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Research Article

See related editorial by Mayne, p. 2145, and article by Goyal et al., p. 2202

Specialty Supplement Use and Biologic Measures of Oxidative Stress and DNA Damage

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Abstract

Background: Oxidative stress and resulting cellular damage have been suggested to play a role in the etiology of several chronic diseases, including cancer and cardiovascular disease. Identifying factors associated with reduced oxidative stress and resulting damage may guide future disease-prevention strategies.

Methods: In the VITamins And Lifestyle (VITAL) biomarker study of 209 persons living in the Seattle area, we examined the association between current use of several specialty supplements and oxidative stress, DNA damage, and DNA repair capacity. Use of glucosamine, chondroitin, fish oil, methylsulfonylmethane (MSM), coenzyme Q10 (CoQ10), ginseng, ginkgo, and saw palmetto was ascertained by a supplement inventory/interview, whereas the use of fiber supplements was ascertained by questionnaire. Supplements used by more than 30 persons (glucosamine and chondroitin) were evaluated as the trend across number of pills/week (non-use, <14 pills/week, 14+ pills/week), whereas less commonly used supplements were evaluated as use/non-use. Oxidative stress was measured by urinary 8-isoprostane and PGF2α concentrations using enzyme immunoassays (EIA), whereas lymphocyte DNA damage and DNA repair capacity were measured using the Comet assay. Multivariate-adjusted linear regression was used to model the associations between supplement use and oxidative stress/DNA damage.

Results: Use of glucosamine (P_{trend}: 0.01), chondroitin (P_{trend}: 0.003), and fiber supplements (P: 0.01) was associated with reduced PGF2α concentrations, whereas CoQ10 supplementation was associated with reduced baseline DNA damage (P: 0.003).

Conclusions: Use of certain specialty supplements may be associated with reduced oxidative stress and DNA damage.

Impact: Further research is needed to evaluate the association between specialty supplement use and markers of oxidative stress and DNA damage. *Cancer Epidemiol Biomarkers Prev; 22(12); 2312–22. ©2013 AACR.*

Introduction

In a state of oxidative stress, excess reactive species can damage various cellular components, including membrane lipids and DNA (1, 2). This resulting damage has been suggested to play a role in several adverse health outcomes, including cardiovascular disease and certain cancers, though prospective human evidence linking oxidative stress and DNA damage to these diseases remains limited (3, 4). Although there is much interest in identifying modifiable factors associated with oxidative stress, little is known about the association between use of non-vitamin, non-mineral specialty supplements, and oxidative stress/DNA damage.

Specialty supplements are often used for disease prevention, and while some of these supplements have been associated with reduced risk of cancer and other diseases (5–10), the mechanisms underlying these associations are not well studied in humans. Laboratory studies suggest that glucosamine (11, 12), chondroitin (13, 14), and coenzyme Q10 (CoQ10; refs. 15–17) may reduce oxidative stress and/or DNA damage. However, the association between glucosamine and chondroitin and oxidative stress/DNA damage has not been studied in humans, and evidence of an effect of CoQ10 on oxidative stress/DNA damage in humans is conflicting (18–26).

Although little research has been conducted on the associations between specialty supplement use and oxidative stress, several of these supplements have been suggested to have anti-inflammatory properties, including glucosamine (27–29), chondroitin (27, 29, 30), fish oil (29, 31, 32), CoQ10 (33), methylsulfonylmethane (MSM; ref. 34), garlic (35), ginseng (36), ginkgo (37), saw palmetto (38), and fiber (39). Because inflammation and oxidative

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stress can act to propagate one another (2), one might hypothesize that supplements with posited anti-inflammatory properties may also be associated with reduced oxidative stress and DNA damage. Using data on 209 individuals in the VITamins And Lifestyle (VITAL) biomarker study, we examined the associations between oxidative stress, DNA damage, and DNA repair capacity and use of the following specialty supplements posited to have antioxidant and/or anti-inflammatory properties: glucosamine, chondroitin, fish oil, CoQ10, MSM, garlic, ginseng, ginkgo, saw palmetto, and fiber.

Materials and Methods

Study population

VITAL biomarker study participants were drawn from the overall VITAL cohort, a prospective study of 77,719 western Washington residents of ages 50 to 76 years (40). VITAL participants who completed the VITAL baseline questionnaire between October 2000 and February 2001 were eligible for participation in the VITAL biomarker study. Persons living outside the Seattle metropolitan area were excluded from the biomarker study, as were persons with Alzheimer's disease, insulin-dependent diabetes, or any conditions preventing the collection of fasting blood. Of the 290 persons contacted, 220 (76%) agreed to participate and completed the study protocol. The sample was stratified to obtain an equal sex distribution, and the final sample included 149 randomly selected VITAL study respondents and 71 oversampled high users of vitamin C ($n = 26$), vitamin E ($n = 23$), or calcium ($n = 22$). Study participants completed a second mailed questionnaire and participated in an in-home visit, at which blood and urine were collected. Study procedures were approved by the Fred Hutchinson Cancer Research Center (FHCRC; Seattle, WA) Institutional Review Board and all study participants provided written informed consent.

Exposure

Supplement use was ascertained by a supplement inventory/interview conducted at the time of home visit. For all currently used supplements, participants were asked to report on the frequency (times/week) of use, the number of pills taken/occasion of use, and the date at which each supplement was last used. Persons reporting use of a given supplement within the 2 weeks before interview were classified as current users, whereas those reporting no use or last use more than 2 weeks prior were classified as non-users. This home interview provided the source of information on glucosamine, chondroitin, fish oil, CoQ10, MSM, garlic, ginseng, ginkgo, and saw palmetto (men only). Use of fiber supplements was assessed using the mailed biomarker study questionnaire; participants reporting use of fiber-containing supplements at least once a week over the prior month were classified as current users. Supplements used by more than 30 persons (glucosamine and chondroitin) were classified in terms of average pills/week of use [non-use, low use (<14 pills/week), or high use (14+ pills/week)]. Persons missing information on the number of pills/occasion of use ($n = 7$ for glucosamine; $n = 5$ for chondroitin) were assumed to use 1 pill/time, as the majority of glucosamine and chondroitin supplement users reported use of 1 pill/occasion. To preserve power in analyses of less commonly used supplements, the remaining supplements were classified as current use or non-use.

Outcomes

Oxidative stress was measured by urinary 8-isoprostane and PGF2α. 8-Isoprostane, an F2-isoprostane commonly used to measure lipid peroxidation, is formed when reactive species initiate the peroxidation of arachidonic acid stored in the cell membrane in a COX-independent process (41). Recent work has shown that urinary PGF2α is formed by a similar COX-independent isoprostane pathway, and can be used as a measure of oxidative stress (42). 8-Isoprostane and PGF2α were measured in unacidified spot urine collected at the time of home interview using enzyme immunoassays (EIA; Cayman Chemical kits #516351 and #516011, respectively). Urine was processed within approximately 2 hours of collection at the FHCRC Specimen Processing Laboratory and was frozen at −80°C thereafter. EIAs were conducted at the German Cancer Research Center (Heidelberg, Germany) and plates were read using the μQuant spectrophotometer from BioTek Instruments (410 nm). For PGF2α, one value fell below the level of quantification and was replaced with one half of the lowest detected value. The 8-isoprostane and PGF2α assays were corrected for urinary creatinine, and values were expressed as pg/mg creatinine.

Reactive species can cause DNA breaks, and such damage can persist either unrepaired or may be repaired via repair mechanisms (43), though these repair mechanisms may also be affected by oxidative stress (44). We therefore measured DNA damage and DNA repair capacity, as it is DNA damage in the presence of diminished repair capacity which has been implicated in disease etiology (45). DNA damage and repair were measured in viable lymphocytes isolated from semi-fasting (more than 6 hours) blood collected at the time of home interview. Blood was processed within approximately 2 hours of collection at the FHCRC Specimen Processing Laboratory. Viable lymphocytes were isolated by Ficoll-gradient centrifugation and were resuspended before undergoing a controlled-step freezing process. Specimens were frozen at −70°C for less than 48 hours before they were transferred to liquid nitrogen for storage. DNA damage and repair was assessed using the Comet assay, a single-cell electrophoresis assay in which damaged DNA fragments migrate toward the anode, giving the appearance of a comet, where the amount of migrated DNA in the tail represents the amount of DNA damage. The Comet assay was conducted at the German Cancer Research Center as previously described (46, 47) with some modifications. Frozen cells were rapidly thawed, washed, and resuspended in RPMI-1640 medium. Trypan blue staining was

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used to assess cell viability and samples were placed on ice until electrophoresis to prevent repair. Analysis and evaluation of cellular damage was conducted by fluorescence microscopy using the automated cell scanning system Metafer-4 (Metasystems) described by Schunck and colleagues (48). Baseline DNA damage was expressed as the Olive tail moment, which was calculated by first subtracting the head mean from the tail mean, after which this difference was then multiplied by the percentage of DNA in the tail/100. To evaluate DNA repair, DNA damage was induced by irradiating cells on ice with 5 Gy using a ^{137}Cs source with a dose rate of 0.54 Gy/min. After irradiation, samples were incubated for 15 and 60 minutes at 37°C, after which the amount of damage at each of these time points was evaluated to calculate the percentage of DNA damage repaired by that time point. DNA repair capacity was calculated as follows: 1 − [mean tail moment at × minutes/mean tail moment immediately after irradiation].

Of the 220 persons included in the biomarker study, 14 were excluded from analyses of 8-isoprostane and PGF2α due to lack of available urine to measure either the marker of interest or creatinine, leaving 206 persons for analyses corresponding to these measures. Persons with self-reported history of cancer ($n = 38$) were excluded from analyses of DNA damage and repair, and an additional 63 persons were excluded from these analyses if lymphocytes were not viable, less than 60 cells were able to be scored, or more than 50% of the cells were determined to be ghost cells (defined as the lack of cell viability 24 hours after being thawed; ref. 49). Twenty-three persons were excluded from DNA repair capacity analyses due to having implausibly higher baseline damage than induced damage, or a higher level of residual damage than induced damage. After making these exclusions, 119 persons remained eligible for analyses of DNA damage and repair and 97 remained for analyses of DNA repair capacity. Of note, 209 persons had data on any of the biomarkers of interest and are therefore included in our analyses.

Table 1. VITAL biomarker study participant characteristics

Characteristics	*N* (%)
Age, y	
50–<55	48 (23.0)
55–<60	53 (25.4)
60–<65	36 (17.2)
65–<70	29 (13.9)
70+	43 (20.6)
Sex	
Female	102 (48.8)
Male	107 (51.2)
Race/ethnicity	
White	197 (94.3)
Non-White	12 (5.7)
Smoking	
Never	105 (50.2)
Low (<18 pack-years)	52 (24.9)
High (18+ pack-years)	52 (24.9)

Statistical analysis and confounder selection

Details on the study population have been presented in Table 1 and unadjusted Pearson correlation coefficients were calculated to assess the correlation between biomarkers (Table 2).

Linear regression was used to evaluate the associations between specialty supplements and measures of oxidative stress and DNA damage. All specialty supplement exposures were modeled as either binary variables or indicator variables. The P_{trend} for exposures with three or more levels was based on a model with the variable of interest modeled as a single variable with values 1, 2, and 3, where the P value from the Wald test corresponds to the P_{trend}.

Because distributions of 8-isoprostane, PGF2α, and baseline DNA damage were skewed, we log-transformed these variables to normalize their distributions; results have been exponentiated to present the average geometric mean per exposure category (Tables 3 and 4). The distributions of DNA repair capacity at 15 and 60 minutes were nearly normally distributed and were not transformed; these variables were instead presented as arithmetic means.

A priori, all regression analyses were adjusted for age, sex, and pack-years smoked. Additional covariates were selected for inclusion by assessing their association with each outcome in this minimally adjusted model. The broad set of potential confounding variables evaluated included various demographic (race/ethnicity, education), lifestyle/anthropometric [body mass index (BMI), physical activity, and alcohol consumption], medical [aspirin use, baby aspirin use, non-aspirin nonsteroidal anti-inflammatory drug (NSAID) use, use of cholesterol-lowering drugs, history of cardiovascular disease, cancer, diabetes, and sunburns], and dietary/supplementary factors (multivitamin use and intake of the following vitamins and minerals from supplements and from diet and supplements combined: β-carotene, vitamin C, α-tocopherol, iron, selenium, and zinc). Additional dietary factors considered include: energy intake, fiber intake, saturated fat intake, and dietary γ-tocopherol intake. In evaluating associations involving dietary variables, energy intake was included in the model. Because baseline DNA damage may reflect long-term exposures from an accumulation of insults, we also tested a selected subset of variables representing long-term exposure for this outcome, including BMI at age 45 years, 10-year physical activity, as well as 10-year supplemental intake of β-carotene, vitamin C, and α-tocopherol.

Variables associated with one of the five outcomes at the $\alpha = 0.10$ level in the minimally adjusted model were included as covariates in analyses of that outcome. This

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Table 2. Pearson correlation coefficients among biomarkers of oxidative stress, DNA damage, and DNA repair capacity

	8-Isoprostane pg/mg creatinine	PGF2α pg/mg creatinine	Baseline DNA damage Olive tail moment	DNA repair capacity-15 min Olive tail moment (% repaired)	DNA repair capacity-60 min Olive tail moment (% repaired)
8-isoprostane pg/mg creatinine	1.00				
PGF2α pg/mg creatinine	0.22 (*P* = *0.001*)	1.00			
Baseline DNA damage Olive tail moment	−0.03 (*P* = *0.74*)	−0.02 (*P* = *0.83*)	1.00		
DNA repair capacity-15 min Olive tail moment (% repaired)	0.02 (*P* = *0.86*)	0.16 (*P* = *0.12*)	−0.30 (*P* = *0.003*)	1.00	
DNA repair capacity-60 min Olive tail moment (% repaired)	−0.02 (*P* = *0.83*)	0.15 (*P* = *0.15*)	−0.28 (*P* = *0.006*)	0.36 (*P* = *0.003*)	1.00

less stringent α was used for selection of confounders, so as to err on the side of inclusion, rather than exclusion, of potential confounders. We also adjusted for conditions that may have increased use of specific supplements, regardless of association with the outcomes of interest (e.g., for glucosamine and chondroitin use, we adjusted for reported arthritis or chronic pain). Covariates included in our final models are listed in the footnotes of Table 4, where we present the multivariate-adjusted associations between specialty supplement exposures and oxidative stress/DNA damage. To reduce the likelihood of false-positive results, a more stringent α level ($\alpha = 0.01$) was used to assess statistical significance of associations between exposures (specialty supplement use) and markers of oxidative stress, DNA damage, and DNA repair capacity. Analyses were conducted using Stata (version 12).

Results

Our study population was composed of men (51%) and women (49%) of ages 50 to 75 years; persons included in the VITAL biomarker study were predominantly White (94%), and half reported no history of smoking (50%; Table 1). The two measures of oxidative stress, urinary PGF2α and 8-isoprostane, were modestly correlated ($r = 0.22$); neither of these measures were correlated with measures of DNA damage or repair capacity (Table 2). Baseline DNA damage was modestly negatively correlated with repair capacity at 15 and 60 minutes ($r = -0.30$ and -0.28, respectively) and DNA repair capacity at 15 and 60 minutes were positively correlated ($r = 0.36$).

In multivariate-adjusted analyses, none of the specialty supplements studied were significantly associated with 8-isoprostane (Table 4). Current glucosamine use was associated with reduced PGF2α concentrations (P_{trend}: 0.01): persons consuming more than 14 pills/week had a 40% lower adjusted geometric mean PGF2α than non-users [225 pg/mg creatinine; 95% confidence interval (CI), 163–312) vs. 374 pg/mg creatinine; 95% CI, 326–429). Chondroitin use was also associated with reduced PGF2α concentrations (P_{trend}: 0.003): persons using more than 14 pills/week of chondroitin had a geometric mean PGF2α of 196 pg/mg creatinine (95% CI, 131–293), 47% lower than was observed among non-users (371 pg/mg creatinine; 95% CI, 326–422). Because all chondroitin users in our study reported use of glucosamine and two thirds of glucosamine users were taking glucosamine in combination with chondroitin, we conducted a sensitivity analysis in which we examined the association between use of glucosamine alone and markers of oxidative stress and DNA damage. When the exposed group was limited to the 19 persons reporting use of glucosamine alone (without chondroitin or MSM), no association was observed between glucosamine alone and PGF2α (*P*: 0.67; results not shown). Use of fiber products at least once a week in the month prior was associated with 43% lower PGF2α than was observed among non-users (208 pg/mg creatinine; 95% CI, 140–308) vs. 364 pg/mg creatinine; 95% CI, 320–413). None of the other supplements studied were associated with PGF2α concentration.

CoQ10 users had 58% lower baseline DNA damage than non-users (*P*: 0.003), with an adjusted geometric mean Olive tail moment of 1.02 among current users (95% CI, 0.60–1.72) vs. 2.42 among non-users (95% CI, 2.10–2.78). No other supplements were associated with baseline DNA damage. None of the supplements studied were associated with DNA repair capacity at 15 minutes (results not presented). Use of MSM supplements was associated with reduced DNA repair capacity at 60 minutes (*P*: 0.002). On average, non-users had repaired 64% of induced DNA damage at 60 minutes (95% CI, 61.3–67.2), whereas users repaired an average 47% of induced damage at 60 minutes (95% CI, 36.7–56.8).

Discussion

In our study, persons using glucosamine, chondroitin, and fiber supplements had lower levels of oxidative stress, as measured by PGF2α, than non-users. CoQ10 supplementation was associated with decreased baseline

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Table 3. Distribution of inflammatory biomarkers and association between each biomarker and age, sex, and pack-years smoked

	8-Isoprostane pg/mg creatinine ($n = 206$)			PGF2α pg/mg creatinine ($n = 206$)			Baseline DNA damage Olive tail moment ($n = 119$)			DNA repair capacity-60 min Olive tail moment (% repaired) ($n = 97$)		
	N	Adjusted geometric mean	95% CI	*N*	Adjusted geometric mean	95% CI	*N*	Adjusted geometric mean	95% CI	*N*	Adjusted mean	95% CI
Age[a], y												
50–<55	48	779	649–935	48	337	260–436	33	2.11	1.59–2.81	26	65.5	59.8–71.3
55–<60	53	842	708–1,002	53	376	295–480	29	2.36	1.75–3.19	26	61.4	55.8–67.1
60–<65	36	750	607–926	36	322	239–434	16	3.08	2.05–4.61	12	55.7	47.3–64.0
65–<70	29	691	545–875	29	376	269–525	16	2.22	1.48–3.33	14	67.3	59.5–75.1
70+	40	825	672–1,012	40	310	232–413	25	2.02	1.46–2.80	19	61.0	54.3–67.6
		$P_{continuous}$: *0.75*			$P_{continuous}$: *0.44*			$P_{continuous}$: *0.73*			$P_{continuous}$: *0.21*	
Sex[b]												
Female	100	879	775–998	100	362	303–433	54	2.37	1.90–2.96	43	64.3	59.8–68.8
Male	106	705	623–797	106	327	275–389	65	2.21	1.80–2.70	54	61.2	57.2–65.2
		P: *0.02*			*P*: *0.42*			*P*: *0.65*			*P*: *0.32*	
Smoking[c] (pack-years)												
Never smokers	104	711	628–805	104	332	279–396	59	2.31	1.87–2.86	48	63.4	59.1–67.7
Below median (<18)	51	728	610–869	51	316	246–405	33	2.03	1.53–2.69	29	63.2	57.7–68.7
Above median (18+)	51	1,034	867–1,234	51	400	312–513	27	2.55	1.87–3.48	20	59.6	53.0–66.2
		P_{trend}: *0.002*			P_{trend}: *0.30*			P_{trend}: *0.75*			P_{trend}: *0.40*	

[a]Adjusted for sex and smoking (continuous categorical: never, pack-years below median, pack-years above median).
[b]Adjusted for age (continuous) and smoking (continuous categorical: never, pack-years below median, pack-years above median).
[c]Adjusted for age (continuous) and sex.

DNA damage, and use of MSM was associated with decreased DNA repair capacity at 60 minutes. None of the other supplements studied (fish oil, garlic, ginseng, ginkgo, and saw palmetto) were associated with markers of oxidative stress or DNA damage.

We observed that high users of glucosamine (14+ pills/week) had 40% lower levels of PGF2α than non-users, whereas high users of chondroitin had 47% lower levels of PGF2α than non-users. This percentage reduction was similar or greater than observed for use of supplements known to reduce oxidative stress, including β-carotene, vitamin C, vitamin E, and selenium in this study; for example, high users of vitamin E supplements had 40% lower PGF2α than non-users (data not shown). No prior human studies have reported on the association between glucosamine and chondroitin use and oxidative stress, though results from *in vitro* and animal studies support the role of glucosamine and chondroitin in reducing oxidative stress. *In vitro* work has shown that glucosamine inhibits the free-radical oxidation of lipids, proteins, and DNA (11), while also acting to inhibit superoxide generation (12), scavenge free radicals, and improve redox balance (11). Similar work has also shown that chondroitin reduces lipid peroxidation and DNA damage (13). Several, but not all, *in vitro* studies have reported that glucosamine and/or chondroitin suppress the production of nitric oxide (NO; refs. 50–53), a free radical that can contribute to both lipid and DNA damage (54). Animal work has further shown that chondroitin reduces oxidative bursts of neutrophils (55) and lipid peroxidation (14, 56).

Furthermore, evidence from *in vitro* (28, 57), animal (58–60) and human (27, 29) studies suggests that glucosamine and chondroitin have anti-inflammatory effects resulting from inhibition of NF-κB activity. It has been suggested that chondroitin may act to block NF-κB activation via the inhibition of reactive oxygen species (61). However, given that reactive species can induce inflammation via NF-κB activation and that the inflammatory process can conversely generate reactive species (2), the inverse associations observed between glucosamine and chondroitin use and PGF2α in this study could reflect either or both of these closely related biologic processes.

Because glucosamine is often, but not always, taken with chondroitin in a single daily supplement, our glucosamine and chondroitin findings may not be independent of one another. We conducted an exploratory analysis in which we examined the association between use of glucosamine alone and PGF2α, and found that the association was not statistically significant. Although this may be due to limited power, it is also possible that the observed association between glucosamine use (with or

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Table 4. Association between current specialty supplement use, and measures of oxidative stress and DNA damage

	8-Isoprostane[a] pg/mg creatinine ($n = 206$)			PGF2α[b] pg/mg creatinine ($n = 206$)			Baseline DNA damage[c] Olive tail moment ($n = 119$)			DNA repair capacity-60 min[d] Olive tail moment (% repaired) ($n = 97$)		
Supplement	***N***	**Adjusted geometric mean[e]**	**95% CI**	***N***	**Adjusted geometric mean[e]**	**95% CI**	***N***	**Adjusted geometric mean[e]**	**95% CI**	***N***	**Adjusted mean[e]**	**95% CI**
Glucosamine[f,g]												
No use	158	779	705–859	158	374	326–429	87	2.32	1.97–2.74	72	62.6	59.1–66.1
Low (<14 pills/wk)	18	788	581–1,070	18	334	220–507	12	3.56	2.20–5.76	11	58.6	49.5–67.7
High (14+ pills/wk)	30	822	650–1,039	30	225	163–312	20	1.79	1.25–2.57	14	67.1	59.3–74.8
			P_{trend} *0.84*			P_{trend}: *0.01*			P_{trend}: *0.42*			P_{trend}: *0.47*
Chondroitin[f,h]												
No use	174	797	725–875	174	371	326–422	96	2.23	1.90–2.62	81	62.6	59.4–65.7
Low (<14 pills/wk)	13	697	492–989	13	294	177–489	9	3.30	1.93–5.65	8	55.4	45.1–65.6
High (14+ pills/wk)	19	749	563–998	19	196	131–293	14	2.37	1.52–3.68	8	72.4	62.4–82.5
			P_{trend}: *0.55*			P_{trend}: *0.003*			P_{trend}: *0.52*			P_{trend}: *0.26*
Fish oil[i]												
No use	181	772	704–847	181	339	297–386	110	2.32	2.01–2.69	90	62.9	59.9–66.0
Use	25	891	683–1,163	25	390	273–559	9	2.25	1.35–3.77	7	61.1	50.1–72.0
			P: 0.33			*P: 0.47*			*P: 0.91*			*P: 0.75*
CoQ10[j]												
No use	185	791	722–867	185	344	302–391	110	2.42	2.10–2.78	90	62.5	59.4–65.5
Use	21	737	557–976	21	349	234–523	9	1.02	0.60–1.72	7	66.5	55.5–77.6
			P: 0.64			*P: 0.94*			*P: 0.003*			*P: 0.49*
MSM[f]												
No use	186	795	726–870	186	354	311–402	110	2.32	2.00–2.68	89	64.3	61.3–67.2
Use	20	700	524–936	20	267	178–401	9	2.29	1.31–4.03	8	46.8	36.7–56.8
			P: 0.42			*P: 0.20*			*P: 0.97*			*P: 0.002*
Garlic												
No use	183	800	731–876	183	342	301–389	109	2.42	2.09–2.79	89	62.9	59.8–66.0
Use	23	677	519–884	23	364	252–526	10	1.49	0.92–2.41	8	61.4	51.2–71.6
			P: 0.25			*P: 0.75*			*P: 0.06*			*P: 0.79*
Ginseng[k]												
No use	188	773	707–846	188	342	301–388	107	2.36	2.03–2.73	88	62.9	59.9–66.0
Use	18	928	694–1,241	18	372	243–569	12	2.01	1.28–3.15	9	61.1	50.9–71.3
			P: 0.24			*P: 0.71*			*P: 0.51*			*P: 0.74*
Ginkgo[k]												
No use	183	792	722–868	183	346	305–394	107	2.39	2.06–2.77	87	63.5	60.5–66.5

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Table 4. Association between current specialty supplement use, and measures of oxidative stress and DNA damage (Cont'd)

	8-Isoprostane[a] pg/mg creatinine ($n = 206$)			PGF2α[b] pg/mg creatinine ($n = 206$)			Baseline DNA damage[c] Olive tail moment ($n = 119$)			DNA repair capacity-60 min[d] Olive tail moment (% repaired) ($n = 97$)		
Supplement	***N***	**Adjusted geometric mean[e]**	**95% CI**	***N***	**Adjusted geometric mean[e]**	**95% CI**	***N***	**Adjusted geometric mean[e]**	**95% CI**	***N***	**Adjusted mean[e]**	**95% CI**
Use	23	740	569–961	23	329	224–484	12	1.77	1.14–2.76	10	56.1	46.5–65.6
		P: 0.63			*P: 0.81*			*P: 0.22*			*P: 0.15*	
Saw palmetto[l]												
No use	91	708	622–806	91	330	272–400	57	2.38	1.95–2.89	47	60.7	56.6–64.7
Use	15	644	461–898	15	317	192–522	8	1.43	0.83–2.46	7	66.6	56.2–77.1
		P: 0.61			*P: 0.88*			*P: 0.10*			*P: 0.30*	
Fiber[m]												
No use	184	769	703–842	184	364	320–413	107	2.35	2.03–2.73	86	62.5	59.4–65.6
Use	20	937	709–1,238	20	208	140–308	12	2.00	1.24–3.22	11	65.0	55.7–74.3
		P: 0.19			*P: 0.01*			*P: 0.52*			*P: 0.63*	

[a]Analyses of 8-isoprostane adjusted for age (continuous), gender, education [continuous categorical: high school grad/GED (General Educational Development) certificate or less, some college/tech college grad, advanced degree], pack-years smoked (continuous categorical: none, below median, above median), BMI (kg/m^2, calculated using self-reported height and weight at baseline; continuous categorical: underweight/normal weight, overweight, obese), supplementary β-carotene (continuous categorical: none, low, and high), supplementary α-tocopherol (continuous categorical: none, low, and high), dietary γ-tocopherol (continuous), energy intake (continuous).

[b]Analyses of PGF2α adjusted for age (continuous), gender, pack-years smoked (continuous categorical: none, below median, and above median), any moderate or vigorous physical activity (no/yes), current multivitamin use (no/yes), supplementary β-carotene (continuous categorical: none, low, and high), supplementary vitamin C (continuous categorical: none, low, and high), supplementary α-tocopherol (continuous categorical: none, low, and high), supplementary selenium (continuous categorical: none, low, and high), supplementary iron (continuous categorical: none, low, and high), supplementary zinc (continuous categorical: none, low, and high).

[c]Analyses of baseline DNA damage adjusted for age (continuous), gender, pack-years smoked (continuous categorical: none, below median, and above median), alcohol consumption (categorical: tertiles), any moderate or vigorous physical activity over 10 years before baseline (no/yes), current use of non-aspirin NSAIDs (continuous categorical: none, low, and high).

[d]Analyses of DNA repair capacity at 60 minutes adjusted for age (continuous), gender, pack-years smoked (continuous categorical: none, below median, and above median), current HRT (no/yes).

[e]Means are presented for untransformed variables (DNA repair capacity); geometric means are presented for variables that have been log-transformed to normalize their distributions (baseline DNA damage; 8-isoprostane; PGF2α).

[f]Analyses of glucosamine, chondroitin, and MSM additionally adjusted for the indication of arthritis or chronic pain.

[g]Glucosamine defined by use of glucosamine (with or without chondroitin).

[h]Chondroitin defined by current use of chondroitin, though it should be noted that all chondroitin users also reported use of glucosamine.

[i]Analyses of fish oil additionally adjusted for indications of cardiovascular disease and memory loss.

[j]Analyses of CoQ10 were additionally adjusted for the indication of cardiovascular disease.

[k]Analyses of ginseng and ginkgo additionally adjusted for the indication of memory loss.

[l]Analyses of saw palmetto additionally adjusted for the indication of benign prostatic hyperplasia; analyses of saw palmetto were limited to men.

[m]Analyses of fiber additionally adjusted for the indication of constipation.

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without chondroitin) and PGF2α is driven by chondroitin or that glucosamine and chondroitin may act together, as suggested by some biologic studies (50, 62). We were unable to examine the association between chondroitin alone and PGF2α, as all chondroitin users in this study reported use of glucosamine.

Persons using fiber supplements had 43% lower PGF2α levels than non-users (*P*: 0.01). Limited work has been conducted on the association between fiber and oxidative stress in humans. In a cross-sectional study of 246 healthy adults, fiber intake from fruits and vegetables was associated with increased total antioxidant capacity (63). The association between fiber and oxidative stress has also been indirectly addressed in small human trials, with results showing decreases in lipid peroxidation (64, 65), as well as decreases in the production of superoxide and hydrogen peroxide (66); however, the interventions in these trials were multipronged (e.g., high fiber plus low-fat diet and/or exercise) and do not allow the effect of dietary fiber to be singled out. Animal studies on the association between fiber and oxidative stress have been inconsistent, with some, but not all, studies suggesting an effect (67–69). If fiber reduces oxidative stress, the mechanism has not been fully elucidated. Because dietary fiber intake has been inversely associated with inflammation (39), it is possible that fiber may act indirectly to reduce oxidative stress via decreased inflammation.

We also observed that CoQ10 supplement users had 58% lower levels of DNA damage than non-users (*P*: 0.003). Studies evaluating the association between CoQ10 and DNA damage in humans have been mixed. Two small intervention studies reported that CoQ10 supplementation decreased oxidative DNA damage (23, 24). However, other intervention studies have reported no evidence of an association between CoQ10 supplementation and DNA damage (18, 25, 26, 70), possibly due to small sample size or use of doses insufficient to yield biologic effects (19). Despite inconsistent human studies, *in vitro* research suggests that CoQ10 may reduce DNA damage (15, 16). If CoQ10 does reduce DNA damage, it is likely through a reduction in oxidative stress (23), as CoQ10 is an essential cofactor in the electron transport chain, and acts to stabilize free radicals (17, 20, 71). However, in our study, CoQ10 use was not associated with measures of oxidative stress, and other human research has been inconsistent about the effect of CoQ10 on oxidative stress (18, 22).

We also observed MSM use to be associated with reduced DNA repair capacity at 60 minutes. No other human research has reported on the association between MSM use and DNA repair capacity. Although limited, some *in vitro* (34) animal (72) and human research (73) suggests that MSM may reduce oxidative stress. Because reduced oxidative stress would be expected to *improve*, not diminish, DNA repair capacity (44), there is little support for our finding.

Results of this study suggest that glucosamine and chondroitin supplements may be associated with reduced oxidative stress, providing a biologic mechanism to support findings from the VITAL study, which have shown glucosamine and chondroitin to be associated with reduced risk of colorectal cancer (5), lung cancer (5), and total mortality (6). We also observed fiber supplement use to be associated with reduced oxidative stress, adding support to prior epidemiologic studies which suggest that fiber might reduce risk of cardiovascular disease (7) and cancer (8, 9).

A noteworthy strength of this study is that the VITAL biomarker study oversampled supplement users; therefore, the majority of non-users of any given supplement were using other supplements. The use of a comparison group largely composed of supplement users may reduce concern about potential residual confounding by healthy behaviors associated with supplement use. Other advantages of our study include our extensive assessment of and control for potential confounders, as well as the fact that all supplements except fiber were ascertained by home interview and supplement inventory, likely increasing the accuracy of exposure measurement.

This study is not without limitation. Our ability to detect associations may have been limited by the small sample size and by the fact that biomarker study participants were instructed not to use the supplement on the day of interview/specimen collection; it should be noted, though, that the majority of supplement users reported use the day prior. Furthermore, in our analyses of DNA damage and DNA repair capacity, we made additional exclusions (exclusion of those with prior cancer or nonviable lymphocytes), further limiting power, potentially explaining the small number of factors associated with these outcomes. Furthermore, with 50 statistical tests (10 exposures and five outcomes), we cannot rule out the presence of false positives, although less than one false-positive association would be expected with our use of $\alpha = 0.01$.

Another limitation in the interpretation of our results is that it is unclear why the factors associated with the two biomarkers of oxidative stress, PGF2α and 8-isoprostane, differed in our study and why these two biomarkers were only modestly correlated with one another. This may be due to differences in biologic pathways reflected, sensitivity of biomarkers, or measurement error. We observed modest intraclass correlation coefficients (ICC) when assessing the between-plate reliability of 8-isoprostane and PGF2α on 22 duplicate pairs. The ICC for PGF2α was 0.80 and the ICC for 8-isoprostane was 0.85. However, the ICCs for the creatinine-corrected biomarkers decreased to 0.32 and 0.46, respectively. The ICCs for the unadjusted measures are likely higher than the creatinine-corrected measures due to substantial differences in the dilution of urine between subjects, increasing the between-person variation relative to the within-person variation; therefore, adjusting for the dilution of the urine would act to decrease the ICCs, as has been observed elsewhere (74). It should also be noted that assessment of 8-isoprostane via EIA has been shown to differ from gas chromatography/mass spectrometry (GS/MS), as antibodies may bind

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to more than one lipid molecule (75). Despite these limitations, several expected predictors of oxidative stress were associated with measures of 8-isoprostane and PGF2α, increasing our confidence that these were well measured. For example, supplements known to be associated with reduced oxidative stress, such as β-carotene, vitamin C, vitamin E, and selenium, were associated with reduced PGF2α concentrations this study, whereas supplementary β-carotene and α-tocopherol were associated with reduced 8-isoprostane concentrations (data not shown).

It is also unclear why factors associated with oxidative stress were not reflected in measures of DNA damage. We observed no correlation between measures of oxidative stress and measures of DNA damage/DNA repair capacity in our study, and others have reported only modest correlation between 8-isoprostane and oxidative DNA damage (76). It may be that factors associated with oxidative stress do not translate well to measures of DNA damage, as DNA damage may result from factors beyond oxidative stress. Therefore, the relative contribution of any one factor (such as oxidative stress) may be too small to see effect on DNA damage. Measurement error in the measures of DNA damage is also a concern. A control sample was included within each batch of assays, which was then used to calculate a between-batch coefficient of variation (CV). These CVs are as follows: baseline damage (41%), damage immediately after irradiation (24%), damage 60 minutes after irradiation (30%), and DNA repair capacity at 60 minutes (11%). These values are comparable with those observed in the literature (77).

In summary, our results suggest that glucosamine, chondroitin, and fiber supplements are associated with reduced oxidative stress, whereas CoQ10 supplementation was associated with reduced DNA damage. Further research is needed to better understand the associations between use of these popular supplements and oxidative stress/DNA damage (78), as oxidative stress and DNA damage have been suggested to play a role in several diseases. Our results provide evidence of a potential mechanism by which these supplements may affect disease risk, warranting further research on these potential preventives.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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