Associations between nut consumption and inflammatory biomarkers[1,2]

*Zhi Yu,[3,8] Vasanti S Malik,[4,8] NaNa Keum,[4] Frank B Hu,[4–6] Edward L Giovannucci,[4–6] Meir J Stampfer,[4–6] Walter C Willett,[4–6] Charles S Fuchs,[6,7] and Ying Bao[6]**

[3]Division of Rheumatology, Allergy and Immunology, Section of Clinical Sciences, Brigham and Women's Hospital, Boston, MA; Departments of [4]Nutrition and [5]Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA; [6]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; and [7]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

ABSTRACT

Background: Increased nut consumption has been associated with reduced risk of cardiovascular disease and type 2 diabetes, as well as a healthy lipid profile. However, the associations between nut consumption and inflammatory biomarkers are unclear.

Objective: We investigated habitual nut consumption in relation to inflammatory biomarkers in 2 large cohorts of US men and women.

Design: We analyzed cross-sectional data from 5013 participants in the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS) who were free of diabetes. Nut intake, defined as intake of peanuts and other nuts, was estimated from food-frequency questionnaires, and cumulative averages from 1986 and 1990 in the NHS and from 1990 and 1994 in the HPFS were used. Plasma biomarkers were collected in 1989–1990 in the NHS and 1993–1995 in the HPFS. Multivariate linear regression was used to assess the associations of nut consumption with fasting plasma C-reactive protein (CRP, n = 4941), interleukin 6 (IL-6, n = 2859), and tumor necrosis factor receptor 2 (TNFR2, n = 2905).

Results: A greater intake of nuts was associated with lower amounts of a subset of inflammatory biomarkers, after adjusting for demographic, medical, dietary, and lifestyle variables. The relative concentrations (ratios) and 95% CIs comparing subjects with nut intake of ≥5 times/wk and those in the categories of never or almost never were as follows: CRP: 0.80 (0.69, 0.90), P-trend = 0.0003; and IL-6: 0.86 (0.77, 0.97), P-trend = 0.006. These associations remained significant after further adjustment for body mass index. No significant association was observed with TNFR2. Substituting 3 servings of nuts/wk for 3 servings of red meat, processed meat, eggs, or refined grains/wk was associated with significantly lower CRP (all $P < 0.0001$) and IL-6 (P ranges from 0.001 to 0.017).

Conclusion: Frequent nut consumption was associated with a healthy profile of inflammatory biomarkers. *Am J Clin Nutr* 2016;104:722–8.

Keywords: inflammatory biomarkers, nuts, peanuts, substitution, tree nuts, peanut butter

INTRODUCTION

Nuts are nutrient-dense, rich in unsaturated fatty acids, high-quality plant protein, fiber, minerals, vitamins, and other bioactive compounds such as phytosterols and phenolic antioxidants (1, 2). Epidemiologic studies have consistently shown that intake of nuts is associated with reduced risk of cardiovascular disease (CVD)[9] and type 2 diabetes (3), and short-term trials have demonstrated beneficial effects on intermediate markers of cardiovascular disease risk, particularly lipids with a dose-related reduction in LDL cholesterol (4, 5). Moreover, a variety of healthful components in nuts such as magnesium, fiber, α-linolenic acid, L-arginine, antioxidants, and unsaturated fatty acids may also confer protection against inflammation (6).

Inflammation is a key process in the development of atherosclerosis associated with future CVD events (7). Chronic inflammation is also closely related to the pathogenesis of type 2 diabetes (8). Although several trials have investigated the associations between nut consumption and inflammatory biomarkers, results have been inconsistent and limited by small sample size ($n \leq 50$), short duration, targeting subjects with certain health conditions, and combined effects with other dietary factors (9, 10). Few prospective cohort studies have examined the associations between nut intake and inflammatory biomarkers (11, 12). In a cross-sectional analysis of the Multi-Ethnic Study of Atherosclerosis study, frequent nut consumption

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[2] Supplemental Figure 1 and Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

[8] These authors contributed equally to this study.

*To whom correspondence should be addressed. E-mail: ying.bao@channing.harvard.edu.

[9] Abbreviations: CRP, C-reactive protein; CVD, cardiovascular disease; FFQ, food-frequency questionnaire; HPFS, Health Professionals Follow-Up Study; ICAM-1, intracellular adhesion molecule-1; NHS, Nurses' Health Study; PREDIMED, Primary Prevention of Cardiovascular Disease with a Mediterranean Diet; TNFR2, TNF receptor 2.

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was associated with lower concentrations of C-reactive protein (CRP), IL-6, and fibrinogen (12). Among participants with high cardiovascular disease risk in the Primary Prevention of Cardiovascular Disease with a Mediterranean Diet (PREDIMED) trial, those with the highest consumption of nuts at baseline had the lowest concentrations of CRP, IL-6, and intracellular adhesion molecule-1 (11). In both of these studies, however, nut intake was only measured at one point in time, and thus the studies were less able to capture habitual intake. Therefore, in the current study, we aimed to investigate the associations between usual nut intake and biomarkers of inflammation in 2 large prospective cohorts of US adults using repeated measurements of nut intake including separate data on peanuts, other nuts, and peanut butter. We also examined the associations between substituting nuts for sources of animal protein, refined grains, potatoes, and potato chips with concentrations of inflammatory biomarkers.

METHODS

Study population

Our cross-sectional analysis was conducted in 2 ongoing prospective cohort studies: the Nurses' Health Study (NHS), which consists of 121,700 female registered nurses aged 30–55 y at baseline in 1976, and the Health Professionals Follow-Up Study (HPFS), which consists of 51,529 male health professionals aged 40–75 y at baseline in 1986. For each cohort, mailed questionnaires were administered biennially to collect data on lifestyle factors and health, with a follow-up rate exceeding 90% for each 2-y cycle. Between 1989 and 1990, 32,826 women in the NHS provided a blood sample and between 1993 and 1995, 18,159 men from the HPFS provided a blood sample. As previously reported, participants who provided a blood specimen were generally similar to those who did not in terms of diet and lifestyle (13). For the current study, we included participants who provided a blood sample and were previously selected as controls for nested case-control analyses of type 2 diabetes, ischemic heart disease, stroke, colon cancer, colon polyps, pancreatic cancer, breast cancer (NHS), and prostate cancer (HPFS). Participants with self-reported prevalent diabetes at blood draw were excluded, as were those who fasted less than 8 h before blood collection. After these exclusions, a total of 5013 individuals with valid nut intake data were included in the current analysis (**Supplemental Figure 1**). Because different combinations of biomarkers were measured by substudies, the sample sizes for each biomarker varied: CRP (n = 4941), IL-6 (n = 2859), and TNF receptor 2 (TNFR2) (n = 2905). The study protocol was approved by the Institutional Review Board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of the Harvard T.H. Chan School of Public Health.

Dietary assessment

Dietary intake was measured by use of validated food-frequency questionnaires (FFQs) administered every 4 y as described in detail previously (14). For the current study, we used the last 2 FFQs before blood collection for each cohort (1986 and 1990 for NHS and 1990 and 1994 for HPFS). Participants were asked to report how often, on average, they consumed a standard portion of foods and beverages, by use of 9 possible responses ranging from "never or less than once per month" to "6 or more times per day." Nutrient intakes were calculated by multiplying the frequency of consumption of each unit of food and beverage by nutrient contents and summing across all items. Values were obtained with the use of the USDA food composition database. FFQ items on nut consumption included "peanuts," "other nuts," and "peanut butter." "Other nuts" was regarded as all types of tree nuts. Total nut consumption was defined as the intake of peanuts and other nuts and did not include peanut butter. One serving of nuts was equivalent to 28 g (1 oz) of peanuts or other nuts and was equivalent to 1 tablespoon peanut butter. A validation study of the FFQ indicated that nut intake correlated well with intakes assessed by multiple dietary records (r = 0.75) (15).

Biochemical analysis

Blood sample collections for both cohorts were described in detail previously (16, 17). Briefly, a phlebotomy kit and instructions were sent to participants willing to provide blood specimens between 1989 and 1990 in NHS and between 1993 and 1995 in HPFS. Samples were returned by overnight mail with a frozen water bottle and processed immediately on arrival. Whole blood samples were separated into plasma, buffy coat, and erythrocytes and stored in the vapor phase of liquid nitrogen freezers at −150°C until analysis. Quality control samples were routinely frozen with study samples to monitor potential changes caused by long-term storage and to assess assay stability. All biomarkers were measured in the Clinical Chemistry Laboratory at the Children's Hospital in Boston. Plasma CRP was measured with the use of a highly sensitive immunoturbidimetric assay with reagents and calibrators from Denka Seiken. IL-6 and TNFR2 were measured with the use of enzyme-linked immunosorbent assays (R&D Systems). The mean intra-assay CVs were <10% for all assays. Our outcomes included CRP because it is the best characterized and most used biomarker of inflammation, and an elevated CRP concentration has been shown to predict the development of both cardiovascular events and type 2 diabetes (18); our outcomes also included IL-6 and TNFR2 because they are key cytokines that mediate both acute and chronic inflammation and have both been shown to be associated with cardiometabolic risk (19, 20).

Covariates

In biennial follow-up questionnaires, we updated information on lifestyle factors and medical history, including age, body weight, smoking status, physical activity, medication use, and history of chronic diseases. For nondietary covariates in this analysis, we used questionnaires administered closest in time to blood draw (1990 for NHS and 1994 for HPFS). BMI (in kg/m^2) was calculated using height measured in 1976 for the NHS and 1986 for the HPFS and weight measured closest to blood draw (1990 for NHS and 1994 for HPFS). Information on dietary factors was obtained from the last 2 FFQs before blood collection for each cohort (1986 and 1990 for NHS and 1990 and 1994 for HPFS), and cumulative averages were used. The inflammatory diet score was calculated as previously described (21), with a higher score indicating a more inflammatory diet.

This pattern represents a diet relatively high in intakes of fish (other than dark meat fish), tomatoes, processed meat, sugar-sweetened beverages, other vegetables (i.e., vegetables other than green leafy vegetables and dark yellow vegetables), red meat, artificially sweetened beverages, refined grains, and organ meats but low in pizza, wine, green leafy vegetables, dark yellow vegetables (comprising carrots, yellow squash, and yams), beer, coffee, fruit juice, snacks, and tea. The reason for the apparently contradictory observation that tomatoes increase the score but pizza decreases it is because tomato paste, often used as pizza sauce, contains a 2.5–4-fold higher concentration of lycopene (a nutrient with anti-inflammatory properties) compared with fresh tomatoes (22), and previous studies have generally found no associations between fresh tomatoes and concentrations of inflammatory biomarkers (23). Nut intake was not included in the calculation of the inflammatory diet score.

Statistical analysis

Distributions of continuous biomarkers were assessed for normality, and if skewed they were natural log-transformed in analyses. To better reflect recent nut consumption, we calculated the cumulative average of intakes from the last 2 FFQs before blood collection for each cohort (1986 and 1990 FFQs of NHS and 1990 and 1994 FFQs of HPFS). The distribution of nut intake in each cycle was examined, and nut consumption remained constant in both cohorts. To minimize the impact of potential outliers and to facilitate pooling of results from the 2 cohorts, we used the same categories for nut intake based on the FFQs: never or almost never, <1 time/wk, 1 time/wk, 2–4 times/wk, and ≥ 5 times/wk.

Generalized linear models were used to evaluate associations between total nut intake and plasma biomarker concentrations. To account for variation in sample handing and laboratory drift among batches, all biomarkers were recalibrated by use of the method of Rosner et al. (24). We tested for interactions by sex and found no evidence that the associations of interest were different in men and women (P-interaction > 0.05 for all 3 markers), so we combined primary data from the 2 cohorts. Model 1 was adjusted for demographic information including sex and age at blood draw (continuous). Model 2 was additionally adjusted for medical history and lifestyle variables including history of hypertension (yes or no), history of hypercholesterolemia (yes or no), smoking status (current, former, or never), physical activity (continuous), alcohol intake (NHS: 0, 0.1–4.9, 5.0–14.9, or ≥ 15 g/d; HPFS: 0, 0.1–4.9, 5.0–29.9, or ≥ 30 g/d), total energy intake (continuous), inflammatory diet score (continuous), and menopausal status and postmenopausal hormone use (premenopausal, postmenopausal without hormone use, or postmenopausal with hormone use) in NHS. Model 3 was further adjusted for BMI, a potential mediator of the association between nut intake and inflammation. For continuous covariates, we assigned corresponding medians to the missing values. For categorical covariates, subjects with missing data were assigned to corresponding reference groups. Least-squares means of biomarkers were estimated in frequency categories of nut intake, and linear trends were tested. For better illustration, we presented the results using relative concentrations (ratios) with 95% CIs of the biomarker amounts among subjects with greater nut consumption to those with the lowest intake category (i.e., never or almost never as reference). Potential interactions with BMI and medical history were tested by adding an interaction term of nut intake (continuous) with BMI (<25 compared with ≥ 25), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), or inflammatory diet score (quartiles). We also performed the analysis by type of nut (peanuts or other nuts), as well as peanut butter because it is a popular source of nuts in the diet.

The effect of substituting 3 servings of nuts/wk for 3 servings of animal protein food sources or poor quality carbohydrate foods/wk was estimated by including both terms as continuous variables in the same multiple regression model (25, 26). The differences in their β coefficients, variances, and covariance were used to estimate β coefficients $\pm$ SEs and P values of the substitution effect. Both animal protein foods and poor quality carbohydrates have been previously associated with cardiovascular disease and/or diabetes in our cohorts. Individual substitution foods included (1 oz = 28 g, 1 cup = 227 g): red meat (serving size: 4–6 oz), processed meat (serving size: piece or slice), poultry (serving size: 4–6 oz), seafood (serving size: 3–5 oz), eggs (serving size: 1), potatoes (including boiled or mashed potatoes and French fries) (serving size: 1 cup), potato chips (serving size: small bag or 1 oz), and refined grains (serving size: 1 cup cooked).

In a sensitivity analysis, we examined the associations between most recent nut consumption (1990 for NHS and 1994 for HPFS) and amounts of inflammatory biomarkers to test the robustness of our main results. For all statistical analyses, 2-sided $P < 0.05$ was considered to be statistically significant. All data analyses were performed with the use of SAS software, version 9.3 for UNIX (SAS 170 Institute).

RESULTS

The age-adjusted characteristics of study participants according to their frequency of nut intake are shown in **Table 1**. Participants from both cohorts who had a higher intake of nuts tended to be older, were more likely to exercise, drank more alcohol, and had lower CRP and IL-6 concentrations than with those with a lower intake. In addition, women in the NHS who had a higher intake of nuts also had lower BMI and were more likely to use postmenopausal hormones than with those with a lower intake.

Associations between nut intake and inflammatory biomarkers

Relative concentrations of inflammatory biomarkers by frequency of nut consumption are shown in **Table 2**. Greater total nut consumption was associated with lower plasma CRP and IL-6 concentrations in age- and sex-adjusted models (model 1); relative concentrations (95% CIs) comparing subjects with nut intake of ≥ 5 times/wk and those in the categories of never or almost never were CRP: 0.81 (0.71, 0.93), P-trend = 0.001; and IL-6: 0.87 (0.78, 0.97), P-trend = 0.005. Similar results were found in multivariate models adjusting for demographic, medical history, and lifestyle variables (model 2); relative concentrations (95% CIs) comparing subjects with nut intake of ≥ 5 times/wk and those in the never or almost never categories were CRP: 0.80 (0.69, 0.90), P-trend = 0.0003; and IL-6: 0.86 (0.77,

TABLE 1
Age-adjusted characteristics of participants in the NHS and HPFS according to frequency of nut consumption[1]

	NHS (n = 3654)			HPFS (n = 1359)		
Characteristic	Never or almost never (n = 957)	1 time/wk (n = 749)	≥5 times/wk (n = 163)	Never or almost never (n = 225)	1 time/wk (n = 340)	≥5 times/wk (n = 129)
Age at blood draw, y	57.7 ± 7.1	58.0 ± 6.7	59.2 ± 6.3	63.7 ± 8.4	64.0 ± 8.2	64.0 ± 8.3
BMI, kg/m^2	25.4 ± 4.6	25.2 ± 4.1	24.6 ± 4.1	25.5 ± 3.2	25.9 ± 3.2	25.5 ± 2.8
Hypertension, %	29.2	30.4	23.6	28.5	28.4	29.1
Hypercholesterolemia, %	42.6	42.2	35.2	39.9	46.1	41.6
Postmenopausal hormone use, %	32.1	36.6	40.1	NA	NA	NA
Smoking status, %						
Never	42.0	47.4	44.5	45.6	47.3	42.5
Former	42.4	40.1	41.8	45.5	46.5	46.3
Current	15.6	12.5	13.7	8.9	6.2	11.3
Alcohol intake, g/d	5.2 ± 10.2	5.5 ± 9.2	7.8 ± 13.2	9.5 ± 13.3	11.6 ± 14.4	16.7 ± 21.1
Physical activity, MET-h/wk	15.4 ± 18.8	15.7 ± 15.9	15.9 ± 18.3	30.9 ± 27.5	39.6 ± 37.3	41.6 ± 44.6
Inflammatory diet score[2]	−0.1 ± 0.3	−0.1 ± 0.3	−0.1 ± 0.3	0.1 ± 0.5	0.1 ± 0.6	0.2 ± 0.7
C-reactive protein,[3] mg/L	1.9 (3.2)	1.6 (2.5)	1.6 (2.4)	1.2 (1.6)	1.1 (1.8)	0.8 (1.3)
IL-6,[3] pg/mL	1.3 (1.1)	1.2 (1.1)	1.1 (1.0)	1.4 (1.3)	1.2 (1.3)	1.2 (0.9)
TNF receptor 2,[3] pg/mL	2569 (863)	2521 (794)	2614 (859)	2653 (1028)	2552 (847)	2701 (872)

[1]Values are means ± SDs or medians (interquartile ranges) unless otherwise indicated. One serving is 28 g. HPFS, Health Professionals Follow-Up Study; MET, metabolic equivalent; NHS, Nurses' Health Study.

[2]Inflammatory diet score represents a diet relatively high in intakes of fish (other than dark-meat fish), tomatoes, processed meat, sugar-sweetened beverages, other vegetables (i.e., vegetables other than green leafy vegetables and dark yellow vegetables), red meat, artificially sweetened beverages, refined grains, and organ meats but low in pizza, wine, green leafy vegetables, dark yellow vegetables (comprising carrots, yellow squash, and yams), beer, coffee, fruit juice, snacks, and tea. Nut intake was not included in the calculation of the inflammatory diet score. The range of the score is −2.27 to 1.28 in the NHS and −4.23 to 3.89 in the HPFS.

[3]Not age-adjusted.

0.97), P-trend = 0.006. These associations were attenuated but remained statistically significant after further adjustment for BMI (model 3); relative concentrations (95% CIs) comparing subjects with nut intake of ≥5 times/wk and those in the categories of never or almost never were CRP: 0.84 (0.74, 0.95), P-trend = 0.006; and IL-6: 0.88 (0.79, 0.99), P-trend = 0.016.

TABLE 2
Relative concentrations (95% CIs) of inflammatory biomarkers by frequency of nut consumption among participants in the NHS and HPFS[1]

	Frequency of nut consumption					
Fasting biomarker relative concentration	Never or almost never	<1 time/wk	1 time/wk	2–4 times/wk	≥5 times/wk	P-trend
C-reactive protein						
N	1143	1497	1082	927	292	
Model 1	Reference	0.95 (0.90, 1.01)	0.93 (0.87, 1.00)	0.86 (0.81, 0.93)	0.81 (0.71, 0.93)	0.001
Model 2	Reference	0.96 (0.90, 1.02)	0.93 (0.86, 0.99)	0.86 (0.79, 0.93)	0.80 (0.69, 0.90)	0.0003
Model 3	Reference	0.96 (0.90, 1.02)	0.93 (0.87, 0.99)	0.90 (0.84, 0.97)	0.84 (0.74, 0.95)	0.006
IL-6						
N	658	856	639	539	167	
Model 1	Reference	0.94 (0.90, 0.99)	0.94 (0.89, 0.99)	0.86 (0.81, 0.92)	0.87 (0.78, 0.97)	0.005
Model 2	Reference	0.94 (0.89, 0.99)	0.95 (0.89, 1.01)	0.86 (0.81, 0.92)	0.86 (0.77, 0.97)	0.006
Model 3	Reference	0.95 (0.90, 1.00)	0.95 (0.90, 1.01)	0.88 (0.83, 0.94)	0.88 (0.79, 0.99)	0.016
TNF receptor 2						
N	673	866	646	551	169	
Model 1	Reference	0.98 (0.97, 1.00)	0.96 (0.94, 0.98)	0.95 (0.93, 0.98)	0.97 (0.93, 1.01)	0.058
Model 2	Reference	0.99 (0.97, 1.01)	0.97 (0.95, 0.99)	0.97 (0.94, 0.99)	0.98 (0.94, 1.02)	0.258
Model 3	Reference	0.99 (0.97, 1.01)	0.97 (0.95, 0.99)	0.97 (0.95, 1.00)	0.99 (0.94, 1.03)	0.478

[1]Biomarker sample sizes vary: C-reactive protein (n = 4941), IL-6 (n = 2859), and TNF receptor 2 (n = 2905). Values were determined by use of general linear models. Model 1 was adjusted for sex and age at blood draw (continuous). Model 2 was adjusted for the variables in model 1 plus a history of hypertension (yes or no), history of hypercholesterolemia (yes or no), smoking status (current, former, or never), physical activity (continuous), alcohol intake (NHS: 0, 0.1–4.9, 5.0–14.9, or ≥15 g/d; HPFS: 0, 0.1–4.9, 5.0–29.9, or ≥30 g/d), total energy intake (continuous), inflammatory diet score (nuts were not included in the calculation; continuous), and in women, menopause status and postmenopausal hormone use (premenopausal, postmenopausal without hormone use, or postmenopausal with hormone use). Model 3 was adjusted for the variables in model 2 plus BMI (continuous). HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

We did not observe statistically significant association between nut consumption and TNFR2 amount. In a sensitivity analysis, similar results were observed with the use of most recent nut consumption instead of cumulative averages (**Supplemental Table 1**). The associations between nut intake and inflammatory biomarkers did not differ by sex. Relative concentrations (95% CIs) comparing subjects with nut intake of ≥5 times/wk and those in the never or almost never categories were CRP: NHS 0.87 (0.73, 1.01), HPFS 0.73 (0.61, 0.86), *P* for heterogeneity = 0.16; IL-6: NHS 0.89 (0.75, 1.03), HPFS 0.88 (0.75, 1.01), *P* for heterogeneity = 0.91; and TNFR2: NHS 0.99 (0.92, 1.05), HPFS 0.99 (0.94, 1.04), *P* for heterogeneity = 0.94. The associations were also not different across groups defined by BMI, hypertension, hypercholesterolemia status, or inflammatory diet score (all *P*-interaction ≥ 0.23) (data not shown). Similarly, intakes of peanuts and other nuts were both inversely associated with inflammatory markers. Higher intake of other nuts was significantly associated with lower CRP and IL-6, and higher intake of peanuts was associated with lower CRP and TNFR2 (**Supplemental Table 2**). However, the tests for heterogeneity between peanuts and other nuts were not statistically significant (*P*-heterogeneity ≥ 0.48 for all 3 biomarkers). No associations were observed for peanut butter.

Substitution analyses of nuts for other food sources

Substituting 3 servings of nuts/wk for 3 servings of red meat, processed meat, eggs, or refined grains/wk was associated with significantly lower CRP (all $P < 0.0001$) and IL-6 (*P* ranges from 0.001 to 0.017). Lower CRP concentrations were also observed when substituting 3 servings of nuts/wk for potatoes ($P = 0.003$) and potato chips ($P = 0.001$) (**Table 3**). Similar results were found for peanuts and other nuts (**Supplemental Table 3**).

TABLE 3
Effect estimates for 1 unit change in biomarkers corresponding to substitution of 3 servings of total nuts/wk for alternative foods among participants in the NHS and HPFS[1]

Alternative foods	C-reactive protein		IL-6	
	β ± SE	*P* value	β ± SE	*P* value
Red meat	−0.205 ± 0.033	<0.0001	−0.084 ± 0.028	0.002
Processed meat	−0.241 ± 0.036	<0.0001	−0.102 ± 0.030	0.001
Poultry	−0.034 ± 0.040	0.392	−0.001 ± 0.034	0.971
Seafood	−0.058 ± 0.038	0.122	−0.003 ± 0.031	0.912
Eggs	−0.180 ± 0.040	<0.0001	−0.088 ± 0.032	0.006
Potatoes[2]	−0.116 ± 0.039	0.003	−0.039 ± 0.032	0.230
Potato chips	−0.147 ± 0.046	0.001	−0.035 ± 0.037	0.349
Refined grains	−0.124 ± 0.027	<0.0001	−0.055 ± 0.023	0.017

[1]Biomarkers were log-transformed. The values were determined by including both nuts and other food sources as continuous variables in the same multiple regression models. The differences in their β coefficients, variances, and covariance were used to estimate the substitution effect. Models were adjusted for sex, age at blood draw (continuous), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), smoking status (current, former, or never), physical activity (continuous), alcohol intake (NHS: 0, 0.1–4.9, 5.0–14.9, or ≥15 g/d; HPFS: 0, 0.1–4.9, 5.0–29.9, or ≥30 g/d), total energy intake (continuous), inflammatory diet score (nuts and alternative food were not included in the calculation; continuous), and in women, menopause status and postmenopausal hormone use (premenopausal, postmenopausal without hormone use, or postmenopausal with hormone use). HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[2]Included boiled or mashed potatoes and French fries.

DISCUSSION

In these 2 large prospective cohorts, nut consumption was inversely associated with concentrations of the inflammatory biomarkers CRP and IL-6. Substituting nuts for red meat, processed meat, eggs, refined grains, potatoes, or potato chips was associated with a healthier inflammatory biomarker profile. To our knowledge, this is the first study that has examined habitual nut intake in relation to inflammatory markers with the use of repeated measurements of diet and has examined the substitution of nuts for animal sources of protein and low-quality carbohydrate foods on inflammation.

Our results are consistent with previous observational studies that have found inverse associations between nuts and inflammatory markers. In a cross-sectional analysis of the Multi-Ethnic Study of Atherosclerosis study, nut and seed consumption was inversely associated with CRP, IL-6, and fibrinogen; relative concentrations comparing ≥5 times/wk with never or almost never were CRP: 0.87; IL-6: 0.92; and fibrinogen: 0.97 (all *P*-trend ≤ 0.004) (12). Similar to our study, comparable associations were observed for tree nuts and peanuts. We did not observe associations with peanut butter, possibly because we underestimated intake amounts. In our analysis, 1 tablespoon peanut butter was considered a serving, but the standard serving size is 2 tablespoons. The lack of association could also be due to loss of healthful components or addition of ingredients that may be unhealthy during processing. In a baseline cross-sectional analysis of the PREDIMED trial, those with the highest nut intake had the lowest concentrations of CRP, IL-6, and intracellular adhesion molecule-1 (11). However, because participants were at high risk of developing CVD, results could be skewed by already elevated concentrations of inflammatory markers. In addition, in both of these studies, nut intake was only measured once, which is less able to capture habitual intake.

In contrast, findings from trials have been inconsistent (9, 10, 27–30). Potential reasons for this include: small sample size, short duration, targeting unhealthy subgroups, use of single nut types, and combined effects with other dietary factors. For example, two 4-wk crossover trials of almonds ($n = 27$) and walnuts ($n = 21$) conducted among individuals with hypercholesterolemia did not find significant changes in CRP (30, 31). The PREDIMED trial observed a reduction in IL-6 but not CRP in participants consuming a Mediterranean diet with 30 g mixed nuts, compared with the low-fat diet group (29). However, the trial of Rajaram et al. (28) found that a diet enriched with 2 doses of almonds/d reduced CRP but not IL-6, relative to a healthy nut-free diet.

Previous studies have shown that nut consumption is inversely associated with BMI despite being an energy-dense food, and BMI is a strong determinant of inflammatory biomarkers. Moreover, weight loss has been repeatedly observed to be associated with a decrease in amounts of inflammatory biomarkers (32–34). Thus the associations between nut intake and inflammatory markers could be mediated in part through BMI (35, 36). Similar to other observational studies (12), in our analysis, associations between nut intake and amounts of CRP and IL-6

were attenuated after adjusting for BMI but remained statistically significant, suggesting that BMI accounts for a proportion of the associations.

Nuts are rich in unsaturated fatty acids, and in several previous trials and cohort studies PUFAs, particularly α-linolenic acid, which is high in walnuts, were inversely associated with CRP, IL-6, TNFR2, and other inflammatory markers (37–43). Other constituents of nuts, such as dietary fiber, vitamin E, L-arginine, and phenolic compounds, have also been suggested to have anti-inflammatory effects (44–49). L-Arginine, an amino acid with a high content in peanuts and hazelnuts, is a precursor for endothelium-derived nitric oxide synthesis, which may also explain the beneficial effects of nut intake on vascular reactivity (10).

Our findings suggest that the inverse associations between nut consumption and risk of CVD and type 2 diabetes may be partly due to association with vascular inflammation. Low-grade systemic inflammation is a well-established common antecedent for both CVD and type 2 diabetes (50), possibly through inducing endothelial dysfunction. Inflammation plays a major role in all phases of atherosclerosis that predicts future CVD events. Both CRP and IL-6 have been identified as independent predictors of CVD (7), and CRP and IL-6 concentrations are associated with quantitative measures of insulin resistance (51). In our cohorts, CRP and IL-6 were associated with increased risk for CHD, whereas TNFR2 was related to increased risk among women only, suggesting that this biomarker may not be as predictive, which may partly explain our null findings with TNFR2 (52).

Our study has several strengths. A large sample size and detailed diet and lifestyle information provided us with sufficient power to detect associations with nut intake and finely control for potential confounding. By use of the cumulative mean nut intake from multiple FFQs, we were able to reduce within-person variability (53). Having detailed information on medical history allowed us to reduce recall bias by excluding participants with chronic diseases that may influence diet. Although our study was conducted among predominantly white health professionals, which increases internal validity, there is no reason to expect that these biological relationships would be different in other populations. Other limitations include the cross-sectional nature of our analysis; although we adjusted for many potential confounders, we cannot exclude the possibility of residual confounding. In addition, some measurement error with the use of FFQs to assess diet and a single measure of inflammatory markers is likely, and such nondifferential misclassification may have attenuated the results. However, the FFQ has been validated, and the correlations for nuts are among the most accurately reported (14, 15), and the long-term stability of plasma biomarkers from our cohorts has been previously reported (54).

In conclusion, we found that greater nut intake was associated with lower concentrations of CRP and IL-6, and these associations were independent of BMI. Substituting nuts for red and processed meat, eggs, refined grains, potatoes, or potato chips was associated with a healthier profile of inflammatory markers. These data support an overall healthful role of nuts in the diet and suggest an inverse association with inflammation as one of the potential mechanisms underlying inverse associations between nuts and cardiometabolic diseases, although confirmation in large trials is warranted.

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