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Almond Consumption Reduces Oxidative DNA Damage and Lipid Peroxidation in Male Smokers[1,2]

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Abstract

Smoking increases the risk of several chronic diseases associated with elevated oxidative stress status. Almonds are a good source of antioxidant nutrients and may diminish smoking-related biomarkers of oxidative stress. We investigated whether almond consumption decreases biomarkers of oxidative stress in young male smokers. We conducted a randomized, crossover clinical trial with 60 healthy male soldiers (18–25 y) who were habitual smokers (5–20 cigarettes/d) and supplemented their diet with 84 g almonds or 120 g pork (to control for calories) daily for 4 wk with a 4-wk washout period between treatment periods. In addition, 30 healthy nonsmoking men were provided the same daily serving of pork as reference comparison. Blood and urine were collected and assessed for biomarkers of oxidative stress. Baseline values of urinary 8-hydroxy-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) and peripheral lymphocyte DNA strand breaks were significantly higher by 185, 64, and 97% in smokers than nonsmokers, whereas activities of plasma superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase were significantly lower by 15, 10, and 9%, respectively. After the almond intervention, serum α-tocopherol, SOD, and GPX increased significantly in smokers by 10, 35, and 16%, respectively and 8-OHdG, MDA, and DNA strand breaks decreased significantly by 28, 34, and 23%. In smokers, after almond supplementation, the concentration of 8-OHdG remained significantly greater than in nonsmokers by 98%. These results suggest almond intake can enhance antioxidant defenses and diminish biomarkers of oxidative stress in smokers. J. Nutr. 137: 2717–2722, 2007.

Introduction

According to the 2002 World Health Report (1), smoking is among the 10 greatest risks to health due to its strong association with several forms of cancer and cardiovascular and pulmonary diseases (2). Smokers between 45 and 64 y have a mortality rate 3 times greater than nonsmokers and this rate is doubled among those 65–84 y (3). Cigarettes contain numerous reactive oxygen and nitrogen species in their smoke and tar and additional oxidants are generated endogenously following exposure to them (4). These reactive species can induce oxidative stress and are a putative source of the etiology and/or progression of smoking-related pathologies (2). Animal model and human studies indicate smoking enhances oxidative damage to DNA as revealed by increased DNA strand breaks and 8-hydroxy-deoxyguanosine (8-OHdG)[5] (5–7) as well as lipid peroxidation reactions evidenced by elevations in malondialdehyde (MDA) and $F_{2\alpha}$-isoprostanes (8–10). Further, smoking decreases the concentration of small molecular antioxidants such as vitamins C and E, β-carotene, and glutathione, as well as measures of total antioxidant capacity (9–11).

Clinical trials have shown that supplementation with dietary antioxidants such as vitamin C, vitamin E, and/or β-carotene can increase their plasma concentration and decrease several biomarkers of oxidative stress (12,13). Importantly, some studies indicate that harm can result from high-dose antioxidant supplements, particularly β-carotene (14,15). However, there are a limited number of studies with dietary interventions of antioxidant-rich foods in smokers; e.g., Hakim et al. (16) found consumption of green tea, a rich source of epigallocatechin gallate, reduced the urinary excretion of 8-OHdG in smokers.

Nut consumption is inversely associated with the incidence of cardiovascular disease, diabetes, and some forms of cancers (17–19). Reflecting their contribution to health promotion, nuts have been incorporated into recommended dietary guidelines in the United States, Canada, and Spain (20) and a qualified health claim for reducing the risk of heart disease is provided by the U.S. FDA (21). Almonds (*Prunus dulcis*) contain an array of

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[5] Abbreviations used: GPX, glutathione peroxidase; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-deoxyguanosine; SOD, superoxide dismutase.
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essential nutrients, including arginine, calcium, potassium, niacin, α-tocopherol, fiber, and monounsaturated fatty acids (22) as well as phytochemicals like phenolic acids and flavonoids (23). We and others have found that almond flavonoids are bioavailable and act as antioxidants, increasing the resistance of LDL to oxidation and decreasing oxidative damage to DNA (24–26). Interestingly, Davis et al. (27) found both almond meal and almond oil reduced aberrant crypt foci in azoxymethane-treated rats. In a small pilot study, we found a 4-wk intervention with 84 or 168 g/d of almonds reduced plasma MDA and lymphocyte DNA strand breaks in young male smokers (26). To confirm these results, we undertook a randomized, placebo-controlled, cross-over clinical trial in a similar cohort to determine the effect of an intake of 84 g/d of almonds on biomarkers of oxidative stress, specifically lymphocyte DNA strand breaks, urinary 8-OHdG and MDA, and plasma, superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase.

Materials and Methods

Sixty male soldiers (aged 21.8 ± 0.2 y) who were habitual smokers (5–20 cigarettes/d with a history of smoking for ≥5 y) and living in a single military camp were recruited from the Chinese Army in Beijing (**Table 1**). In addition, 30 nonsmoking volunteers (aged 21.5 ± 0.4 y) from the same camp were recruited to serve as a reference group. All participants were in good health based on a medical history questionnaire as well as fulfilling the following eligibility criteria: *1*) no history of cardiovascular, hepatic, gastrointestinal, or renal disease; *2*) no alcoholism; and *3*) no use of antibiotic drugs, functional foods, or dietary supplements for ≥4 wk before the start of the study. All participants received the same meals every day in the same canteen. All the volunteers performed only light physical activity throughout the study. All nuts, including peanuts, were omitted from the diet during the study period. All smokers maintained their usual smoking habits throughout the trial. Dietary intakes were assessed the week prior to the trial and the first and the last week of each treatment period using self-administered questionnaires (28). Intakes of protein, fat, and carbohydrate were calculated based on the China Food Composition 2002 database (29). Body weight and height were also recorded at this time. All subjects participated in the protocol concurrently. The study protocol was approved by the Ethical Committee of the National Institute for Nutrition and Food Safety at the Chinese Center for Disease Control and Prevention and written informed consent was obtained from each participant.

Study design. Sixty smokers were enrolled in a placebo-controlled, cross-over clinical trial and randomly assigned to 2 equal-sized subject groups (A and B) (**Fig. 1**). In addition to the standard diet served in the canteen, whole almond powder (84 g), generously provided by the Almond Board of California (Modesto, CA) or pork (120 g) was provided daily to participants in their respective groups for 4 wk. All subjects refrained from consuming nuts and dietary supplements during run-in and washout periods. The total amount of almonds or pork for a day was divided into 3 equal portions and added to the breakfast, lunch, and dinner meals. Other than the almonds and pork, the amount of other foods consumed in the meals was not restricted. The almond powder was consumed with steamed bread made of white flour, steamed rice, or water and the pork was consumed with steamed bread or vegetables. To generally control for the added energy [2.03 MJ (485 kcal)] and protein [1.96 MJ (18 g)] from the almonds, fresh boneless pork was selected as the alternate added food to provide similar energy (468 kcal) and protein (19.8 g) (29) and because it is a common ingredient in the diet of this region. The pork was cooked with soy sauce and salt. Body weights of all smokers did not differ after 4 wk supplementation of almonds or pork. The nonsmoking reference group was supplemented with 120 g pork/d during the 4-wk study period. Compliance with the treatments was achieved by close monitoring from a ranked officer. All subjects were asked to refrain from participation in rigorous physical activities 3 d prior to the collection of blood and urine samples.

TABLE 1 Characteristics of the study subjects[1,2]

Variables	Smokers	Nonsmokers	*P*
n	60	30	—
Age, *y*	21.8 ± 0.2	21.5 ± 0.4	0.55
Smoking history, *y*	6.8 ± 0.3	0	—
Cigarette use, *n/d*	10.2 ± 0.6	0	—
Urinary cotinine, *μmol/mol creatinine*	750 ± 35	10.8 ± 0.9	≤0.0001
Body weight, *kg*	66.7 ± 0.9	66.0 ± 1.2	0.66
BMI, *kg/m²*	22.6 ± 0.3	22.8 ± 0.4	0.77
Energy intake, *kJ/d*	9584 ± 113	9638 ± 75	0.46
Protein, *% energy*	14.8 ± 1.0	14.7 ± 1.2	0.89
Fat, *% energy*	43.2 ± 1.5	44.1 ± 1.4	0.48
Carbohydrate, *% energy*	38.6 ± 2.1	37.7 ± 1.8	0.52
Vegetables and fruits, *g/d*	345.4 ± 4.1	352.3 ± 6.1	0.59
Vitamin E intake, *mg/d*	21.5 ± 0.8	21.4 ± 1.0	0.76

[1] Results are means ± SEM.
[2] Comparisons between smokers and nonsmokers performed using a Student's *t* test.

Blood and urine samples. Four overnight fasted blood samples and 4 24-h urine samples were collected from all the smokers and 2 sets of samples were collected from the nonsmokers before and after the intervention. After volume measurement, aliquots of urine were stored at −80°C until use. Plasma, serum, and lymphocytes were isolated from whole blood within 2 h of collection. All samples were coded, divided into aliquots, and stored at −80°C. Samples from the same individual were batched for analysis in the same assay analysis.

α-Tocopherol in almond powder and serum. α-Tocopherol was measured by the HPLC method of Nomura et al. (30). Briefly, following deproteinization with ethanol, α-tocopherol in the almond powder or serum samples was extracted twice with hexane. The combined supernatant was evaporated under nitrogen, reconstituted with aqueous mobile phase, and separated by a C-18 analytical Ultrasphere ODS column (5 μm, 250 × 4.6 mm; Beckman Coulter) with a guard Ultrasphere ODS column (10 μm, 50 × 4.6 mm). Benzo(a)pyrene was used as an internal standard. The intra- and inter-day CV were 5.2 and 6.5%, respectively. The concentration of vitamin E in the almond powder did not differ before and after 4 wk of storage at 4°C with α-tocopherol at 27.7 ± 0.4 and 29.1 ± 0.6 mg/100 g and with γ-tocopherol at 1.1 ± 0.1 and 1.1 ± 0.1 mg/100 g at the 2 time points, respectively.

Blood clinical chemistries and urinary cotinine. Alanine aminotransferase, aspartate aminotransferase, total protein, albumin, creatinine, and urea nitrogen in serum and creatinine in urine were analyzed with a Hitachi 7060 Automatic Analyzer using commercial kits (Zhongsheng Beikong Bio-technology and Science). Urinary cotinine was determined with the barbituric acid colorimetric method described by Barlow et al. (31) and expressed relative to creatinine.

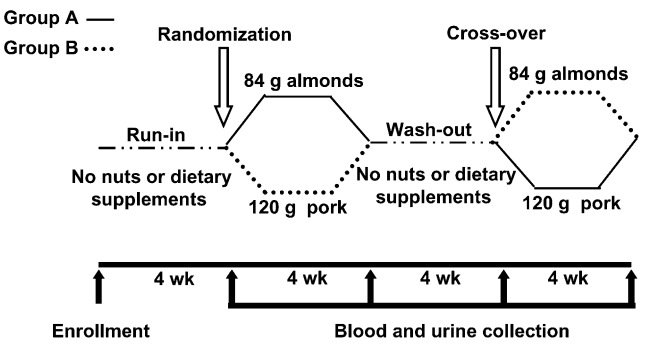

FIGURE 1 Study design.

Urinary 8-OhdG. Urinary 8-OHdG was measured by HPLC with electrochemical detection according to the method of De Martinis et al. (32). The separation of 8-OHdG was achieved using a HPLC system equipped with Hewlett-Packard 1050 pumps, EcoCart analytical columns (125 × 3 mm, Merck), and electrochemical detector. Detection was performed using a glass carbon electrode operated at +0.6 V vs. an Ag/AgCl reference electrode and 50-nA deflection. Urinary 8-OHdG is calculated based on standard curves of an authentic standard (Sigma) and expressed relative to creatinine. The intra- and inter-day CV were 4.5 and 5.7%, respectively.

Comet assay for DNA strand breaks. The comet assay was performed according to the method of Collins et al. (33). Briefly, isolated lymphocytes were embedded in a layer of low-melting point agarose on a microscope slide. After alkaline electrophoresis (pH >13), neutralization, and ethidium bromide staining, DNA migration was evaluated under a Nikon fluorescence microscope. DNA breaks, expressed as percent tail DNA, were quantified using image analysis software (IMI1.0, Shenzhen, China).

Urinary MDA. Urinary MDA was measured using the HPLC method of Olga et al. (34). Briefly, following a reaction with 2,4-dinitrophenylhydrazine, MDA in pentane was dried under nitrogen, reconstituted in 50% acetonitrile, and measured using a Hewlett-Packard HPLC equipped with a 4.6- × 250-mm Hypersil ODS column (Agilent Technologies) and UV detection at 340 nm. MDA concentration is expressed relative to creatinine. The intra- and inter-day CV were 4.6 and 5.1%, respectively.

Plasma SOD, GPX, and catalase. Heparinized plasma was collected from blood after centrifugation at 1300 × *g*; 15 min and 4°C and stored at −80°C. The activity of SOD, GPX, and catalase in plasma was measured by standard colorimetric assays using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis. All results are reported as means ± SEM. Two-factor repeated-measures ANOVA of subject group [A vs. B (almond-pork vs. pork-almond)] and treatment (almonds vs. pork) and their interaction was included in the model to compare differences of pre- and posttreatment in measured variables in smokers fed almonds and pork. Significant differences between smokers and nonsmokers at baseline, as well as between smokers consuming the almond diet and nonsmokers consuming the pork diet at the end of intervention, were assessed by a Student's *t* test. Pearson correlation between urinary cotinine or number of cigarettes smoked and biomarkers of oxidative biomarkers was also examined. Differences with $P \leq 0.05$ were considered significant. The SAS program (Version 8.2, SAS Institute) was used to perform all statistical analyses.

Results

Oxidative stress in smokers vs. nonsmokers. Smoking 10 cigarettes per day resulted in a 70-fold greater urinary excretion of cotinine in smokers than nonsmokers ($P \leq 0.0001$) (Table 1). Smoking was not associated with a difference in the daily fruit and vegetable intake or BMI in this cohort (Table 1). Neither serum vitamin E nor clinical chemistry variables were different between smokers and nonsmokers (**Table 2**). However, lymphocyte DNA strand breaks and urinary 8-OHdG were 1- and 2-fold greater in smokers than nonsmokers, respectively ($P \leq 0.0001$). Urinary MDA was 64% higher in smokers than nonsmokers ($P = 0.005$). The activity of plasma SOD, GPX, and catalase was 15, 10, and 9% lower, respectively, in smokers than in nonsmokers ($P \leq 0.01$).

Urinary cotinine and clinical chemistry. The clinical chemistry variables did not differ after the 4 wk of each food intervention (data not shown). Similarly, urinary cotinine in smokers remained unchanged before and after the almond and pork intervention (750 ± 35 vs. 723 ± 35 μmol/mol creatinine).

TABLE 2 Baseline values of biomarkers in plasma and urine of the study subjects[1,2]

Variables	Smokers	Nonsmokers	*P*
n	60	30	
Serum α-tocopherol, *μmol/L*	14.2 ± 0.4	14.6 ± 0.4	0.43
α-Tocopherol:total cholesterol, *μmol/mmol*	4.3 ± 0.2	3.9 ± 0.2	0.16
Comet assay, *% tail DNA*	14.2 ± 0.9	7.2 ± 1.0	≤0.0001
Urinary 8-OHdG, *μmol/mol creatinine*	18.0 ± 1.0	6.3 ± 0.5	≤0.0001
Urinary MDA, *μmol/mol creatinine*	2.1 ± 0.2	1.3 ± 0.2	0.005
Plasma SOD, *kU/L*	62.0 ± 0.8	73.0 ± 1.6	≤0.0001
Plasma GPX, *U/L*	158.8 ± 0.9	175.7 ± 1.5	≤0.0001
Plasma catalase, *kU/L*	10.1 ± 0.3	11.1 ± 0.2	0.01
Clinical chemistry			
Alanine aminotransferase, *U/L*	14.6 ± 0.7	15.2 ± 1.6	0.68
Aspartate aminotransferase, *U/L*	24.8 ± 1.0	21.9 ± 1.4	0.10
Total protein, *g/L*	76.6 ± 0.5	78.2 ± 0.8	0.07
Albumin, *g/L*	47.6 ± 0.2	48.1 ± 0.4	0.20
Urea nitrogen, *mmol/L*	4.9 ± 0.1	5.4 ± 0.2	0.06
Creatinine, *mmol/L*	93.6 ± 1.8	87.8 ± 3.8	0.12

[1] Results are means ± SEM.
[2] Comparisons between smokers and nonsmokers performed using a Student's *t* test.

Vitamin E and antioxidant enzymes. The 4-wk almond intervention increased daily vitamin E intake by 23 mg α-tocopherol. Serum α-tocopherol in smokers increased 8.6% after the almond diet compared with no change in smokers after the pork diet ($P \leq 0.05$) (**Table 3**), which provided <0.5 mg/d α-tocopherol (35).

Smoking was associated with significantly lower activities of the antioxidant enzymes than those found in the nonsmokers

TABLE 3 Serum α-tocopherol and plasma antioxidant enzymes in young male smokers who consumed diets containing almonds or pork for 4 wk[1]

	Pork	Almonds
Serum α-tocopherol, *μmol/L*		
Pretreatment	14.43 ± 0.37	14.10 ± 0.33
Posttreatment	14.25 ± 0.36	15.48 ± 0.34
Change[2]	−0.18 ± 0.01	1.38 ± 0.01*
SOD, *kU/L*		
Pretreatment	60.0 ± 1.1	56.2 ± 1.1
Posttreatment	63.5 ± 1.8	75.9 ± 1.5
Change[2]	3.5 ± 1.5	19.7 ± 1.5*
GPX, *U/L*		
Pretreatment	163.6 ± 1.0	161.2 ± 1.1
Posttreatment	161.7 ± 1.5	187.8 ± 1.3
Change[2]	−1.9 ± 2.9	26.5 ± 1.5*
Catalase, *kU/L*		
Pretreatment	10.0 ± 0.3	9.0 ± 0.2
Posttreatment	9.7 ± 0.2	8.7 ± 0.2
Change[2]	−0.3 ± 0.4	−0.4 ± 0.2

[1] Values are means ± SEM, $n = 60$. *Different from pork, $P < 0.05$ (2-factor repeated measures ANOVA with group × treatment interaction).
[2] Change = posttreatment − pretreatment.

(Table 2). The almond treatment raised SOD and GPX activity in smokers by 35.0 and 16.4%, respectively ($P \leq 0.05$), whereas pork had no effect (Table 3). Antioxidant enzymes in nonsmokers given pork for 4 wk were not altered (data not shown). SOD activity at 75.9 kU/L in smokers after 4 wk of almond consumption was 6.8% higher than that of the nonsmokers fed pork ($P \leq 0.05$); similarly, GPX activity at 187.8 U/L was 6.3% higher ($P \leq 0.05$). Neither almond nor pork intake affected catalase activity.

Biomarkers of oxidative stress. Biomarkers of oxidative stress in nonsmokers given pork for 4 wk were not altered (data not shown). Using the Comet assay, DNA strand breaks in lymphocytes were higher in smokers than nonsmokers ($P \leq 0.0001$) (Table 2). Daily almond consumption reduced DNA strand breaks by 34% in smokers compared with pretreatment value, whereas pork intake in smokers did not modify this variable ($P \leq 0.05$) (**Table 4**). The magnitude of DNA strand breaks in smokers after the 4-wk almond intervention was not significantly different from that of the nonsmokers. An oxidatively modified nucleic acid, 8-OHdG at the baseline was higher in smokers vs. nonsmokers ($P \leq 0.0001$) (Table 2). The almond diet reduced urinary 8-OHdG in smokers by 28%, whereas the pork intervention had no influence on this outcome in smokers (Table 4). In contrast to DNA strand breaks, urinary 8-OHdG in smokers following the almond intervention remained greater than that of nonsmokers by 98% ($P \leq 0.05$). Urinary MDA was higher in smokers than nonsmokers ($P \leq 0.0001$) and was reduced by 23% in smokers following the almond diet, whereas the pork treatment had no effect in smokers ($P \leq 0.05$). Following almond consumption, urinary MDA was not significantly different from that of the nonsmokers.

Correlation between urinary cotinine and biomarkers of oxidative stress. Urinary cotinine was strongly correlated with the number of cigarettes smoked ($r = 0.74$; $P \leq 0.0001$). However, there were no correlations between cotinine and 8-OHdG ($r = -0.15$), DNA strand breaks (0.04), MDA (0.02), SOD (0.12), GPX (0.03), or catalase (0.09). Among biomarkers of oxidative stress, only urinary 8-OHdG and MDA were correlated ($r = 0.27$; $P = 0.04$).

TABLE 4 Biomarkers of oxidative stress in young male smokers who consumed diets containing almonds or pork for 4 wk[1]

	Pork	Almonds
Comet assay, *% tail DNA*		
Pretreatment	15.3 ± 1.3	15.1 ± 1.1
Posttreatment	14.3 ± 1.1	9.8 ± 0.9
Change[2]	−1.0 ± 1.5	−5.2 ± 1.3*
Urinary 8OHdG, *μmol/mol creatinine*		
Pretreatment	17.0 ± 0.8	18.2 ± 1.0
Posttreatment	17.2 ± 0.8	13.2 ± 0.6
Change[2]	0.2 ± 0.7	−5.0 ± 0.8*
Urinary MDA, *μmol/mol creatinine*		
Pretreatment	2.1 ± 0.1	1.9 ± 0.2
Posttreatment	2.1 ± 0.2	1.5 ± 0.1
Change[2]	0.1 ± 0.2	−0.4 ± 0.1*

[1] Results are means ± SEM, $n = 60$. *Different from pork, $P < 0.05$ (2-factor repeated measures ANOVA with group × treatment interaction).
[2] Change = posttreatment − pretreatment.

Discussion

Smoking, a potent but modifiable risk factor of cancer and cardiovascular disease, acts substantially via reactive species to initiate and promote pathology and is consistently associated with elevated oxidative damage to DNA, lipids, and proteins (2,5,36–39). Although smoking cessation represents the only safe and reliable approach to risk reduction, dietary antioxidant interventions to decrease oxidative stress in smokers and former smokers may contribute to chemoprevention and, by analogy, suggest mechanisms of action for other pathogenic processes associated with oxidative stress and inflammation (40). We conducted a pilot study employing a daily diet containing 84 or 168 g almonds for 4 wk and observed a decrease in urinary 8-OHdG and plasma MDA (26). Because the lower dose was effective and represented a lower energy and more realistic dietary intake, we conducted a fully powered clinical trial of almonds in a stable environment that allowed substantial control and monitoring of food intake and other environmental factors with all the volunteers living in the same place and participating in the study at the same time.

In our cohort of healthy young men with an average 6.8-y history of smoking, we found a mean of 10 cigarettes daily was associated with enhanced lymphocyte DNA strand breaks as well as greater urinary excretion of 8-OHdG and MDA compared with nonsmokers. Further, smoking was correlated with lower activities of the principal antioxidant enzymes in plasma, catalase, GPX, and SOD. Some laboratories have reported increased utilization and turnover rate of vitamin E in smokers compared with nonsmokers (10,41). However, we found comparable serum α-tocopherol in both groups, possibly due to the their general good health or young age, as smoking-induced decreases in α-tocopherol appear more marked in older adults (9,10,37). The absence of a difference between the smokers and nonsmokers in this regard may also partly be a result of their otherwise identical diets, environment, and lifestyle. Nonetheless, a limitation of this study is the lack of a direct assessment of the status of other antioxidants, including vitamin C, glutathione, and uric acid, which reside in a dynamic interrelationship with vitamin E.

Baseline serum α-tocopherol status (14.2 μmol/L) (42) increased 8.6% after the 4-wk almond intervention providing 23 mg/d α-tocopherol compared with the pork diet containing <0.5 mg/d α-tocopherol (35). This increment was smaller than the 19% increment observed by Jambazian et al. (43) who found 56 g/d almonds for 4 wk increased plasma α-tocopherol from 26.3 to 31.2 μmol/L in healthy subjects (aged 41 y; BMI 25.2). This contrast may result from differences in the food matrix in which the almonds were consumed, age of the subjects, smoking status, and/or different baseline status of vitamin E.

Although there are a variety of essential nutrients and other bioactive compounds in almonds, including low concentrations of β-carotene, lutein, and zeaxanthin, we hypothesize the vitamin E, phenolic acids, flavonoids, and other antioxidant polyphenols (23) are responsible for the reduction of lymphocyte DNA strand breaks and urinary 8-OHdG in smokers. However, caution is always warranted when interpreting the outcomes of these biomarkers, because the DNA damage in lymphocytes may not accurately reflect similar injury in other tissues and urinary 8-OHdG is dependent on the balance between its systemic production and DNA repair reactions (44). However, urinary 8-OHdG appears to be a more reproducible and reliable biomarker than its determination in cells due to fewer artifacts being produced during its isolation and analysis (45). The concentration of urinary 8-OHdG excretion in this study is consistent with

results from some clinical interventions with vitamin C supplementation (48), green tea (39), and red ginseng (49) on oxidative DNA damage. However, it is worth contrasting the efficacy of our whole food intervention with the null impact of several studies testing single antioxidant vitamins on protection of DNA against smoking-induced oxidative stress (12,46,47). Although some reports of antioxidant supplementation demonstrate efficacy (48,49) or equivocal results (50), the complex array of essential nutrients and phytochemical antioxidants, as well as other ingredients, such as mono-unsaturated fatty acids and PUFA in almonds may provide opportunities for additive and synergistic interactions.

Several biomarkers of lipid peroxidation, including plasma $F_{2\alpha}$-isoprostanes and MDA, have been consistently and positively associated with the pathogenesis of several chronic diseases linked to smoking (44). However, the relationship between specific constituents of cigarette smoke and tar, including reactive species and aldehydes, and these individual biomarkers has not been established. Consistent with our observation of decreased urinary MDA, consumption of pistachios (51) and hazelnuts (52), other tree nuts containing tocopherols and polyphenols, has also been reported to decrease plasma MDA in vivo. Compared with the control pork diet, we found the almond diet decreased lymphocyte DNA strand breaks by 34.4%, urinary MDA by 22.6%, and urinary 8-OHdG by 27.8%, with the latter 2 outcomes being correlated with one another ($r = 0.27$; $P = 0.03$). Interestingly, these 2 biomarkers in urine may better reflect systemic oxidative stress than reactions in lymphocytes.

In contrast to the absence of an effect on antioxidant enzymes in our pilot, 4-wk almond study (26), we found the almond diet increased GPX and SOD activity not only relative to pretreatment values but compared with the nonsmokers consuming the pork diet. These contrasting results may be due to the larger sample size and the greater control associated with a crossover design in this protocol. Catalase was not affected in either study, possibly due to its very low concentration in plasma vs. that found in erythrocytes, kidney, and liver (53). The increased activity of GPX and SOD may be due to the antioxidant ingredients in almonds, because similar changes have been reported using 280 mg/d vitamin E for 10 wk and 400 mg α-tocopherol for 6 wk in smokers (54,55) and by 400 mg/d naringin for 8 wk in hypercholesterolemic subjects (56). The enhancement in GPX activity is not likely attributable to selenium because of the negligible amount of this mineral provided in the intervention of 2.4 μg/84 g almonds, providing 4.4% of the DRI.

Consistent with its use as an index of smoking status, we found a strong correlation between urinary cotinine and magnitude of cigarette smoking ($P \leq 0.0001$). However, neither cotinine nor daily cigarette number was associated with the biomarkers of oxidative stress we measured. Although Fracasso et al. (57) found basal DNA damage and urinary cotinine to be highly correlated, compared with our trial, they had examined older people (42 vs. 22 y) who were heavier smokers (20 vs. 10 cigarettes/d) and had a longer history of smoking (>20 vs. 6.8 y).

In summary, consuming a diet containing 84 g/d almonds for 4 wk decreased oxidative stress in young male smokers. However, the effects of almonds on the antioxidant defense system and oxidative stress in nonsmokers remains to be explored. Whereas the ingredients responsible for this action were not specifically explored, further research is warranted regarding the contributions of vitamin E and the antioxidant phenolic acids and polyphenols found in almonds to diminishing the adverse impact of oxidative stress.

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