3) than did the NEC group after 2 y. In subjects who consumed the ALA-rich margarine, dietary intakes of ALA and LA were 2.3% of energy (6.3 g/d) and 9.5% of energy (26.3 g/d), compared with 0.4% of energy (1.0 g/d) and 10.6% of energy (26.8 g/d), respectively, in subjects who consumed the LA-rich margarine. The ratios of LA to ALA were 4.7 with the ALA-rich margarine and 29.1 with the LA-rich margarine ($P < 0.01$).

Consumption of the ALA-rich margarine for 1 y increased the content of ALA and eicosapentaenoic acid in the CE and decreased

TABLE 4
Changes in the fatty acid composition of the serum cholesteryl ester during 52 wk of follow-up after adjustment for sex and baseline concentrations of fatty acids[1]

		NEC group[3]		NEI group[3]		P (main effects and interaction)		
Fatty acid	Baseline (n = 265)[2]	LA (n = 100)	ALA (n = 54)	LA (n = 48)	ALA (n = 49)	NEI − NEC	ALA − LA	Interaction
14:0 (mol%)	0.94 ± 0.29	−0.16 ± 0.03	−0.24 ± 0.04	−0.21 ± 0.04	−0.19 ± 0.04	NS	NS	NS
16:0 (mol%)	12.54 ± 1.18	−0.29 ± 0.17	−0.14 ± 0.23	0.21 ± 0.24	0.45 ± 0.24	0.016	NS	NS
16:1n−7 (mol%)	3.53 ± 1.52	−1.08 ± 0.08	−1.10 ± 0.11	−0.95 ± 0.12	−0.77 ± 0.12	0.043	NS	NS
18:0 (mol%)	1.64 ± 0.53	0.53 ± 0.12	0.48 ± 0.17	0.66 ± 0.18	0.50 ± 0.18	NS	NS	NS
cis 18:1n−9 (mol%)	15.23 ± 2.23	−0.96 ± 0.30	−1.06 ± 0.40	−0.20 ± 0.43	−1.01 ± 0.42	NS	NS	NS
18:2n−6 (mol%)	54.41 ± 4.61	1.03 ± 0.43	0.46 ± 0.59	−0.74 ± 0.63	−0.24 ± 0.62	0.033	NS	NS
18:3n−3 (mol%)	0.52 ± 0.14	−0.11 ± 0.03	0.36 ± 0.04	−0.06 ± 0.04	0.47 ± 0.04	0.021	<0.01	NS
20:4n−6 (mol%)	6.55 ± 1.40	0.98 ± 0.13	0.54 ± 0.18	0.70 ± 0.19	0.16 ± 0.19	NS	<0.01	NS
20:5n−3 (mol%)	1.02 ± 0.73	−0.17 ± 0.06	0.35 ± 0.08	0.14 ± 0.09	0.35 ± 0.09	NS	<0.01	NS
22:6n−3 (mol%)	0.58 ± 0.23	0.20 ± 0.03[b]	0.25 ± 0.03[a,b]	0.33 ± 0.04[a]	0.24 ± 0.04[a,b]	NS	NS	0.036
n−6:n−3	34.27 ± 12.91	2.41 ± 1.01[c]	−11.39 ± 1.37[b]	−5.70 ± 1.46[a]	−11.90 ± 1.44[b]	<0.01	<0.01	<0.01
18:2n−6:18:3n−3	112.60 ± 33.44	52.47 ± 6.31	−41.56 ± 8.57	32.41 ± 9.11	−50.79 ± 9.00	NS	<0.01	NS

[1] LA, linoleic acid–rich margarine; ALA, α-linolenic acid–rich margarine; NEC, nutritional education control; NEI, nutritional education intervention. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni adjustments).
[2] $\bar{x}$ ± SD.
[3] $\bar{x}$ ± SEM.

TABLE 5
Changes in cardiovascular risk factors during 104 wk of follow-up[1]

Risk factor	Baseline (n = 265)[2]	NEC group[3]		NEI group[3]		P (main effect of ALA compared with LA)[4]
		LA (n = 93)	ALA (n = 49)	LA (n = 48)	ALA (n = 47)	
Estimated IHD risk (%)[5,6]	15.4 ± 9.1	−2.6 ± 0.3	−2.1 ± 0.5	−2.4 ± 0.5	−2.1 ± 0.5	NS
Serum total cholesterol (mmol/L)[6]	6.70 ± 0.74	−0.39 ± 0.07	−0.25 ± 0.10	−0.38 ± 0.10	−0.31 ± 0.10	NS
HDL cholesterol (mmol/L)[6]	1.27 ± 0.36	0.13 ± 0.02	0.09 ± 0.03	0.15 ± 0.03	0.10 ± 0.03	0.04
LDL cholesterol (mmol/L)[6]	4.57 ± 0.72	−0.53 ± 0.07	−0.39 ± 0.09	−0.53 ± 0.09	−0.50 ± 0.10	NS
Total cholesterol:HDL cholesterol[6]	5.66 ± 1.48	−0.89 ± 0.08	−0.50 ± 0.12	−0.86 ± 0.12	−0.57 ± 0.12	<0.01
Serum triacylglycerol (mmol/L)[6]	1.98 ± 1.04	0.05 ± 0.09	0.18 ± 0.12	−0.03 ± 0.12	0.33 ± 0.12	0.03
Systolic blood pressure (mm Hg)[7]	145 ± 22	−0.2 ± 1.5	−2.9 ± 2.1	−0.1 ± 2.1	1.1 ± 2.1	NS
Diastolic blood pressure (mm Hg)[7]	87 ± 15	1.9 ± 1.2	0.7 ± 1.7	2.7 ± 1.7	4.3 ± 1.7	NS
Fibrinogen (g/L)[7,8]	3.46 ± 0.63	0.46 ± 0.05	0.32 ± 0.07	0.53 ± 0.08	0.32 ± 0.08	0.01
von Willebrand factor (%)[7,8]	145.1 ± 52.6	0.3 ± 3.2	−2.1 ± 4.3	−7.2 ± 4.6	0.7 ± 4.6	NS
BMI (kg/m^2)[7]	29.8 ± 4.5	0.5 ± 0.1	0.2 ± 0.2	0.7 ± 0.2	0.5 ± 0.2	NS

[1] LA, linoleic acid–rich margarine; ALA, α-linolenic acid–rich margarine; IHD, ischemic heart disease; NEC, nutritional education control; NEI, nutritional education intervention.
[2] $\bar{x}$ ± SD.
[3] $\bar{x}$ ± SEM.
[4] There was no significant main effect of nutritional education and no significant interaction between margarine type and nutritional education.
[5] According to the Framingham 10-y risk calculation for ischemic heart disease based on systolic blood pressure and adjusted for increased age during the study (17).
[6] Adjusted for baseline values, sex, and use of lipid-lowering medication.
[7] Adjusted for baseline values and sex.
[6] Data from 1 y of follow-up.

the ratios of LA to ALA and of n−6 to n−3 fatty acids (**Table 4**). The NEI+LA subgroup had a significantly higher docosahexaenoic acid (22:6n−3) content in the CE and a significantly lower ratio of n−6 to n−3 fatty acids than did the NEC+LA subgroup; this result was explained by the increased consumption of fish by the NEI+LA subgroup. These variables were not significantly different between the NEI+ALA and NEC+ALA subgroups.

Estimated risk, cardiovascular risk factors, and cardiac events

After adjustment for sex, baseline estimated risk, and use of lipid-lowering drugs, the effect on the 10-y estimated IHD risk after 2 y of follow-up was not significantly different between the NEI and NEC groups or between the LA and ALA subgroups (**Table 5**); the mean (±SEM) 10-y estimated IHD risk decreased 2.1 ± 0.3% and 2.5 ± 0.3% in the ALA and LA subgroups, respectively (data not shown). After adjustment for sex, baseline estimated risk, and use of lipid-lowering drugs, consumption of the ALA-rich margarine resulted in a higher ratio of total to HDL cholesterol (net difference: 0.34; 95% CI: 0.12, 0.56), a lower HDL-cholesterol concentration (net difference: −0.05 mmol/L; 95% CI: −0.10, 0), a higher serum triacylglycerol concentration (net difference: 0.24 mmol/L; 95% CI: 0.02, 0.46), and a lower plasma fibrinogen concentration (net difference: −0.18 g/L; 95% CI: −0.31, −0.04; based on 1 y of follow-up) than did consumption of the LA-rich margarine after 2 y (Table 5). During the 2-y study period, there were 2 cardiovascular deaths and 4 cases of nonfatal myocardial infarction (**Table 6**). There tended to be a higher proportion of cardiac deaths and myocardial infarction in the LA subgroups, but the differences between the subgroups were not significant because of the small numbers.

DISCUSSION

The design of the MARGARIN study was inspired by the LDHS. This secondary prevention trial showed a 70% reduction in cardiac events in the intervention group, who obtained nutritional

TABLE 6
Number of cardiovascular events and deaths and use of medication during 104 wk of follow-up[1]

	NEC group		NEI group	
	LA (n = 105)	ALA (n = 58)	LA (n = 52)	ALA (n = 51)
Cardiovascular death	—	1	1	—
Nonfatal myocardial infarction	1	—	3	—
Stroke	1	—	1	—
PTCA or revascularization	2	—	—	1
Noncardiovascular death	—	1	—	1
Use of medication (%)[2]				
Lipid-lowering	7	6	19	6
Acetylsalicylic acid	9	2	8	10
Antihypertensive	47	46	53	67

[1] LA, linoleic acid–rich margarine; ALA, α-linolenic acid–rich margarine; PTCA, percutaneous transluminal coronary angioplasty; NEC, nutritional education control; NEI, nutritional education intervention.
[2] At baseline, subjects who took lipid-lowering medications, acetylsalicylic acid, or both were excluded.

education and consumed an ALA-rich margarine (4). In a population with multiple cardiovascular risk factors, we focused on investigating the effect of long-term increased intake of ALA on IHD risk factors. The intake of ALA in the present study was 3-fold higher than that in the LDHS, and the fatty acid composition of the CE showed significant and considerable changes in the expected direction. Hence, the outcome of the MARGARIN study may indicate the effects of dietary ALA enrichment on IHD risk factors. Contrary to the LDHS, the present study was designed to investigate the effect of the nutritional education program separately. However, with respect to the effect on IHD risk factors, this investigation may be limited due to lack of power. The difference in serum total cholesterol between the NEI and NEC groups had to be 0.18 mmol/L to be significant (power: 51%; calculation based on baseline data from the present study), whereas a review of intervention studies in primary care showed a mean difference between intervention and control groups of 0.14 mmol/L (19). Nevertheless, the effect of the education program on dietary habits was established independently of the type of margarine, and an indication of the effect on the IHD risk factors is provided.

In the LDHS, not only the dietary intake of ALA but also that of oleic acid differed between the intervention and control groups because the composition of the project margarine was rich in monounsaturated fatty acids. The Mediterranean diet is rich in oleic acid, which may be cardioprotective in itself (20). In the present study, the possibilities for increasing the intake of oleic acid in the NEI group were limited because the margarines rich in polyunsaturated fatty acids were used for baking as well. Hence, in all subgroups, the intake of LA increased, and there was no significant difference in oleic acid intake between the NEI and NEC groups. Other components of the Mediterranean diet that may be cardioprotective are fiber (21) and fish (12). The nutritional education program did not significantly increase the intake of fiber, although it tended to be more succesful in women than in men. Women in the NEI group increased their intake of vegetables and had higher intakes of bread and fruit after 2 y than did women in the NEC group. The nutritional education program succesfully increased the consumption of fish, which was verified by the fatty acid composition of the CE. Epidemiologic studies showed that a high intake of fish is associated with a lower incidence of IHD (22, 23). Because fish contain a high amount of long-chain n−3 fatty acids (24), the cardioprotective effect of fish may be due to an antiarrhythmic mechanism. Hence, the success of the educational program in increasing fish intake is important in IHD prevention. Additionally beneficial in this respect is the lower intake of total and saturated fats in the NEI group during 2 y of follow-up.

Concerning the effect of increased ALA intake, our results showed a higher concentration of serum triacylglycerol in the ALA subgroups than in the LA subgroups. Previous short-term studies with healthy volunteers (25, 26) and secondary prevention trials (4, 10, 21) showed no effect of ALA supplementation (≤9.2 g/d) on serum triacylglycerol. It has been shown, however, that the n−3 fatty acids in fish lower serum triacylglycerol more effectively than does ALA (25). At baseline, we found an independent positive association between the ALA content in the CE and the triacylglycerol concentration ($r = 0.13$, $P < 0.05$), whereas the LA content in the CE was inversely associated with the triacylglycerol concentration ($r = -0.32$, $P < 0.01$) (27). This agrees with our longitudinal results in the present study, but the mechanism behind this result is hard to explain.

During the study the serum HDL-cholesterol concentration increased in the LA subgroups and to a lesser extent in the ALA subgroups. This increase may be unexpected because a previous short-term study showed a decrease in serum HDL cholesterol when the diet was enriched with LA or ALA (25). However, our results agree with those of the LDHS, in which the HDL-cholesterol concentration increased during a 2-y study period and tended to increase less in the intervention group (4).

The concentration of plasma fibrinogen increased less in the ALA subgroups than in the LA subgroups. In general, n−3 fatty acids have been shown to lower plasma fibrinogen (28), although results are inconsistent (29). Short-term studies in healthy subjects showed no effect of ALA supplementation on plasma fibrinogen (30, 31) and also showed that the effect of ALA was equal to that of fish oil (31). The Atherosclerosis Risk in Communities study showed an inverse association between the usual intake of n−3 fatty acids (ALA or fish oil) and plasma fibrinogen (32), but another study showed no such association in young adults (33). An experimental study showed that a diet rich in n−6 fatty acids increases plasma fibrinogen (34). This may partly explain the increase in plasma fibrinogen we observed in all subgroups because the LA intake increased in all subgroups. However, an increase of 0.4–0.6 g/L was also observed after supplementation with either fish oil or corn oil (29). The authors suggested that this was because of the oxidative stress induced by supplementation with polyunsaturated fatty acids. As an acute phase reactant, fibrinogen may respond to oxidative stress. These experiments were all short-term studies, and our results suggest a relatively beneficial effect of ALA (mean intake of 6 g/d) on plasma fibrinogen in the longer term compared with the effect of LA.

There were no significant differences in estimated IHD risk between the ALA and LA subgroups. Serum total and HDL-cholesterol concentrations are included in the Framingham calculation, and the less favorable effect of the ALA-rich margarine on the ratio of total to HDL cholesterol apparently did not result in a higher estimated IHD risk. The Framingham score may be limited because few risk factors are included and because the score has not yet been validated in the Dutch population. However, in English and German subjects with hypertension, the Framingham score was similar to risk calculations that were developed in those populations (35). Meta-analyses showed that the 0.24-mmol/L higher concentration of serum triacylglycerol in the ALA subgroup would increase the cardiovascular risk by ≈3% in men and 8% in women (36) and that a 0.18-g/L lower plasma fibrinogen concentration would decrease the IHD risk by ≈11% (37).

A recent study showed that substituting common foods with ALA-rich foods can increase the intake of ALA by up to 9 g/d (38). In the present study, the free provision of an ALA-rich margarine increased the ALA intake substantially (from 2 to 6 g/d), and the group nutritional education succesfully increased the consumption of fish during a 2-y period. Our results show that the effects of ALA supplementation on estimated IHD risk are at least similar to, or even more beneficial than, those of a standard LA-rich diet because the lower plasma fibrinogen concentration may outweigh the increase in serum triacylglycerol. Hence, from this perspective increased ALA intakes can be recommended for the primary prevention of IHD.

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