Dietary linolenic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study[1–3]

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ABSTRACT

Background: Dietary intake of linolenic acid is associated with a decreased risk of cardiovascular disease mortality. However, the mechanisms by which dietary linolenic acid affects cardiovascular disease risk are not clearly understood.

Objective: We examined the association between dietary linolenic acid and plasma triacylglycerol concentrations.

Design: In a cross-sectional design, we studied 4440 white subjects (2036 men and 2404 women) aged 25–93 y who participated in the National Heart, Lung, and Blood Institute Family Heart Study. We used generalized linear models to estimate adjusted mean triacylglycerol concentrations according to categories of total dietary linolenic acid (α- and γ-linolenic acid) intake.

Results: The mean dietary linolenic acid intakes were 0.81 and 0.69 g/d for the men and the women, respectively. High consumption of dietary linolenic acid was associated with young age; high intakes of energy, fat, carbohydrates, fruit, vegetables, and fish; low HDL cholesterol; current smoking; and frequent consumption of creamy salad dressing. High consumption of dietary linolenic acid was also associated with low plasma triacylglycerol concentrations. From the lowest to the highest quintile of linolenic acid intake, the multivariate-adjusted mean triacylglycerol concentrations were 1.75 (95% CI: 1.65, 1.85), 1.74 (1.66, 1.82), 1.69 (1.61, 1.77), 1.66 (1.58, 1.74), and 1.54 (1.44, 1.64) mmol/L, respectively (P for linear trend = 0.007). When linolenic acid was used as a continuous variable, the corresponding regression coefficient was −0.2811 (−0.4922, −0.07001).

Conclusions: Consumption of total linolenic acid is inversely related to plasma triacylglycerol concentrations in both white men and white women. This suggests a pathway by which dietary linolenic acid might reduce cardiovascular disease risk. *Am J Clin Nutr* 2003;78:1098–1102.

KEY WORDS Linolenic acid, n−3 fatty acids, triacylglycerol, cardiovascular disease risk factors

INTRODUCTION

Coronary artery disease (CAD) remains the leading cause of death in the United States and other industrialized nations. Epidemiologic studies indicate that high triacylglycerol concentrations alone or in combination with low HDL-cholesterol concentrations are independent predictors of CAD (1–5). Strategies directed at primary or secondary CAD prevention have used pharmacologic agents as well as dietary approaches to lower triacylglycerol concentrations. In particular, researchers showed that fish-oil intake lowers triacylglycerol concentrations (6–11). The triacylglycerol-lowering power of fish oil has been attributed to the eicosapentaenoic acid and docosahexaenoic acid that are found in fish.

α-Linolenic acid (ALA) is a precursor of long-chain n−3 fatty acids contained in fish (especially eicosapentaenoic acid), and a high consumption of ALA is associated with a low incidence of CAD and cardiovascular disease mortality (12, 13). The reported association between dietary linolenic acid and triacylglycerol has been inconsistent. Although Singer et al (14) reported that low triacylglycerol concentrations are related to high consumption of linolenic acid, most epidemiologic studies failed to show effects of ALA on triacylglycerol in humans (8, 15–18). In a randomized clinical trial of healthy male volunteers, ALA did not have a significant effect on triacylglycerol (19). In the Mediterranean Alpha-linolenic Enriched Groningen Dietary Intervention study, consumption of ALA-rich margarine resulted in a significant net increase in triacylglycerol concentrations after 2 y of intervention (20). We used data collected from 4440 white participants in the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study to assess whether dietary consumption of total linolenic acid (α- and γ-linolenic acid) is associated with triacylglycerol concentrations in men and women.

SUBJECTS AND METHODS

Study population

Participants in this project were members of the NHLBI Family Heart Study, which is a multicenter, population-based

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study designed to identify and evaluate genetic and nongenetic determinants of CAD, preclinical atherosclerosis, and cardiovascular disease risk factors. A detailed description of the NHLBI Family Heart Study was published previously (21). Briefly, families in the study were chosen randomly (a random group) or on the basis of a higher-than-expected risk of CAD (a high-risk group) from previously established population-based cohort studies. A family risk score, which related the family's age- and sex-specific incidence of CAD to the incidence expected in the general population (22), was used to identify families for the high-risk group. During a clinic visit at one of the study centers, a detailed medical and lifestyle history was obtained through interview, and laboratory measurements were performed.

The present analyses are based on 4440 white participants (from both random and high-risk groups) who had complete dietary and laboratory examination data. Each participant gave written informed consent, and the study protocol was reviewed and approved by each of the participating institutions.

Dietary assessment

A staff-administered semiquantitative food-frequency questionnaire developed by Willett et al (23) was used to collect data on dietary linolenic acid intake and other dietary information. The reproducibility and validity of this food-frequency questionnaire have been documented previously (24, 25). The intake of specific nutrients was computed by multiplying the frequency of consumption of an item by the nutrient content of specified portions. Composition values for total linolenic acid and other nutrients were obtained from the Harvard University Food Composition Database, which is derived from US Department of Agriculture sources (26), and from manufacturers' information.

Blood collection and assays

All participants were asked to fast for 12 h before their arrival at the study center. Evacuated tubes without additives were used to collect blood samples for lipids, and the samples were then spun at 3000 $\times$ *g* for 10 min at 4 °C. Sera were stored at −70 °C until they were shipped periodically to a central laboratory at the Fairview-University Medical Center in Minneapolis for processing.

Triacylglycerol concentrations were measured by using triacylglycerol GB reagent on a Roche COBAS FARA centrifugal analyzer (Boehringer Mannheim Diagnostics, Indianapolis). In this method, free glycerol is eliminated in an initial blank reaction that differs from the final reaction only in the omission of lipase and 4-aminophenazone. The initial reaction is followed by enzymatic hydrolysis of triacylglycerol with lipase and determination of the liberated glycerol by an enzymatic, colorimetric reaction of peroxide and 4-aminophenazone to form color that is directly proportional to the amount of triacylglycerols present in the sample.

Serum total cholesterol concentrations were measured by using a commercial cholesterol oxidase method on a Roche COBAS FARA centrifugal analyzer (Boehringer Mannheim Diagnostics). HDL-cholesterol quantification was performed by using the above-described cholesterol method after precipitation of non-HDL cholesterol with magnesium-dextran. For subjects with a triacylglycerol concentration $<$ 4.5 mmol/L (400 mg/dL), LDL-cholesterol concentrations were calculated by using the Friedewald formula (27). For subjects with higher triacylglycerol concentrations, LDL-cholesterol quantitation was performed with the use of EDTA plasma samples and ultracentrifugation.

Other variables

Information on cigarette smoking, alcohol intake, and education was obtained by interview during the clinic visit. The type of salad dressing consumed and the frequency of fish intake and fruit and vegetable consumption were obtained from the food-frequency questionnaire. The level of physical activity during the previous year was estimated through self-reports. Anthropometric data were collected from the participants while they wore scrub suits. The presence of chronic conditions such as diabetes mellitus and CAD was assessed through medical histories and laboratory measurements and tests. Subjects were considered to have diabetes mellitus if they were taking hypoglycemic agents or had been told by a physician that they had diabetes mellitus. CAD was assessed from the medical history and a 12-lead electrocardiogram. Subjects were defined as having CAD if they had a self-reported history of myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass graft that could be validated by review of medical records or if abnormal Q waves (Minnesota codes 1.1–1.2) were detected on a resting 12-lead electrocardiogram.

Statistical analyses

Of the 4818 white participants who had a clinic examination and data on dietary linolenic acid intake, 378 subjects were excluded because of the following reasons: *1*) probable errors on food-frequency questionnaires (n = 287) [(*i*) answers on the food-frequency questionnaire were judged by the interviewer as unreliable, or $>$ 18 items were left blank on the dietary questionnaires (n = 127), or (*ii*) energy intake was outside the a priori range [3347.2–17 572.8 kJ for men (25) and 2510.4–14 644.0 kJ for women (28)] (n = 160)], *2*) current treatment for hyperlipidemia (n = 63), and *3*) missing data on covariates (n = 28). The total number of nonwhites with available data was inadequate for ethnicity-specific analyses, so the nonwhites were excluded from the present study.

Because energy intake and dietary patterns differ between men and women, we created quintiles of total linolenic acid intake within each sex. We initially conducted sex-specific analyses, but because we observed no significant interaction between sex and quintile of linolenic acid intake (P = 0.98), we have presented the data from the men and the women combined. We used a generalized linear model to compare adjusted mean triacylglycerol concentrations across quintiles of linolenic acid intake. This model corrects the variance for familial clustering. The multivariate model controlled for age; age squared; field center; risk group for CAD; body mass index; waist-to-hip ratio; intakes of energy, total fat, carbohydrates, long-chain n−3 fatty acids, and fruit and vegetables; HDL-cholesterol concentration; current alcohol consumption (yes or no); current smoking (yes or no); physical activity; and history of diabetes mellitus and CAD.

We also used total linolenic acid intake as a continuous variable to assess its relation to triacylglycerol in a data set in which men and women were combined. The significance

TABLE 1
Baseline characteristics of participants in the National Heart, Lung, and Blood Institute Family Heart Study by quintile (Q) of total linolenic acid intake[1]

	Q1 (0.38 g/d)[2] (n = 881)	Q2 (0.55 g/d) (n = 893)	Q3 (0.69 g/d) (n = 911)	Q4 (0.86 g/d) (n = 856)	Q5 (1.24 g/d) (n = 899)	P for trend[3]
High-risk group (%)	53.6	50.1	51.8	54.0	53.0	0.47
Age (y)	52.7 ± 13.5[4]	52.6 ± 14.0	52.4 ± 13.3	50.8 ± 14.0	50.9 ± 14.3	0.0001
Sex (% male)	45.1	45.6	46.4	46.6	45.6	0.96
Any college education (%)	55.3	52.4	54.0	53.6	47.5	0.012
BMI (kg/m^2)	27.0 ± 5.0	26.9 ± 4.8	27.8 ± 5.6	27.5 ± 5.5	28.1 ± 6.0	< 0.0001
Waist-to-hip ratio	0.91 ± 0.10	0.91 ± 0.09	0.92 ± 0.09	0.91 ± 0.09	0.92 ± 0.09	< 0.0001
Coronary artery disease (%)	12.2	12.9	9.8	9.1	8.5	0.0007
Diabetes mellitus (%)	6.0	4.0	5.8	7.4	6.6	0.05
Energy intake (kJ)	4824 ± 1304	6117 ± 1413	7112 ± 1607	8284 ± 1876	10 232 ± 2485	< 0.0001
HDL cholesterol (mmol/L)	1.34 ± 0.41	1.30 ± 0.36	1.30 ± 0.41	1.29 ± 0.40	1.28 ± 0.37	< 0.0001
Total fat intake (g/d)	32.1 ± 10.5	45.6 ± 11.4	57.1 ± 13.7	69.7 ± 16.6	95.6 ± 27.3	< 0.0001
Carbohydrate intake (g/d)	161.5 ± 63.0	196.1 ± 72.3	218.0 ± 75.5	247.8 ± 84.8	287.9 ± 95.8	< 0.0001
Fruit and vegetable intake (servings/d)	1.5 ± 1.0	1.6 ± 1.0	1.7 ± 0.9	1.8 ± 1.0	1.8 ± 1.0	< 0.0001
EPA and DHA intake (g/d)	0.18 ± 0.17	0.21 ± 0.19	0.24 ± 0.21	0.25 ± 0.23	0.29 ± 0.26	< 0.0001
Use of creamy salad dressing (%)	67.6	70.5	73.0	73.1	80.6	< 0.0001
Fish intake ≥ 1 serving/wk (%)	71.8	69.6	72.1	72.6	77.3	0.007
Exercise (min/d)	30.5 ± 34.9	29.0 ± 37.5	30.4 ± 41.6	30.0 ± 36.1	28.2 ± 36.4	0.22
Current smoker (%)	14.1	11.3	12.1	15.2	19.0	< 0.0001
Current alcohol drinker (%)	60.3	53.4	54.1	55.8	50.7	0.0013

[1] From the lowest to the highest quintile, the ranges of linolenic acid intake were 0.19–0.52, 0.53–0.66, 0.67–0.82, 0.83–1.03, and 1.04–3.48 g/d, respectively, in the men and 0.13–0.44, 0.45–0.57, 0.58–0.71, 0.72–0.89, and 0.90–2.45 g/d, respectively, in the women. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
[2] $\bar{x}$.
[3] The midpoint of each quintile was used as a continuous variable in a generalized linear model to obtain P values for trend for the continuous variables. We used chi-square tests for P values for the categorical variables.
[4] $\bar{x}$ ± SD.

level was set at 0.05. All analyses were performed by using SAS, release 8.02, WINDOWS version 5.1 (SAS Institute Inc, Cary, NC).

RESULTS

Of the 4440 white participants included in the analyses, 2036 were men and 2404 were women. The mean (± SD) ages of the men and the women were 51.5 ± 14.0 and 52.2 ± 13.7 y, respectively. The mean daily consumption of total dietary linolenic acid was 0.81 ± 0.35 g (range: 0.19-3.48 g) in the men and 0.69 ± 0.29 g (range: 0.13–2.45 g) in the women. The baseline characteristics of the subjects by quintile of linolenic acid intake are shown in **Table 1**. High intakes of dietary linolenic acid were associated with young age; high body mass index and waist-to-hip ratio; high intakes of energy, fruit and vegetables, fish, eicosapentaenoic and docosahexaenoic acids, total fat, and carbohydrates; and frequent consumption of creamy salad dressing. In addition, the subjects with high intakes of linolenic acid were less likely than those with low intakes to have received any college education, to have CAD, and to be current drinkers but were more likely to be current smokers.

Total linolenic acid intake was inversely related to triacylglycerol concentration. From the lowest to the highest quintile of linolenic acid intake, the mean triacylglycerol concentrations were 1.75 ± 0.05, 1.74 ± 0.04, 1.69 ± 0.04, 1.66 ± 0.04, and 1.54 ± 0.05 mmol/L, respectively (**Table 2**), in a model adjusted for age; age squared; sex; field center; risk group for CAD; body mass index; waist-to-hip ratio; intakes of energy, total fat, carbohydrates, long-chain n−3 fatty acids, fruit and vegetables, and alcohol; smoking; physical activity; HDL-cholesterol concentration; and history of CAD and diabetes

TABLE 2
Adjusted triacylglycerol concentrations by quintile (Q) of linolenic acid intake in the National Heart, Lung, and Blood Institute Family Heart Study[1]

Quintile of linolenic acid intake	Model 1[2]	Model 2[3]
	mmol/L	
Q1 (0.38 g/d)[4] (n = 881)	1.79 ± 0.05	1.75 ± 0.05
Q2 (0.55 g/d) (n = 893)	1.79 ± 0.04	1.74 ± 0.04
Q3 (0.69 g/d) (n = 911)	1.72 ± 0.04	1.69 ± 0.04
Q4 (0.86 g/d) (n = 856)	1.67 ± 0.04	1.66 ± 0.04
Q5 (1.24 g/d) (n = 899)	1.49 ± 0.04	1.54 ± 0.05
P for trend	< 0.0001	0.007

[1] $\bar{x}$ ± SE. From the lowest to the highest quintile, the ranges of linolenic acid intake were 0.19–0.52, 0.53–0.66, 0.67–0.82, 0.83–1.03, and 1.04–3.48, respectively, in the men and 0.13–0.44, 0.45–0.57, 0.58–0.71, 0.72–0.89, and 0.90–2.45, respectively, in the women.
[2] Adjusted for age, age squared, sex, field center, and energy intake.
[3] Adjusted for age; age squared; field center; sex; risk group for coronary artery disease; BMI; waist-to-hip ratio; intakes of energy, total fat, carbohydrates, eicosapentaenoic acid and docosahexaenoic acid, fruit and vegetables, and alcohol; smoking; HDL-cholesterol concentration; physical activity; and history of coronary artery disease and diabetes mellitus in a generalized linear model (MIXED procedure in SAS; SAS Institute Inc, Cary, NC).
[4] $\bar{x}$.

mellitus. When analyzed as a continuous variable, total linolenic acid intake was inversely related to triacylglycerol concentration. The regression coefficient from a multivariate regression model was −0.2811 (SE = 0.1077). Dietary linolenic acid was not associated with LDL cholesterol, HDL cholesterol, or total cholesterol in this study (data not shown).

DISCUSSION

Plasma triacylglycerol has often been found to be a determinant of atherosclerosis (29) and CAD (30, 31). Although fish-oil intake has been repeatedly shown to reduce plasma triacylglycerol concentrations (6, 8, 32), data remain inconsistent on the effects of linolenic acid on plasma triacylglycerol. In this cross-sectional study, we found that dietary linolenic acid was inversely associated with plasma triacylglycerol in both men and women and that this association was independent of fish consumption. The men and the women in the highest quintile of linolenic acid intake had triacylglycerol concentrations that were 26.0% and 14.6% lower, respectively, than those in the men and the women in the lowest quintile of linolenic acid intake.

A limited number of studies have reported an inverse association between dietary linolenic acid intake and plasma triacylglycerol concentration. Singer et al (14) reported a reduction in triacylglycerol concentration among healthy volunteers, male hypertensive subjects, and subjects with primary hyperlipoproteinemia after 2 wk of a diet supplemented with linseed oil (38 mL linolenic acid added to the diet/d). In that study, the magnitude of the reduction in triacylglycerol concentration was 21.8% in the hypertensive subjects and 34.8% in the hypercholesterolemic subjects. In an animal experiment, rats fed perilla oil—which includes as much as 60% ALA in its total fatty acid composition—had decreased triacylglycerol concentrations after 4 wk of intervention (33).

In contrast, in a double-blind crossover study, a linseed oil supplement had no effect on plasma triacylglycerol after a 3-mo intervention period among diabetic patients (18). Other studies failed to show a decrease in triacylglycerol concentrations after dietary supplementation with ALA in normal (8) and diabetic (9, 10, 18) subjects. Most of these negative studies used ALA doses that were much higher (3–10 times) than those consumed in the present study. In the Mediterranean Alpha-linolenic Enriched Groningen Dietary Intervention study (20), which was a randomized, double-blind trial, consumption of margarine rich in ALA produced a net increase in triacylglycerol concentration of 0.24 mmol/L (95% CI: 0.02, 0.46) after 2 y among subjects with multiple cardiovascular disease risk factors. In that study, the average ALA intake was 6.3 g/d.

We do not have a plausible explanation for these discrepancies between studies and can only speculate on possible reasons. The heterogeneity of study participants across studies, the wide range of amounts of ALA consumed, differences in the duration of intervention (from a few weeks to a few years), residual confounding, and inadequate power could partially account for the differences in study findings. In our study, we had large numbers of men and women and a wide range of covariates to control residual confounding. In addition, we estimated the usual dietary intake of linolenic acid over the previous year.

Our study has some limitations. First, because nutrient intakes were derived from a food-frequency questionnaire—an instrument that has been shown to underestimate energy intake when compared with the doubly labeled water technique (34)—our estimate of the daily intakes of linolenic acid and other nutrients may have been biased. Second, the cross-sectional design of our study limits our ability to infer causality between linolenic acid intake and triacylglycerol concentration. Third, the inability to separate ALA from γ-linolenic acid is a weakness of our study.

Physiologic mechanisms by which n−3 fatty acids may lower plasma triacylglycerol concentrations have been suggested. n−3 Fatty acids from fish oil might exert their effects on triacylglycerol through *1*) reduced endogenous production of triacylglycerol-rich lipoproteins (a transport medium for triacylglycerol in blood), *2*) increased elimination of triacylglycerol-rich lipoproteins, or *3*) both (6). n−3 Fatty acids from fish oil have been shown to increase lipoprotein lipase activity (35).

In conclusion, our study indicates that dietary linolenic acid intakes (range: 0.13–3.48 g/d) are inversely related to plasma triacylglycerol concentrations in white men and women. If confirmed by other studies, this finding should be explored as an additional dietary approach to lower elevated triacylglycerol.

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LD designed the project, completed the data analyses, and prepared the manuscript; SCH, DKA, and MAP participated in the study design and data collection and critically reviewed the manuscript; JHE participated in the study design, data collection, and measurement of triacylglycerol concentrations and critically reviewed the manuscript; RCE participated in the study design, data collection, and data analyses and critically reviewed the manuscript. None of the authors had any conflicts of interest.

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