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Original Contribution

Nut and Seed Consumption and Inflammatory Markers in the Multi-Ethnic Study of Atherosclerosis

Rui Jiang[1], David R. Jacobs, Jr.[2,3], Elizabeth Mayer-Davis[4], Moyses Szklo[5], David Herrington[6], Nancy S. Jenny[7], Richard Kronmal[8], and R. Graham Barr[1,9]

[1] Division of General Medicine, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY.
[2] Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN.
[3] Department of Nutrition, Institute of Basal Medical Sciences, University of Oslo, Oslo, Norway.
[4] Center for Research in Nutrition and Health Disparities and Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC.
[5] Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.
[6] Department of Internal Medicine/Cardiology, School of Medicine, Wake Forest University, Winston-Salem, NC.
[7] Department of Pathology, College of Medicine, University of Vermont, Colchester, VT.
[8] Department of Biostatistics, School of Public Health, University of Washington, Seattle, WA.
[9] Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY.

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Nuts and seeds are rich in unsaturated fat and other nutrients that may reduce inflammation. Frequent nut consumption is associated with lower risk of cardiovascular disease and type 2 diabetes. The authors examined associations between nut and seed consumption and C-reactive protein, interleukin-6, and fibrinogen in the Multi-Ethnic Study of Atherosclerosis. This 2000 cross-sectional analysis included 6,080 US participants aged 45–84 years with adequate information on diet and biomarkers. Nut and seed consumption was categorized as never/rare, less than once/week, 1–4 times/week, and five or more times/week. After adjustment for age, gender, race/ethnicity, site, education, income, smoking, physical activity, use of fish oil supplements, and other dietary factors, mean biomarker levels in categories of increasing consumption were as follows: C-reactive protein—1.98, 1.97, 1.80, and 1.72 mg/liter; interleukin-6—1.25, 1.24, 1.21, and 1.15 pg/ml; and fibrinogen—343, 338, 338, and 331 mg/dl (all p's for trend < 0.01). Further adjustment for hypertension, diabetes, medication use, and lipid levels yielded similar results. Additional adjustment for body mass index moderately attenuated the magnitude of the associations, yielding borderline statistical significance. Associations of nut and seed consumption with these biomarkers were not modified by body mass index, waist:hip ratio, or race/ethnicity. Frequent nut and seed consumption was associated with lower levels of inflammatory markers, which may partially explain the inverse association of nut consumption with cardiovascular disease and diabetes risk.

Arachis hypogaea; C-reactive protein; fibrinogen; inflammation; interleukin-6; nuts; seeds

Abbreviations: FFQ, food frequency questionnaire; IRAS, Insulin Resistance Atherosclerosis Study; MESA, Multi-Ethnic Study of Atherosclerosis.

Inflammation appears to be a key process in the development of atherosclerosis, cardiovascular disease, insulin resistance, and type 2 diabetes mellitus (1–3). The inflammatory markers C-reactive protein, interleukin-6, and fibrinogen

Correspondence to Dr. R. Graham Barr, PH-9 East, Room 105, Columbia University Medical Center, 622 West 168th Street, New York, NY 10032 (e-mail: rgb9@columbia.edu).

are independent predictors of cardiovascular disease and type 2 diabetes in prospective studies (4–7). Clinical and epidemiologic studies suggest that dietary factors such as n-3 polyunsaturated fatty acids, antioxidant vitamins, dietary fiber, and L-arginine may play an important role in modulating inflammation (8–18).

Nuts and seeds are rich in unsaturated fatty acids, antioxidant vitamins (especially vitamin E), dietary fiber, and plant protein (19). Frequent nut consumption has been associated with reduced risk of cardiovascular disease and type 2 diabetes in prospective studies (20–28). Several possible mechanisms for these associations have been proposed, including reduced serum concentrations of total and low density lipoprotein cholesterol and platelet aggregation, increased antioxidant activity, and improved insulin sensitivity (22, 27). Two recent randomized clinical trials demonstrated that a Mediterranean-style diet and a conventional cholesterol-lowering diet high in almonds, plant sterols, soy proteins, and fiber reduced plasma C-reactive protein and interleukin-6 levels (29, 30). These studies suggested that nut and seed consumption may decrease levels of inflammatory factors, but it is not known whether the effects were specifically for nuts.

Therefore, we examined the associations of nut and seed consumption with levels of C-reactive protein, interleukin-6, and fibrinogen in a well-characterized multiethnic population, hypothesizing that frequent consumption of nuts and seeds would be associated with lower levels of inflammatory markers. Since levels of C-reactive protein and fibrinogen have been noted to be higher among African Americans than among White Americans (31–34) and it is not clear whether ethnic background modifies the relation between nut and seed consumption and markers of inflammation, we also examined effect modification by race/ethnicity.

MATERIALS AND METHODS

Study population

The Multi-Ethnic Study of Atherosclerosis (MESA) was initiated in July 2000 to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease in a population-based sample of 6,814 persons aged 45–84 years who were free of clinical cardiovascular disease at baseline. The cohort was recruited from six US communities. It included 38.5 percent Whites, 27.8 percent African Americans, 21.9 percent Hispanics, and 11.8 percent Asians, who were predominantly of Chinese decent (35). The baseline examination included comprehensive measurements of subclinical cardiovascular disease and assessment of standard cardiovascular disease risk factors, sociodemographic factors, diet, lifestyle, and psychosocial factors. Blood samples were obtained from each participant. For the present analysis, we excluded participants without information on nut and seed consumption ($n = 577$) and participants with extremely low (<600 kcal/day) or high (>6,000 kcal/day) total energy intakes ($n = 157$). The MESA protocol was approved by institutional review boards at all participating sites, and all participants gave informed consent.

Dietary assessment

We used a self-administered food frequency questionnaire (FFQ) and dietary supplement form to assess the participants' diet and supplement use. The FFQ was modified from an FFQ used in the triethnic (non-Hispanic Whites, African Americans, and Hispanics) Insulin Resistance Atherosclerosis Study (IRAS). The FFQ was modified for the Diabetes Prevention Program to include foods typically consumed by a Chinese population and to collect supplemental information (35, 36). Although this FFQ was not specifically validated in MESA, the IRAS questionnaire on which it was based demonstrated reasonable validity and reproducibility in a diverse cohort (37). For validity, the mean correlation coefficients for correlation between nutrient intake estimated from the FFQ and intake from the average of eight 24-hour recalls were 0.61–0.62 for non-Hispanic Whites, 0.50 for African Americans, and 0.41 for Hispanics. For reproducibility, the mean correlation coefficient for nutrients evaluated was 0.62 and did not differ by ethnic subgroup.

The FFQ asked participants how often, on average, they had consumed listed items (e.g., nuts, seeds, peanuts and peanut butter) during the previous year and the average serving size of items consumed. Nine frequency responses were listed, ranging from "never/rare" to "2+ per day," and three serving sizes were specified: small, medium, and large. We calculated daily servings of nuts, seeds, and peanuts and peanut butter by converting the consumption frequency to servings per day and adjusting for serving size (small = 0.5 × medium; medium = standard age- and gender-specific serving size; large = 1.5 × medium). Total nut and seed consumption was calculated as the sum of the daily intakes for nuts, seeds, and peanuts and peanut butter.

Blood collection and assessment of biomarkers

Blood samples were collected, processed, and stored at −80°C using a standardized protocol based on that used for the Cardiovascular Health Study (38).

Levels of C-reactive protein, interleukin-6, and fibrinogen were measured in baseline blood samples at the Laboratory for Clinical Biochemistry Research at the University of Vermont (Colchester, Vermont). C-reactive protein and fibrinogen concentrations were measured using the BNII nephelometer (N high sensitivity C-reactive protein and N antiserum to human fibrinogen; Dade Behring, Inc., Deerfield, Illinois). Interleukin-6 concentrations were measured by ultrasensitive enzyme-linked immunosorbent assay (Quantikine HS human interleukin-6 immunoassay; R&D Systems, Minneapolis, Minnesota). Analytical coefficients of variation for these assays were 3.6 percent, 2.7 percent, and 6.3 percent, respectively.

Levels of total cholesterol, high density lipoprotein cholesterol, and triglyceride were measured at the Collaborative Studies Clinical Laboratory at Fairview-University Medical

TABLE 1. Baseline characteristics of the study population according to frequency of total nut and seed consumption, Multi-Ethnic Study of Atherosclerosis, United States, 2000

Characteristic	Frequency of total nut and seed consumption			
	Never/rare ($n = 925$)	Less than once/week ($n = 2,288$)	1–4 times/week ($n = 2,197$)	≥5 times/week ($n = 670$)
Mean age (years)	63.1 (10.6)*	61.4 (10.1)	62.3 (10.2)	63.5 (10.2)
Male gender (%)	47.4	45.4	48.7	53.3
Race/ethnicity (%)				
Caucasian	23.6	36.9	47.0	50.2
African-American	22.0	26.9	25.4	27.6
Hispanic	41.1	23.1	14.5	14.0
Chinese	13.3	13.1	13.1	8.2
Education (%)				
Less than high school	34.7	18.0	13.2	9.9
High school	19.6	18.3	17.6	14.8
<4 years of college	24.1	29.1	28.3	28.2
≥4 years of college	21.7	34.6	40.9	47.1
Annual family income (%)				
<$25,000	44.7	29.3	28.1	25.7
$25,000–$50,000	28.3	31.4	26.9	27.6
>$50,000	27.0	39.3	45.0	46.7
Mean body mass index†	28.6 (5.7)	28.4 (5.4)	28.0 (5.4)	27.9 (5.1)
Mean waist circumference (cm)	99.3 (14.5)	98.0 (14.3)	97.2 (14.2)	98.1 (14.0)
Mean physical activity (metabolic equivalent-minutes/week)	4,566 (5,463)	5,051 (5,469)	4,871 (5,711)	5,004 (5,942)
Current smoking (%)	13.9	13.2	12.0	10.0
Mean pack-years of cigarette smoking	11.3 (29.9)	11.0 (20.1)	11.2 (20.4)	12.9 (23.5)
Mean alcohol consumption (g/day)	3.2 (9.4)	3.7 (9.0)	4.5 (9.6)	5.3 (11.0)
History of hypertension (%)	49.1	44.7	43.1	42.2
History of diabetes (%)	17.3	15.3	12.7	13.3
Low density lipoprotein cholesterol level (mg/dl)	116 (31.7)	118 (31.8)	117 (29.7)	116 (32.5)
Mean ratio of total cholesterol to high density lipoprotein cholesterol	4.2 (1.2)	4.1 (1.3)	4.0 (1.2)	3.9 (1.2)
Mean triglyceride level (mg/dl)	143 (77.9)	137 (81.3)	132 (72.2)	126 (72.7)
Mean daily dietary intake				
Polyunsaturated fat (% of calories)	7.7 (3.3)	7.7 (2.9)	7.9 (2.6)	8.7 (2.3)
α-Linoleic acid (% of calories)	0.63 (0.24)	0.64 (0.23)	0.66 (0.23)	0.72 (0.22)
Eicosapentaenoic acid plus docosahexaenoic acid (g/day)	0.11 (0.10)	0.12 (0.10)	0.15 (0.13)	0.18 (0.17)
Saturated fat (% of calories)	10.6 (3.3)	10.7 (3.0)	10.7 (2.9)	10.6 (3.2)
Trans-fat (% of calories)	1.6 (0.8)	1.7 (0.8)	1.7 (0.7)	1.7 (0.7)
Total energy (kcal/day)	1,491 (708)	1,535 (683)	1,766 (767)	2,173 (962)
Vegetables (servings/day/1,000 kcal)	1.5 (1.0)	1.5 (0.9)	1.6 (0.8)	1.5 (0.8)
Fruits (servings/day/1,000 kcal)	1.5 (1.3)	1.5 (1.2)	1.5 (1.1)	1.6 (1.2)
Fish (servings/day/1,000 kcal)	0.04 (0.07)	0.04 (0.07)	0.05 (0.08)	0.06 (0.10)
Use of fish oil supplements (%)	2.5	3.2	4.4	5.5

* Numbers in parentheses, standard deviation.
† Weight (kg)/height $(m)^2$.

TABLE 2. Adjusted mean levels of inflammatory markers according to frequency of total nut and seed consumption, Multi-Ethnic Study of Atherosclerosis, United States, 2000

Inflammatory marker†	Frequency of total nut and seed consumption				*p* for trend
	Never/rare	Less than once/week	1–4 times/week	≥5 times/week	
C-reactive protein (mg/liter)	(*n* = 917)	(*n* = 2,273)	(*n* = 2,183)	(*n* = 666)	
Age-adjusted	2.06	2.00	1.77***	1.69***	<0.001
Model 1‡	1.98	1.97	1.80**	1.72**	0.003
Model 2§	1.97	1.96	1.81*	1.71**	0.003
Model 3¶	1.91	1.94	1.82	1.78	0.06
Interleukin-6 (pg/ml)	(*n* = 898)	(*n* = 2,229)	(*n* = 2,133)	(*n* = 654)	
Age-adjusted	1.30	1.24*	1.19***	1.15***	<0.001
Model 1‡	1.25	1.24	1.21	1.15**	0.004
Model 2§	1.25	1.24	1.21	1.14**	0.003
Model 3¶	1.23	1.24	1.21	1.17	0.05
Fibrinogen (mg/dl)	(*n* = 915)	(*n* = 2,274)	(*n* = 2,182)	(*n* = 669)	
Age-adjusted	348	339***	335***	329***	<0.001
Model 1‡	343	338	338*	331***	0.003
Model 2§	343	338*	338*	331***	0.003
Model 3¶	342	338	338	332**	0.03

* $0.05 \leq p < 0.10$; ** $0.01 \leq p < 0.05$; *** $p < 0.01$ (in comparison with participants who reported never or rarely eating nuts and seeds). Tests of significance were based on log-transformed values.

† Antilogs of adjusted mean levels of log-transformed inflammatory markers.

‡ Results were adjusted for age, gender, race/ethnicity, site, educational attainment, family income, smoking status (never, past, or current), pack-years of smoking, alcohol consumption, total physical activity, use of fish oil supplements, and dietary intake of fruits, vegetables, *trans*-fat, fish, and total energy. Numbers of observations removed from the analyses because of missing values: C-reactive protein, 68; interleukin-6, 66; fibrinogen, 69.

§ Results were adjusted for all of the variables in model 1 plus hypertension, diabetes, levels of total and low density lipoprotein cholesterol, and use of statins, angiotensin-converting enzyme inhibitors, β-blockers, thiazolidinediones, aspirin, and estrogens. Numbers of observations removed from the analyses because of missing values: C-reactive protein, 158; interleukin-6, 154; fibrinogen, 160.

¶ Results were adjusted for all of the variables in model 2 plus body mass index (a strong determinant of inflammatory markers which could be on the causal pathway between nut and seed consumption and systemic inflammation). Numbers of observations removed from the analyses because of missing values: C-reactive protein, 158; interleukin-6, 154; fibrinogen, 160.

Center (Minneapolis, Minnesota). Low density lipoprotein cholesterol level was calculated by means of the Friedewald equation (39).

Assessment of potential confounders

Information collected via questionnaire included age, gender, race/ethnicity, educational attainment, family income, smoking status, medical conditions, and current use of prescription and nonprescription medications. Body weight and height were directly measured. Body mass index was calculated as the ratio of weight (in kg) to squared height (in m). Physical activity was measured using a detailed, semiquantitative questionnaire adapted from the Cross-Culture Activity Participation Study (35). Total physical activity was computed as the total of all light, moderate, and vigorous activities (minutes per week) multiplied by the activities' individual metabolic equivalent values.

Statistical analysis

We categorized participants into four groups according to their frequency of nut and seed consumption (never/rare, less than once/week, 1–4 times/week, and ≥5 times/week), consistent with prior publications and with having an adequate number of participants in each cell for reliable analysis (22, 27). Means and standard deviations or proportions were calculated for selected variables by categories of nut and seed consumption.

For the primary analysis, we modeled the associations between nut and seed consumption and inflammation markers using linear regression. Inflammatory markers were natural log-transformed to improve normality of the distributions. In the multivariate models, we first adjusted for age, gender, race/ethnicity, field site, education, income, smoking status, pack-years of smoking, alcohol consumption, physical activity, and several dietary variables. We further adjusted for hypertension, diabetes, medication use, and

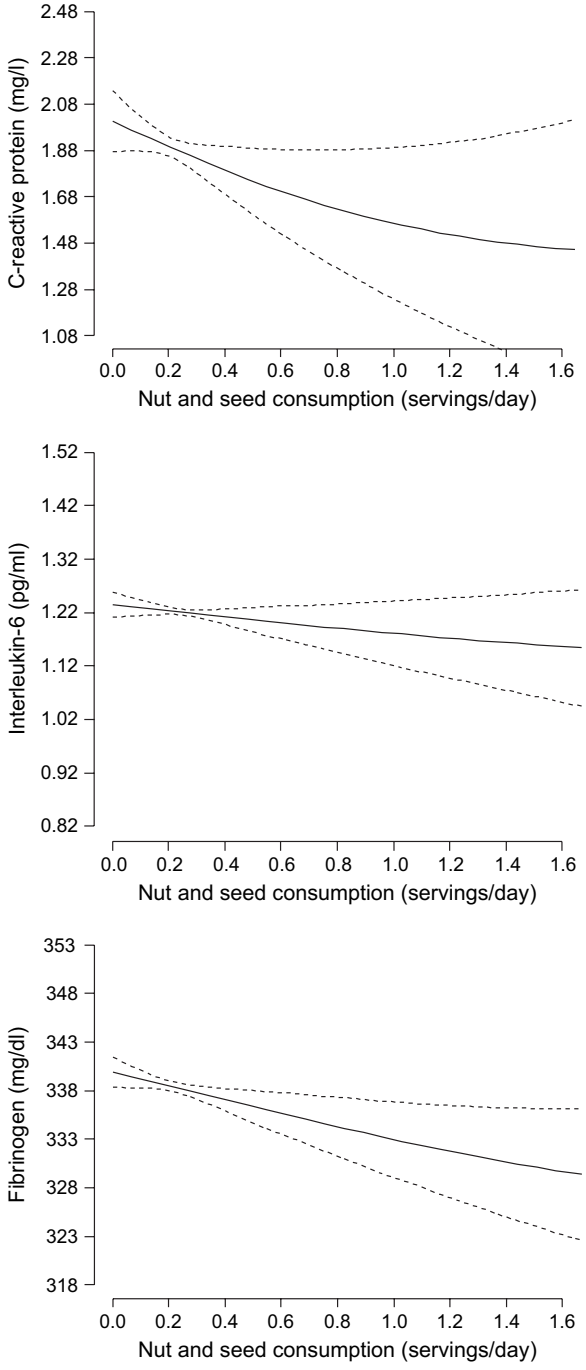

FIGURE 1. Levels of inflammatory markers according to total nut and seed consumption, Multi-Ethnic Study of Atherosclerosis, United States, 2000. The results, obtained from spline regression models, were adjusted for age, gender, race/ethnicity, site, educational attainment, family income, smoking status (never, past, or current), pack-years of smoking, alcohol consumption, total physical activity, use of fish oil supplements, and dietary intake of fruits, vegetables, *trans*-fat, fish, and total energy. Dashed lines, 95% confidence interval.

lipid levels. Finally, we adjusted for body mass index, a strong determinant of inflammatory markers which could be on the causal pathway between nut and seed consumption and systemic inflammation. We used a set of spline terms generated from restricted cubic spline regressions (38–40) for each of the continuous covariates in the analyses in order to reduce residual confounding by the use of categories for continuous confounders. Antilogs of adjusted mean levels of log-transformed inflammatory markers were presented. We conducted tests for trend by using the median values of the categories of nut and seed consumption to form continuous variables. We also used restricted cubic spline regressions with four knots to plot and model flexibly the associations between nut and seed consumption and inflammatory markers. Nonlinearity was tested in nested models using a -2 log-likelihood test.

We further conducted stratified analyses to examine whether the associations between nut and seed consumption and inflammatory markers were modified by body mass index, waist:hip circumference ratio, or race/ethnicity. Interaction terms for interaction between nut and seed consumption and body mass index, waist:hip ratio, or race/ethnicity on the additive scale were entered into the models, and tests for models with and without interaction terms were used to evaluate the significance of the interaction terms.

All p values were two-sided; p values less than 0.05 were considered statistically significant. We used SAS (version 9; SAS Institute, Inc., Cary, North Carolina) and R (version 2.0.1; www.r-project.org) software for analyses.

RESULTS

Among the 6,080 MESA participants with adequate dietary information who had blood drawn, the mean age was 62.2 years; 40.0 percent were White, 25.7 percent were African-American, 21.7 percent were Hispanic, and 12.6 percent were Chinese.

The characteristics of participants are shown in table 1, stratified by frequency of nut and seed consumption. Participants who consumed nuts and seeds more frequently were more likely to be male and Caucasian; generally had more education, a higher family income, and healthier habits; and were leaner than those who rarely or never ate nuts and seeds. Although their average number of pack-years of smoking was higher than that in the other groups, they were less likely to report histories of hypertension and diabetes and had better lipid profiles. Participants who consumed nuts and seeds more frequently had higher intakes of alcohol, polyunsaturated fat, and total energy; however, intakes of vegetables, fruits, saturated fat, and *trans*-fat were similar across categories of nut and seed consumption.

Age-adjusted Spearman partial correlation coefficients showed that total nut and seed consumption was negatively correlated with levels of C-reactive protein ($r = -0.06$, $p < 0.001$), interleukin-6 ($r = -0.05$, $p < 0.001$), and fibrinogen ($r = -0.07$, $p < 0.001$). Levels of C-reactive protein, interleukin-6, and fibrinogen were moderately correlated, ranging from 0.41 for interleukin-6 and fibrinogen to 0.54 for C-reactive protein and interleukin-6.

Frequent total nut and seed consumption was significantly inversely associated with plasma levels of C-reactive protein, interleukin-6, and fibrinogen in age-adjusted models

TABLE 3. Adjusted mean levels of inflammatory markers according to frequency of total nut and seed consumption, by categories of body mass index† and waist:hip circumference ratio, Multi-Ethnic Study of Atherosclerosis, United States, 2000

Inflammatory marker‡	Frequency of total nut and seed consumption				*p* for trend	*p* for interaction
	Never/rare	Less than once/week	1–4 times/week	≥5 times/week		
C-reactive protein (mg/liter)						
Body mass index§						0.71
<28	1.36	1.34	1.27	1.21	0.09	
≥28	2.95	3.05	2.82	2.78	0.19	
Waist:hip ratio§						0.61
<0.94	1.77	1.68	1.56*	1.45**	0.02	
≥0.94	2.24	2.33	2.18	2.09	0.14	
Interleukin-6 (pg/ml)						
Body mass index§						0.62
<28	1.04	1.04	1.00	0.99	0.17	
≥28	1.51	1.53	1.54	1.42	0.09	
Waist:hip ratio§						0.86
<0.94	1.09	1.09	1.07	1.00*	0.04	
≥0.94	1.43	1.44	1.41	1.34	0.08	
Fibrinogen (mg/dl)						
Body mass index§						0.28
<28	328	324	325	321	0.27	
≥28	358	355	354	344**	0.02	
Waist:hip ratio§						0.79
<0.94	336	328**	333	324**	0.14	
≥0.94	350	349	344	339**	0.02	

* $0.05 \leq p < 0.10$; ** $0.01 \leq p < 0.05$; *** $p < 0.01$ (in comparison with participants who reported never or rarely eating nuts and seeds). Tests of significance were based on log-transformed values.

† Weight (kg)/height $(m)^2$.

‡ Antilogs of adjusted mean levels of log-transformed inflammatory markers.

§ Median values were used as the cutoff points. Results were adjusted for age, gender, race/ethnicity, site, educational attainment, family income, smoking status (never, past, or current), pack-years of smoking, alcohol consumption, total physical activity, use of fish oil supplements, and dietary intake of fruits, vegetables, *trans*-fat, fish, and total energy.

(table 2). After controlling for all potentially confounding variables except body mass index, the inverse associations did not change appreciably. After additional adjustment for body mass index, the associations were moderately attenuated. Regression splines demonstrated consistent inverse relations of total nut and seed consumption with levels of C-reactive protein, interleukin-6, and fibrinogen (figure 1). Nonlinear functions did not improve model fit in comparison with linear functions, and observed associations were consistent in the top quartile of inflammatory markers, which may be most relevant to cardiovascular disease risk.

To examine whether the relations between nut and seed consumption and inflammatory markers were modified by obesity, we conducted multivariate analyses within strata defined by categories of body mass index and waist:hip ratio. We found no apparent modification of the relations by obesity (table 3).

We also examined individually the relations between inflammatory markers and consumption of nuts, seeds, or peanuts and peanut butter (table 4). The inverse associations with C-reactive protein, interleukin-6, and fibrinogen were similar for nuts, seeds, and peanuts and peanut butter, although they were not always statistically significant.

We further conducted multivariate analyses within strata defined by ethnic group (table 5). Inverse associations were statistically significant among Caucasians, marginally significant among African Americans, and present but not statistically significant among Hispanics, probably because of a small sample size. These associations remained similar after further adjustment for use of botanicals, and they were attenuated by adjustment for body mass index (data not shown). There was no apparent association between nut and seed consumption and inflammatory markers among Chinese participants.

TABLE 4. Adjusted mean levels of inflammatory markers according to frequency of consumption of nuts, seeds, or peanuts and peanut butter, Multi-Ethnic Study of Atherosclerosis, United States, 2000

Type of nuts, seeds, or peanuts/peanut butter consumed and inflammatory marker†,‡	Frequency of consumption				*p* for trend
	Never/rare	Less than once/week	1–4 times/week	≥5 times/week	
Almonds, walnuts, pecans, or other nuts	(*n* = 2,203)	(*n* = 2,616)	(*n* = 1,051)	(*n* = 210)	
C-reactive protein (mg/liter)	1.98	1.87	1.76***	1.69*	0.02
Interleukin-6 (pg/ml)	1.24	1.22	1.22	1.14*	0.11
Fibrinogen (mg/dl)	341	336**	336**	333	0.11
Sunflower, pinyon, or other seeds	(*n* = 4,857)	(*n* = 927)	(*n* = 245)	(*n* = 51)	
C-reactive protein (mg/liter)	1.90	1.80	1.95	1.29**	0.08
Interleukin-6 (pg/ml)	1.23	1.20	1.24	1.09	0.40
Fibrinogen (mg/dl)	339	334**	333	326	0.06
Peanuts and peanut butter	(*n* = 1,711)	(*n* = 2,733)	(*n* = 1,401)	(*n* = 235)	
C-reactive protein (mg/liter)	1.90	1.91	1.86	1.67	0.10
Interleukin-6 (pg/ml)	1.24	1.24	1.18*	1.11**	0.002
Fibrinogen (mg/dl)	339	338	338	329**	0.07

* $0.05 \leq p < 0.10$; ** $0.01 \leq p < 0.05$; *** $p < 0.01$ (in comparison with participants who reported never or rarely eating nuts and seeds). Tests of significance were based on log-transformed values.

† Antilogs of adjusted mean levels of log-transformed inflammatory markers.

‡ Results were adjusted for age, gender, race/ethnicity, site, educational attainment, family income, smoking status (never, past, or current), pack-years of smoking, alcohol consumption, total physical activity, use of fish oil supplements, and dietary intake of fruits, vegetables, *trans*-fat, fish, and total energy. Numbers of observations removed from the analyses because of missing values: C-reactive protein, 109; interleukin-6, 232; fibrinogen, 109.

In a secondary analysis that excluded participants who reported having a sinus infection ($n = 510$), tooth infection ($n = 166$), or urinary infection ($n = 74$) during the 2 weeks before blood was drawn, results were not substantially altered.

DISCUSSION

In this cross-sectional study, nut and seed consumption was inversely associated with levels of the inflammatory markers C-reactive protein, interleukin-6, and fibrinogen. Qualitatively similar associations were observed for nuts, seeds, and peanuts and peanut butter. There was no significant additive interaction between nut and seed consumption and body mass index, waist:hip ratio, or race/ethnicity for these inflammatory markers.

Although nut and seed consumption has not previously been independently associated with levels of C-reactive protein, interleukin-6, and fibrinogen, various components of nuts and seeds have been shown to have antiinflammatory properties. Investigators in several cross-sectional studies reported that α-linolenic acid (18:3(n-3)), derived from nuts and other plant sources, and the long-chain fatty acids eicosapentaenoic acid (20:5(n-3)) and docosahexaenoic acid (22:6(n-3)), derived from fish or fish oil (the human body is able to convert a portion of α-linolenic acid to eicosapentaenoic and docosahexaenoic acid), were inversely associated with levels of C-reactive protein, interleukin-6, soluble tumor necrosis factor receptors 1 and 2, and fibrinogen in healthy subjects and/or patients with stable coronary artery disease (10, 11, 41, 42). Several intervention studies demonstrated that a diet high in n-3 fatty acids (α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) significantly decreased levels of C-reactive protein, interleukin-6, fibrinogen, tumor necrosis factor-α, and several other inflammatory markers (8, 9, 43, 44). Dietary supplements containing high doses of vitamin E were shown to significantly decrease levels of C-reactive protein and monocyte interleukin-6 in normal volunteers and patients with type 2 diabetes (13). An antiinflammatory effect of dietary fiber and L-arginine has also been suggested (14, 16–18).

Our findings suggest that one mechanism linking nut and seed consumption with the risks of cardiovascular disease and type 2 diabetes may involve influences on the process of vascular inflammation. Inflammation is emerging as an important risk factor for cardiovascular disease and type 2 diabetes. It has been hypothesized that inflammation may contribute to these disease processes by inducing endothelial dysfunction, which could be a key unifying factor in the shared pathogenesis of cardiovascular disease and diabetes (45). Prospective data have shown that markers of endothelial dysfunction are powerful predictors of cardiovascular disease and diabetes (45). However, few studies have evaluated the independent effects of nuts and seeds on inflammation (46). A randomized crossover trial conducted in Spain found that substituting walnuts for monounsaturated fat in a Mediterranean diet did not change C-reactive protein levels significantly, which could be due to the use of an

TABLE 5. Adjusted mean levels of inflammatory markers according to frequency of total nut and seed consumption, by race/ethnicity, Multi-Ethnic Study of Atherosclerosis, United States, 2000

Race/ethnicity and inflammatory marker†,‡	Frequency of total nut and seed consumption				*p* for trend§
	Never/rare	Less than once/week	1–4 times/week	≥5 times/week	
Caucasian	(*n* = 218)	(*n* = 845)	(*n* = 1,032)	(*n* = 336)	
C-reactive protein (mg/liter)	1.86	1.91	1.68	1.57*	0.01
Interleukin-6 (pg/ml)	1.20	1.18	1.16	1.05**	0.004
Fibrinogen (mg/dl)	331	327	329	319**	0.03
African-American	(*n* = 204)	(*n* = 615)	(*n* = 559)	(*n* = 185)	
C-reactive protein (mg/liter)	2.53	2.52	2.37	2.09	0.05
Interleukin-6 (pg/ml)	1.37	1.42	1.32	1.28	0.07
Fibrinogen (mg/dl)	351	354	355	341	0.049
Hispanic	(*n* = 380)	(*n* = 529)	(*n* = 319)	(*n* = 94)	
C-reactive protein (mg/liter)	2.64	2.34	2.22**	2.28	0.31
Interleukin-6 (pg/ml)	1.46	1.38	1.43	1.41	0.99
Fibrinogen (mg/dl)	357	350	343**	349	0.29
Chinese	(*n* = 123)	(*n* = 299)	(*n* = 287)	(*n* = 55)	
C-reactive protein (mg/liter)	0.87	0.98	0.91	1.15	0.30
Interleukin-6 (pg/ml)	0.90	0.92	0.88	0.95	0.84
Fibrinogen (mg/dl)	335	322**	323*	318*	0.35

* $0.05 \leq p < 0.10$; ** $0.01 \leq p < 0.05$; *** $p < 0.01$ (in comparison with participants who reported never or rarely eating nuts and seeds). Tests of significance were based on log-transformed values.

† Antilogs of adjusted mean levels of log-transformed inflammatory markers.

‡ Results were adjusted for age, gender, race/ethnicity, site, educational attainment, family income, smoking status (never, past, or current), pack-years of smoking, alcohol consumption, total physical activity, use of fish oil supplements, and dietary intake of fruits, vegetables, *trans*-fat, fish, and total energy. Numbers of observations removed from the analyses because of missing values: Caucasians—C-reactive protein, 41; interleukin-6, 75; fibrinogen, 40; African Americans—C-reactive protein, 43; interleukin-6, 91; fibrinogen, 43; Hispanics—C-reactive protein, 22; interleukin-6, 55; fibrinogen, 25; Chinese—C-reactive protein, 3; interleukin-6, 11; fibrinogen, 1.

§ *p* values for interaction across categories of race/ethnicity: 0.25 for C-reactive protein, 0.34 for interleukin-6, and 0.85 for fibrinogen.

outpatient intervention diet as opposed to a controlled feeding trial with meals prepared in a metabolic kitchen (46). Our study, to our knowledge, is the first study to show an independent association between intake of nuts and seeds and inflammatory markers. Clearly, more studies are needed to define the role of nuts and seeds in systemic vascular inflammation.

To our knowledge, there are no published results relating nut and seed consumption to inflammatory markers by race/ethnicity. We observed an inverse association between nut and seed consumption and levels of C-reactive protein, interleukin-6, and fibrinogen among Caucasians, African Americans, and Hispanics, although the inverse association did not meet standard thresholds of statistical significance among African Americans and Hispanics, probably because of the relatively small sample sizes. We observed no apparent associations among Chinese Americans. Possible explanations for the lack of associations in Chinese include the relatively small number of Chinese participants and the fact that, in MESA, only 2 percent of Chinese reported consuming nuts and seeds five or more times per week. Another possible explanation is the fact that the FFQ used in this study was based heavily on the IRAS questionnaire, which was validated in Caucasians, African Americans, and Hispanics but not in Chinese. Nondifferential misclassification of dietary assessment in the Chinese population (e.g., due to hidden consumption of nuts and seeds in various dishes and recipes) may have biased the results towards the null. Nevertheless, the possibility of race/ethnicity-specific associations of nut and seed consumption with inflammatory markers warrants further investigation.

The attenuation of the magnitude of the associations between nut and seed consumption and levels of inflammatory markers after adjustment for body mass index should be interpreted cautiously. Body mass index could be an intermediate variable through which nut and seed consumption affects levels of inflammatory markers. Nut and seed consumption was inversely associated with body mass index in this study and in previous epidemiologic studies (47), despite the fact that nuts and seeds are calorie-dense foods. It is possible that frequent nut and seed consumption may lead to weight loss due to incomplete digestion of nuts and seeds and enhancement of satiety (47). Body mass index is also a strong determinant of levels of inflammatory markers.

If body mass index is indeed on a causal pathway between nut and seed consumption and systemic inflammation, conditioning the data on body mass index may introduce additional confounding, which may bias estimates in either direction, leading to unreliable effect estimates.

A main limitation of this study was the cross-sectional design, and we cannot rule out the possibility of reverse causation. Another major concern is that unmeasured aspects of a healthy lifestyle may have caused the lower inflammatory marker levels in persons who ate nuts and seeds frequently. To overcome possible confounding, we adjusted for physical activity, smoking, and several dietary variables considered to be related to a healthy diet in our multivariate analyses. In addition, we used spline terms for each of the continuous variables in the analyses to reduce residual confounding by the use of categories for the continuous covariates. Other concerns also warrant consideration. Dietary intake was self-reported in this study; therefore, there was inevitable measurement error in estimating nut and seed consumption, which, if nondifferential, may have led to underestimation of the true associations. However, nut and seed consumption was probably well reported by this cohort, because it was negatively correlated with serum total cholesterol level, the ratio of total cholesterol to high density lipoprotein cholesterol, and triglyceride level, which is consistent with the published randomized clinical trial literature (48–50).

In conclusion, the results of this study support the hypothesis that frequent nut and seed consumption is associated with lower levels of the inflammatory markers C-reactive protein, interleukin-6, and fibrinogen, although these associations were moderately attenuated by additional adjustment for body mass index.

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