

Dietary Linolenic Acid Is Inversely Associated With Calcified Atherosclerotic Plaque in the Coronary Arteries

The National Heart, Lung, and Blood Institute Family Heart Study

Luc Djoussé, MD, DSc, MPH; Donna K. Arnett, PhD, MSPH; J. Jeffrey Carr, MD;
John H. Eckfeldt, MD, PhD; Paul N. Hopkins, MD, MSPH;
Michael A. Province, PhD; R. Curtis Ellison, MD

Background—High dietary intake of linolenic acid is associated with a lower risk of cardiovascular disease mortality. However, little is known about the association between linolenic acid and subclinical atherosclerosis.

Methods and Results—To examine the association between dietary linolenic acid measured by food frequency questionnaire and calcified atherosclerotic plaque in the coronary arteries (CAC) measured by cardiac CT, we studied 2004 white participants of the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study aged 32 to 93 years. The presence of CAC was defined on the basis of total CAC score of ≥ 100 . We used generalized estimating equations to estimate odds ratios for the presence of CAC across quintiles of linolenic acid. The average consumption of dietary linolenic acid was 0.82 ± 0.36 g/d for men and 0.69 ± 0.29 g/d for women. From the lowest to the highest quintile of linolenic acid, adjusted odds ratios (95% CI) for the presence of CAC were 1.0 (reference), 0.61 (0.42 to 0.88), 0.55 (0.35 to 0.84), 0.57 (0.37 to 0.88), and 0.35 (0.22 to 0.55), respectively (P for trend < 0.0001), after we controlled for age, gender, education, family risk group, smoking, fruit and vegetable intake, history of coronary artery disease, hypertension, diabetes mellitus, and statin use. When linolenic acid was used as a continuous variable, the multivariate adjusted odds ratio was 0.38 (95% CI, 0.24 to 0.46) per gram of linolenic acid intake. Use of different cut points for CAC score yielded similar results.

Conclusions—Consumption of dietary linolenic acid is associated with a lower prevalence of CAC in a dose-response fashion in white men and women. (*Circulation*. 2005;111:2921-2926.)

Key Words: diet ■ atherosclerosis ■ calcium ■ plaque ■ cardiovascular diseases

Coronary artery disease (CAD) is the leading cause of death in the United States. Early identification of people at risk is important for primary and secondary prevention.

See p 2872

Calcium deposition in the arterial walls occurs early during the atherosclerotic process. With the formation of atheroma, the calcified atherosclerosis plaque component is designated as a type Vb lesion in the American Heart Association classification.¹ Histological studies have indicated a high correlation between the extent of calcified atherosclerotic plaque in the coronary arteries (CAC) and total burden of atherosclerotic plaques^{2,3} and the likelihood of vulnerable plaques.⁴ CAC can be measured noninvasively by multidetector CT, which uses ECG gating. Studies have demonstrated that CAC extent measured by cardiac CT provides

more prognostic information on hard CAD end points (ie, myocardial infarction and coronary death) than angiography in symptomatic CAD patients.^{5,6} Dietary α -linolenic acid (ALA) has been associated with a lower rate of fatal and nonfatal coronary events.⁷⁻¹⁰ γ -Linolenic acid has been shown to lower blood pressure^{11,12} and triglycerides, raise HDL, decrease platelet aggregation and serum thromboxane B₂, and enhance vascular production of prostacyclin.¹³⁻¹⁵ It is not known whether total linolenic acid (ALA plus γ -linolenic acid) influences the rate of atherosclerosis progression or is associated with subclinical atherosclerosis as measured by cardiac CT. Previous studies have indicated that ALA might inhibit cytokine production (interleukin-1 β and tumor necrosis factor- α)¹⁶ and therefore slow the progression of atherosclerosis. In addition, ALA is a precursor of eicosapentaenoic acid, which can inhibit arachidonic acid metabolism compet-

Received July 8, 2004; revision received February 3, 2005; accepted February 22, 2005.

From Evans Department of Medicine, Section of Preventive Medicine and Epidemiology, Boston University School of Medicine, Boston, Mass (L.D., R.C.E.); Department of Epidemiology, University of Alabama, Birmingham (D.K.A.); Division of Radiologic Sciences, Wake Forest University School of Medicine, Winston-Salem, NC (J.J.C.); Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis (J.H.E.); Department of Cardiovascular Genetics, University of Utah, Salt Lake City (P.N.H.); and Division of Biostatistics, Washington University, St Louis, Mo (M.A.P.).

Guest Editor for this article is Robert O. Bonow, MD.

Correspondence to Luc Djoussé, MD, DSc, MPH, Boston University School of Medicine, Room B-612, 715 Albany St, Boston, MA 02118. E-mail ldjoussé@bu.edu.

© 2005 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/CIRCULATIONAHA.104.489534

itively via enzymatic pathways, thus preventing the production of proinflammatory markers.¹⁷ The metabolism of γ -linolenic acid yields antiinflammatory products such as prostaglandin E1 and 15-hydroxy-dihomo- γ -linolenic acid.¹⁴ If this is true, one would expect less CAC among subjects consuming higher concentrations of dietary linolenic acid.

In the present study we used data collected on 2004 white participants of the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study (FHS) to determine whether dietary consumption of total linolenic acid (ALA plus γ -linolenic acid) is associated with a lower prevalence of CAC in men and women.

Methods

Study Population

Participants in this project were members of the NHLBI FHS in whom coronary calcified plaque was measured by cardiac gated multidetector CT (cardiac CT). A detailed description of the NHLBI FHS has been published.¹⁸ Briefly, families in the study had been chosen randomly (random group) or on the basis of a higher than expected risk of CAD (high-risk group) from previously established population-based cohort studies. Between 1993 and 1995, groups of individuals participating in each of the parent studies were selected at random and invited to furnish an updated family health history that contained information on their parents, children, and siblings. Of 4679 individuals contacted, responses were obtained from 3150 (67%); their family members were then contacted, and self-reported health data were obtained from a total of 22 908 individuals (86% of those contacted). From the families responding to the health questionnaire, 588 families were chosen at random, and 566 families were selected on the basis of higher than expected risk of CHD. The high-risk group was defined on the basis of a family risk score, which compares the family's age and sex-specific incidence of CHD to that expected in the general population.¹⁹ All members of these families were invited to come to one of the 4 study clinics for an \approx 4-hour clinical evaluation. The evaluation consisted of a detailed medical and lifestyle history, obtained through interview. All interviewers were trained centrally and required periodic certification; standardization of interviews was facilitated by periodic review of taped interviews and by frequent circulation of the distributions of responses obtained by different interviewers and different centers. During the initial clinic visit (1993–1995) at one of the study centers, a detailed medical and lifestyle history was obtained through interview, and laboratory measurements were done.

Follow-Up Examination

Between 2002 and 2003, approximately two thirds of the families (largest families available who also had genome-wide anonymous markers typed by the Mammalian Genotyping Service) of the NHLBI FHS were invited to participate in a clinical examination that included measurement of CAC with cardiac CT. In addition to the initial study centers, an all-black center, University of Alabama, Birmingham, was recruited from the Hypertension Genetic Epidemiology Network study. Participants of the latter center also underwent clinical evaluation including cardiac CT. This report is restricted to the initial NHLBI FHS because dietary data were not available among subjects from the Alabama field center.

Family members were invited to attend a brief clinic visit, where medical and personal history questionnaires were obtained, blood pressure and anthropometric measurements were taken, and blood samples were collected for a lipid profile. After this examination, participants underwent CAC measurements as described below. The current analyses are based on 2004 white participants with complete data on diet and CAC score. Each participant gave informed consent, and the study protocol was reviewed and approved by each of the participating institutions.

Measurement of CAC

Cardiac CT examinations from the 4 FHS CT sites that had previous data on diet are part of this analysis, and cardiac CT devices capable of 4 or 8 slices were utilized. The specific systems were General Electric Health Systems LightSpeed Plus and LightSpeed Ultra, Siemens Volume Zoom, and Philips MX 8000. Examinations were performed with the use of the same protocol as that used in the Multi-Ethnic Study of Atherosclerosis of the NHLBI.²⁰ The scans were performed with the use of prospective ECG gating at 50% of the cardiac cycle, 120 kV, 106 mA, 2.5-mm slice collimation, 0.5-second gantry rotation, and a partial scan reconstruction resulting in a temporal resolution of from 250 to 300 ms. Images were reconstructed with the use of the standard algorithm into a 35-cm display field of view. All participants were imaged with a calcium calibration standard within the imaging field (Image Analysis). For participants weighing \geq 100 kg (\geq 220 lb), the mAs was increased by 25%. The scan through the heart was repeated after a 1-minute pause during the same examination, resulting in 2 sequential scans for measurement of CAC. The effective radiation exposure for the average participant of each coronary scan was 1.5 milli-Sievert (mSv) for men and 1.9 mSv for women.

CT images from all sites were sent electronically to the central CT reading center at Wake Forest University Health Sciences, Winston Salem, NC. Trained CT analysts using dedicated hardware (GE Advantage Windows Workstation) and software (GE SmarScore) identified CAC in the epicardial coronary arteries, and an Agatston score modified to account for slice thickness was calculated with the use of a 130 CT number threshold and a minimum lesion size of 0.9 mm (ie, 2-pixel connectivity filter). In this report the sum of the vessel plaque is reported as the total CAC score. The total CAC scores from the first and second measurements were then averaged.

For these analyses, we dichotomized the distribution of CAC score at an Agatston score of \geq 100 for the following reasons: (1) we desired a level of plaque burden that would likely result in a degree of luminal narrowing as manifest at coronary angiography⁵; and (2) pending results from the Saint Francis Heart Study indicate that an Agatston score threshold of 100 resulted in a 10-fold increase in risk of events (A. Guerci, unpublished data, 2003; data also presented at the American College of Cardiology, 2003). Nonetheless, we conducted sensitivity analyses to assess the robustness of our findings to different cutoff points (CAC score of 0, 50, and 150) to define the presence of calcified atherosclerotic plaque.

Dietary Assessment

We used a semiquantitative food frequency questionnaire developed by Willett et al²¹ to collect data on dietary linolenic acid and other dietary information. The reproducibility and validity of this food frequency questionnaire have been documented elsewhere.^{22,23} 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 derived from US Department of Agriculture sources²⁴ and manufacturer information. Major sources for dietary linolenic acid were salad dressing and canola oil in this population.

Blood Collection and Assays

All participants were asked to fast for 12 hours before their arrival at the study center. Evacuated tubes without additives were used to collect samples for lipids; blood samples were then spun at 3000g for 10 minutes at 4°C. Sera were stored at -70°C until shipped periodically to a central laboratory at the Fairview University Medical Center in Minneapolis, Minn, for processing.

Triglycerides concentrations were measured with the use of triglycerides GB reagent on the Roche COBAS FARA method. 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 triglycerides 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 triglycerides present in the sample.

Serum total cholesterol was measured by a commercial cholesterol oxidase method on a Roche COBAS FARA analyzer. HDL cholesterol quantification was performed with the aforementioned cholesterol method after precipitation of non-HDL cholesterol with magnesium/dextran. For samples with triglycerides concentrations <4.5 mmol/L (400 mg/dL), LDL cholesterol was calculated with the Friedewald formula.²⁵ For subjects with higher levels of triglycerides, LDL cholesterol quantification was performed on EDTA plasma by 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. Level of physical activity during the previous year was estimated through self-reports. Anthropometric data were collected with participants wearing scrub suits. Diabetes mellitus was present if a subject was taking hypoglycemic agents or if a physician had told him/her that he/she has diabetes mellitus or if fasting glucose was >7 mmol/L. Individuals were defined as a case of CAD if there was a self-reported history of myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass graft or if abnormal Q waves (Minnesota codes 1.1 to 1.2) were detected on a resting 12-lead ECG. All variables used in these analyses were ascertained during the follow-up examination except dietary linolenic acid and education, which were only collected during the initial examination.

Statistical Analyses

Of the 5710 white participants in the initial FHS Study, data on linolenic acid were available on 4883; of those, 2268 had data from a cardiac CT. We excluded 264 subjects from the analyses because of (1) history of coronary artery bypass graft surgery (n=143) (because of the likelihood that existing grafts may be occluded and calcified, thus leading to higher CAC score) and (2) probable errors on food frequency questionnaires (n=121) ([a] answers on the food frequency questionnaire judged by the interviewer as unreliable or >18 items left blank on the dietary questionnaires [n=55] or [b] energy intake outside a priori ranges [acceptance range=3347.2 to 17 572.8 kJ for men²³ and 2510.4 to 14 644 kJ for women²⁶] [n=66]). Thus, 2004 white subjects were included in subsequent analyses.

Because energy intake and dietary patterns differ between men and women, we initially conducted sex-specific analyses, but because we observed an inverse association in both sexes, we have presented the data combined for men and women. We used generalized estimating equations to calculate prevalence odds ratios for the presence of CAC across quintiles of linolenic acid. This method corrects the variance for familial clustering. The initial multivariate model controlled for age and sex. A second model controlled for age, sex, risk group (random versus high risk for CAD), smoking, and history of CAD, diabetes mellitus, and hypertension. The final model also controlled for education, fruit and vegetable consumption, and statin use. Additional adjustment for energy intake (quintiles), household income, field center, body mass index, waist-to-hip ratio, physical activity, LDL and HDL cholesterol, ω -6 fatty acids, and alcohol consumption (yes/no) did not alter these findings (data not shown). To assess confounding, we compared different models using partial likelihood ratio tests and change in point estimate, where 10% change in odds ratio was considered meaningful. The final tables present the most parsimonious models beyond which adding additional variables did not change the results. Probability value for linear trend was calculated with the use of a continuous variable in a regression model to which the median value of each quintile of linolenic acid was assigned. We tested for interaction between dietary linolenic acid and risk group, center, sex, age, education, and intake of long-chain ω -3 fatty acids. We tested the sensitivity of our findings to (1) different cut points for defining CAC (using 0, 50, and

150); (2) level of educational attainment (up to high school graduate, some college, college graduate, and more); (3) ratio of n-6 to n-3, using median value as cut point; and (4) reported frequency of weekly fish consumption (0, 1, \geq 2).

We also evaluated whether our findings were influenced by the presence of cardiovascular disease. For these analyses, we excluded subjects with (1) history of myocardial infarction and/or PTCA and (2) history of myocardial infarction and/or PTCA, hypertension, or stroke. Significance level was set at 0.05. All analyses were performed with the use of SAS, release 8.02, Windows version 5.1 (SAS Institute Inc).

Results

Of the 2004 white participants included in the analyses, 845 were men and 1159 were women. The mean age was 56.2 ± 13.1 years for men and 58.2 ± 12.7 years for women. The average daily consumption of total dietary linolenic acid was 0.82 ± 0.36 g for men (range, 0.23 to 3.48 g/d) and 0.69 ± 0.29 g for women (range, 0.17 to 2.29 g/d). Table 1 shows the baseline characteristics by quintiles of linolenic acid. A higher intake of dietary linolenic acid was associated with male gender; younger age; higher waist-to-hip ratio, energy intake, fruit and vegetable consumption; and higher diastolic blood pressure and lower prevalence of hypertension.

Because (1) we observed an inverse association between dietary linolenic acid and CAC in both men and women and in both the random group and the high-risk group and (2) there was no interaction between linolenic acid and gender or risk group, we present combined data in this report.

Our data showed an inverse and dose-dependent relation between dietary linolenic acid and prevalent CAC. From the lowest to the highest quintile of linolenic acid, prevalence odds ratios (95% CI) for CAC were 1.0 (reference), 0.61 (0.42 to 0.88), 0.55 (0.35 to 0.84), 0.57 (0.37 to 0.88), and 0.35 (0.22 to 0.55), respectively, in a model adjusting for age, sex, risk group, education, fruit and vegetable consumption, smoking, history of CAD, hypertension, diabetes mellitus, and statin use (P for linear trend <0.0001; Table 2). Additional adjustment for body mass index, waist-to-hip ratio, exercise, energy intake, household income, field center, LDL and HDL cholesterol, ω -6 fatty acids, alcohol consumption, and history of stroke did not alter these findings (P for linear trend <0.0001; data not shown).

When analyzed as a continuous variable, each additional gram per day of dietary linolenic acid was associated with a 62% lower prevalence of CAC (odds ratio=0.38; 95% CI, 0.24 to 0.46) in a full model, as above.

Sensitivity Analyses

The Figure presents odds ratios of prevalent calcified atherosclerotic plaque according to different cut points of CAC score. Although the observed effect was minimal with a cut point of 0, it was intermediate with a cut point of 50 and was highest for a cut point of either 100 or 150. In addition, an inverse association between linolenic acid and CAC was observed across categories of n-6/n-3 ratio, fish consumption, and educational attainment (Table 3). Furthermore, exclusion of subjects with (1) history of CAD (n=134) or (2) history of CAD, hypertension, or stroke (n=827) did not substantially alter the association (corresponding odds ratios per gram increase of linolenic acid were 0.39 [95% CI, 0.24 to 0.62] and 0.22 [95% CI, 0.10 to 0.46], respectively). Finally, stratification by age,

TABLE 1. Baseline Characteristics of 2004 Study Participants in the FHS Study

Characteristic	Quintiles of Dietary Total Linolenic Acid (Ranges in g/d)					P*
	0.17–0.47 (Low) (n=391)	0.48–0.61 (n=409)	0.62–0.75 (n=394)	0.76–0.95 (n=415)	0.96–3.48 (High) (n=395)	
Dietary linolenic acid, g/d	0.38±0.07	0.55±0.04	0.68±0.04	0.85±0.06	1.25±0.30	<0.0001
Male, %	28.9	37.9	40.9	48.0	54.9	<0.0001
Age, y	59.1±12.4	57.7±12.9	57.6±13.0	56.5±13.0	55.8±13.2	0.005
Less than high school, %	5.1	4.4	4.8	4.6	6.3	0.75
Household income <\$25 000/y, %	14.8	13.9	15.9	11.7	14.2	0.53
Body mass index, kg/m ²	28.5±5.9	28.5±5.3	29.3±5.6	28.9±5.6	29.3±5.9	0.10
Waist/hip ratio	0.89±0.11	0.90±0.09	0.91±0.09	0.91±0.09	0.93±0.09	<0.0001
Strenuous activity, %	23.0	25.2	23.9	27.7	23.8	0.56
Energy intake, kJ	4678±1198	6056±1274	6968±1566	8275.7±1756	10 354±2198	<0.0001
Fruit and vegetable, servings per day	2.88±1.43	3.17±1.70	3.39±1.73	3.47±1.86	3.60±1.96	<0.0001
Regular salad dressing, %	40.8	40.3	46.6	49.6	61.5	<0.0001
Creamy salad dressing, %	65.2	75.1	73.9	73.9	79.1	0.0006
Fish ω-3 (EPA and DHA)	0.16±0.14	0.20±0.19	0.22±0.19	0.24±0.22	0.27±0.28	<0.0001
Fish consumption, %	67.8	68.5	72.1	69.9	73.4	0.36
Linoleic acid, g/d	4.24±1.75	5.99±2.52	6.76±2.26	8.75±3.21	11.51±4.22	<0.0001
Linoleic/linolenic ratio	11.44±4.6	11.0±4.7	9.9±3.3	10.3±3.9	9.4±3.2	<0.0001
Systolic blood pressure, mm Hg	121.0±20.0	120.7±20.3	121.4±20.2	119.6±19.5	119.9±20.5	0.70
Diastolic blood pressure, mm Hg	68.3±9.6	69.9±9.7	69.9±10.0	69.4±9.4	70.2±9.6	0.03
Hypertension, %	38.1	44.0	42.4	33.5	37.0	0.02
CAD, %	5.9	6.4	8.4	7.0	5.8	0.59
Diabetes mellitus, %	4.6	5.6	6.1	6.8	8.4	0.26
Current smoking, %	44.0	40.3	36.6	40.5	42.3	0.28
Statin use, %	19.7	17.7	19.6	19.1	15.0	0.42
Current alcohol drinking, %	56.0	51.8	48.5	50.6	46.3	0.08

Data are mean±SD unless otherwise specified.

*P values were obtained with the use of χ^2 for categorical variables and ANOVA for continuous variables.

energy intake, and household income also showed an inverse association between linolenic acid and CAC within each group (data not shown). When analyses were repeated with the use of covariates obtained during the initial examination (instead of those obtained during the visit when cardiac CT was done), we observed similar results (data not shown).

Discussion

In this study we demonstrated that higher intake of dietary linolenic acid was associated with a lower prevalence of CAC as measured by cardiac CT in both men and women, after adjustment for confounding factors, in a dose-response fashion. This association was independent of age, education,

TABLE 2. Prevalence Odds Ratios of CAC According to Dietary Linolenic Acid

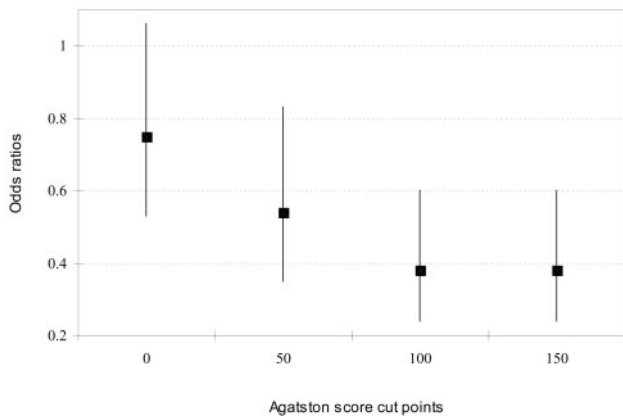
Quintiles of Dietary Linolenic Acid, g/d	n	Cases	Odds Ratios (95% CI)		
			Model 1*	Model 2†	Model 3‡
Q1 (0.17–0.47) (low)	391	126	1.0	1.0	1.0
Q2 (0.48–0.61)	409	113	0.73 (0.51–1.04)	0.64 (0.44–0.93)	0.61 (0.42–0.88)
Q3 (0.62–0.75)	394	108	0.66 (0.44–0.97)	0.60 (0.39–0.91)	0.55 (0.35–0.84)
Q4 (0.76–0.95)	415	113	0.68 (0.46–1.01)	0.64 (0.43–0.98)	0.57 (0.37–0.88)
Q5 (0.96–3.48) (high)	395	89	0.44 (0.29–0.69)	0.40 (0.25–0.63)	0.35 (0.22–0.55)
P for trend			0.0004	0.0003	<0.0001

From the lowest to the highest quintile of dietary linolenic acid, mean±SD values were 0.38±0.07, 0.55±0.04, 0.68±0.04, 0.85±0.06, and 1.25±0.30 g/d, respectively.

*Model 1 controls for age and sex. Coronary calcification was defined as total coronary calcium score of ≥ 100 .

†Model 2 controls for age, sex, risk group (high vs low CAD risk), smoking status, and history of CAD, hypertension, and diabetes.

‡Model 3 controls for variables in model 2 plus education, statin use, and fruit and vegetable intake.



Odds ratios and 95% CIs for prevalent CAC per gram increase of dietary linolenic acid according to different Agatston score cut points.

income, energy intake, ratio of n-6 to n-3 fatty acids, and fish consumption.

Although dietary linolenic acid has been associated with a lower risk of fatal and nonfatal coronary events^{7,9,27} and carotid intima-media thickness,²⁸ limited data are available on the underlying biological mechanisms. To our knowledge, no previous study has examined the relation between dietary linolenic acid and CAC in a community setting. Calcium deposition in the arterial walls occurs in the early stages of atherosclerosis just after fatty streak formation²⁹ and has been shown to correlate closely with the total burden of atheroma, both calcified and noncalcified.^{2,3} Furthermore, calcified atherosclerotic plaque measured by cardiac CT is a prognostic factor for coronary end points.^{5,6} There is substantial evidence that inflammation plays a key role in all phases of atherosclerosis,³⁰ and it is possible that linolenic acid might decrease the risk of coronary calcification through its anti-inflammatory properties. Published data on the effects of

linolenic acid on inflammation have been inconsistent. A diet rich in ALA was shown to reduce interleukin-1 β and tumor necrosis factor- α by \approx 30% among male volunteers.¹⁶ Furthermore, in a randomized trial, dietary supplementation with ALA was associated with a decreased concentration of C-reactive protein (\approx 38%), interleukin-6 (\approx 10%), and serum amyloid A (\approx 23%) after 3 months of intervention.³¹ γ -Linolenic acid has been shown to decrease serum thromboxane B₂ and enhance vascular production of prostacyclin.^{13,14} It is thus possible that the observed lower prevalence of CAC in subjects consuming higher amounts of dietary total linolenic acid might be mediated through inhibition of inflammation. However, contrary to this hypothesis is the lack of an association between dietary ALA and inflammatory markers in healthy men and women in one study.³² It is also possible that linolenic acid might influence the risk of atherosclerosis through established risk factors. Using the same data, we previously described beneficial effects of linolenic acid on blood pressure³³ and triglycerides.¹⁵ Additional studies are necessary to elucidate pathophysiological mechanisms by which linolenic acid might reduce the risk of developing or propagating calcified atherosclerotic plaque.

In the sensitivity analyses, we observed a minimal effect with 0, an intermediate effect with 50, and a similar and strongest effect with 100 and 150 as cut point for CAC score. It is likely that with a lower Agatston score cut point such as 0, cardiac CT might not have been able to accurately discriminate the presence or absence of clinically relevant CAC. This might lead to effect dilution, as observed in this article. Detrano and colleagues⁵ have shown that the use of an Agatston score of 0 as cut point to predict angiographic disease had a poor specificity (ranging from 13% to 31%), whereas the use of a cut point of 100 had a sensitivity and specificity of \approx 70% each. The fact that the use of an Agatston score of either 100 or 150 as cut point yielded a similar effect suggests that beyond a certain threshold, the ability of cardiac CT to detect CAC remains constant and accurate.

Our study has limitations. First, nutrients were derived from a food frequency questionnaire that has been shown to underestimate energy intake compared with the doubly labeled water technique³⁴; therefore, our estimate of daily intake of linolenic acid and other nutrients might have been biased, and such measurement error of linolenic acid might have introduced a bias in our results. Second, we were not able to separate ALA from γ -linolenic acid. However, this seems reasonable because of the following: (1) the relation of ALA to CHD⁸ and (2) γ -linolenic acid in the diet is primarily provided by beef fats or other animal fats, and γ -linolenic concentration in these fats is very low.³⁵ Third, because of the cross-sectional design, we were not able to evaluate the temporal relation between dietary linolenic and CAC. Although this is a weakness, the impact of diet on the atherosclerotic process is likely to become manifest after a reasonable latent period, and the average of \approx 7 years between baseline assessment of dietary linolenic acid and measurement of CAC ensures that the exposure precedes the expected effect. Finally, confounding by unmeasured factors or residual confounding cannot be completely ruled out in our study. Nevertheless, the large sample size, the availability of data on

TABLE 3. Odds Ratios of CAC (per Gram Increase of Linolenic Acid) Stratified by Ratio of n-6 -to n-3, Fish Consumption, and Education Among 2004 Participants in the FHS Study*

	n	Cases	Odds Ratio (95% CI)
n-6/n-3 ratio			
\leq 9.7 (median value)	1003	282	0.45 (0.25–0.80)
$>$ 9.7	1001	267	0.23 (0.11–0.48)
Fish consumption per week			
0	595	116	0.33 (0.12–0.93)
1	915	285	0.44 (0.23–0.85)
\geq 2	494	148	0.30 (0.13–0.68)
Education†			
\leq High school graduate	675	263	0.20 (0.05–0.82)
Some college	699	155	0.45 (0.29–0.70)
College graduate	627	129	0.73 (0.29–1.88)

*Model controls for age, sex, risk group (high vs low CAD risk), education, smoking status, statin use, fruit and vegetable consumption, and history of CAD, hypertension, and diabetes.

†Three subjects had missing data on education.

major CAD risk factors, and the wide range of linolenic acid were major strengths of the study.

In conclusion, our findings indicate that a higher intake of dietary linolenic acid (range, 0.17 to 3.48 g/d) is associated with lower prevalence of CAC (AHA type Vb) in white men and women in a dose-response manner.

Acknowledgments

This study was supported by NHLBI Cooperative Agreement grants U01 HL 67893, U01 HL67894, U01 HL67895, U01 HL67896, U01 HL67897, U01 HL67898, U01 HL67899, U01 HL67900, U01 HL67901, U01 HL67902, and K01-HL70444. This report is presented on behalf of the investigators of the NHLBI FHS. The investigators thank the FHS participants and staff for their valuable contributions.

References

- Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*. 1995;92:1355–1374.
- Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, Schwartz RS. Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using noncalcifying methodology. *J Am Coll Cardiol*. 1998;31:126–133.
- Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area: a histopathologic correlative study. *Circulation*. 1995;92:2157–2162.
- Rumberger JA, Behrenbeck T, Breen JF, Sheedy PF. Coronary calcification by electron beam computed tomography and obstructive coronary artery disease: a model for costs and effectiveness of diagnosis as compared with conventional cardiac testing methods. *J Am Coll Cardiol*. 1999;33:453–462.
- Detrano R, Hsiai T, Wang S, Puentes G, Fallavollita J, Shields P, Stanford W, Wolfkiel C, Georgiou D, Budoff M, Reed J. Prognostic value of coronary calcification and angiographic stenoses in patients undergoing coronary angiography. *J Am Coll Cardiol*. 1996;27:285–290.
- Keelan PC, Bielak LF, Ashai K, Jamjoum LS, Denktas AE, Rumberger JA, Sheedy PF II, Peyser PA, Schwartz RS. Long-term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. *Circulation*. 2001;104:412–417.
- de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet*. 1994;343:1454–1459.
- Hu FB, Stamper MJ, Manson JE, Rimm EB, Wolk A, Colditz GA, Hennekens CH, Willett WC. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *Am J Clin Nutr*. 1999;69:890–897.
- Baylin A, Kabagambe EK, Ascherio A, Spiegelman D, Campos H. Adipose tissue alpha-linolenic acid and nonfatal acute myocardial infarction in Costa Rica. *Circulation*. 2003;107:1586–1591.
- Rastogi T, Reddy KS, Vaz M, Spiegelman D, Prabhakaran D, Willett WC, Stamper MJ, Ascherio A. Diet and risk of ischemic heart disease in India. *Am J Clin Nutr*. 2004;79:582–592.
- Engler MM, Engler MB, Erickson SK, Paul SM. Dietary gamma-linolenic acid lowers blood pressure and alters aortic reactivity and cholesterol metabolism in hypertension. *J Hypertens*. 1992;10:1197–1204.
- Engler MM, Schambelan M, Engler MB, Ball DL, Goodfriend TL. Effects of dietary gamma-linolenic acid on blood pressure and adrenal angiotensin receptors in hypertensive rats. *Proc Soc Exp Biol Med*. 1998;218:234–237.
- Guvernau M, Meza N, Barja P, Roman O. Clinical and experimental study on the long-term effect of dietary gamma-linolenic acid on plasma lipids, platelet aggregation, thromboxane formation, and prostacyclin production. *Prostaglandins Leukotrienes Essent Fatty Acids*. 1994;51:311–316.
- Laidlaw M, Holub BJ. Effects of supplementation with fish oil-derived n-3 fatty acids and gamma-linolenic acid on circulating plasma lipids and fatty acid profiles in women. *Am J Clin Nutr*. 2003;77:37–42.
- Djousse L, Hunt CS, Arnett DK, Province MA, Eckfeldt JH, Ellison RC. Dietary linolenic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr*. 2003;78:1098–1102.
- Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *Am J Clin Nutr*. 1996;63:116–122.
- James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr*. 2000;71:343S–348S.
- Higgins M, Province M, Heiss G, Eckfeldt J, Ellison RC, Folsom AR, Rao DC, Sprafka JM, Williams R. NHLBI Family Heart Study: objectives and design. *Am J Epidemiol*. 1996;143:1219–1228.
- Hunt SC, Williams RR, Barlow GK. A comparison of positive family history definitions for defining risk of future disease. *J Chronic Dis*. 1986;39:809–821.
- Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr, Sidney S, Bild DE, Williams OD, Detrano RC. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of the Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. *Radiology*. 2005;1:35–43.
- Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*. 1985;122:51–65.
- Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semi-quantitative food frequency questionnaire among male health professionals. *Am J Epidemiol*. 1992;135:1114–1126.
- Stein AD, Shea S, Basch CE, Contento IR, Zybert P. Consistency of the Willett semiquantitative food frequency questionnaire and 24-hour dietary recalls in estimating nutrient intakes of preschool children. *Am J Epidemiol*. 1992;135:667–677.
- US Department of Agriculture. *Composition of Foods: Raw, Processed, and Prepared, 1963–1988*. Washington, DC: US Government Printing Office; 1989. Agriculture Handbook No. 8.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
- Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA*. 1997;277:472–477.
- Djousse L, Pankow JS, Eckfeldt JH, Folsom AR, Hopkins PN, Province MA, Hong Y, Ellison RC. Relation between dietary linolenic acid and coronary artery disease in the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr*. 2001;74:612–619.
- Djousse L, Folsom AR, Province MA, Hunt SC, Ellison RC. Dietary linolenic acid and carotid atherosclerosis: the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr*. 2003;77:819–825.
- Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, Rumberger J, Stanford W, White R, Taubert K. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications: a statement for health professionals from the American Heart Association Writing Group. *Circulation*. 1996;94:1175–1192.
- Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
- Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A. Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. *Atherosclerosis*. 2003;167:237–242.
- Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation*. 2003;108:155–160.
- Djousse L, Arnett DK, Pankow JS, Hopkins PN, Province MA, Ellison RC. Dietary linolenic acid is associated with a lower prevalence of hypertension in the NHLBI Family Heart Study. *Hypertension*. 2005;45:1–6.
- Trabulsi J, Schoeller DA. Evaluation of dietary assessment instruments against doubly labeled water, a biomarker of habitual energy intake. *Am J Physiol*. 2001;281:E891–E899.
- Nettleton JA. Omega-3 fatty acids: comparison of plant and seafood sources in human nutrition. *J Am Diet Assoc*. 1991;91:331–337.

Dietary Linolenic Acid Is Inversely Associated With Calcified Atherosclerotic Plaque in the Coronary Arteries: The National Heart, Lung, and Blood Institute Family Heart Study
Luc Djoussé, Donna K. Arnett, J. Jeffrey Carr, John H. Eckfeldt, Paul N. Hopkins, Michael A. Province and R. Curtis Ellison

Circulation. 2005;111:2921-2926; originally published online May 31, 2005;
doi: 10.1161/CIRCULATIONAHA.104.489534

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:

<http://circ.ahajournals.org/content/111/22/2921>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Circulation* is online at:
<http://circ.ahajournals.org/subscriptions/>