THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 00, Number 00, 2020, pp. 1–10

DOI: 10.1089/acm.2020.0283

JACM

Glucosamine and Chondroitin Use in Relation to C-Reactive Protein Concentration: Results by Supplement Form, Formulation, and Dose

Elizabeth D. Kantor, PhD,[1] Kelli O'Connell, MSPH,[1] Mengmeng Du, ScD,[1] Chao Cao, MPH,[2] Xuehong Zhang, ScD,[3,4] Dong Hoon Lee, ScD,[3] Yin Cao, ScD,[2,5,6] and Edward L. Giovannucci, ScD[3,4]

Abstract

Objectives: Glucosamine and chondroitin supplements have been associated with reduced inflammation, as measured by C-reactive protein (CRP). It is unclear if associations vary by formulation (glucosamine alone vs. glucosamine+chondroitin), form (glucosamine hydrochloride vs. glucosamine sulfate), or dose.

Design, Subjects, Setting, Location: The authors evaluated these questions using cross-sectional data collected between 1999 and 2010 on 21,917 US adults, surveyed as part of the National Health and Nutrition Examination Survey (NHANES).

Exposures: Glucosamine and chondroitin use was assessed during an in-home interview; exposures include supplement formulation, form, and dose.

Outcome/Analysis: CRP was measured using blood collected at interview. Survey-weighted linear regression was used to evaluate the multivariable-adjusted association between exposures and log-transformed CRP.

Results: In early years (1999–2004), use of glucosamine (ratio = 0.87; 95% confidence interval [CI] = 0.79–0.96) and chondroitin (ratio = 0.83; 95% CI = 0.72–0.95) was associated with reduced CRP. However, associations significantly varied by calendar time (p-interaction = 0.04 and p-interaction = 0.01, respectively), with associations nonsignificant in later years (ratio = 1.09; 95% CI = 0.94–1.28 and ratio = 1.16; 95% CI = 0.99–1.35, respectively). Consequently, all analyses have been stratified by calendar time. Associations did not significantly differ by formulation in either set of years; however, significant associations were observed for combined use of glucosamine+chondroitin ($\text{ratio}_{\text{early}} = 0.82$; 95% CI = 0.72–0.95; $\text{ratio}_{\text{late}} = 1.16$; 1.00–1.35), but not glucosamine alone. Associations also did not significantly differ by supplement form. Even so, a significant inverse association was observed only for glucosamine sulfate in the early years (ratio = 0.78; 95% CI = 0.64–0.95); no significant association was observed for glucosamine hydrochloride. No significant trends were observed by dose.

Conclusions: Although a significant inverse association was observed for glucosamine and chondroitin and CRP in early years, this association did not hold in later years. This pattern held for combined use of glucosamine+chondroitin as well as glucosamine sulfate, although associations did not significantly vary by supplement form, formulation, or dose. Further study is needed to better understand these associations in the context of calendar time.

[1]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA.
[2]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St. Louis, St. Louis, MO, USA.
[3]Department of Nutrition, Harvard TH Chan School of Public Health, Boston, MA, USA.
[4]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.
[5]Alvin J. Siteman Cancer Center, Washington University School of Medicine, St. Louis, St Louis, MO, USA.
[6]Division of Gastroenterology, Department of Medicine, Washington University School of Medicine, St. Louis, St Louis, MO, USA.

Keywords: Centers for Disease Control and Prevention, chondroitin, dietary supplements, glucosamine, National Health and Nutrition Examination Survey

Introduction

Often taken for osteoarthritis, glucosamine and chondroitin are commonly used nonvitamin nonmineral supplements.[1,2] Although the effectiveness of these supplements for joint pain and function remains controversial,[3–9] a growing body of evidence suggests that glucosamine and chondroitin have anti-inflammatory properties.[10–32] *In vitro* models have shown that glucosamine and chondroitin inhibit the activity of nuclear factor kappa B (NFkB), a transcription factor central to the inflammatory cascade, in a dose-dependent manner; specifically, research suggests that these supplements act to inhibit the degradation of NFkB inhibitory subunit, IkB, blocking NFkB from translocating to the nucleus.[10,11,33] Supporting research from animal studies has demonstrated that administration of glucosamine/chondroitin reduces inflammatory markers downstream of NFkB.[21–26]

Several studies have evaluated whether the anti-inflammatory effect extends to humans.[29–31,34,35] Two prior observational studies suggest glucosamine/chondroitin to be associated with reduced concentration of C-reactive protein (CRP), a nonspecific marker of systemic inflammation.[29,30] These results are consistent with those from a small double-blinded randomized placebo-controlled crossover trial in which the intervention was 1500 mg of glucosamine hydrochloride +1200 mg of chondroitin sulfate, administered over a 28-day period.[31] A proteomics analysis from this randomized controlled trial further revealed that the pathway most different between the intervention and placebo was the ''cytokine activity'' pathway ($p = 2.6 \times 10^{-16}$), supporting the hypothesis that glucosamine/chondroitin may reduce inflammation.

Further, in a trial of 53 osteoarthritic patients, 1500 mg of glucosamine hydrochloride +675 mg of chondroitin significantly reduced concentration of inflammatory marker, prostaglandin E_2.[34] However, in a trial of 51 patients with rheumatoid arthritis, 1500 mg of glucosamine hydrochloride did not reduce CRP.[35] The intervention was glucosamine alone (rather than glucosamine+chondroitin) and study participants were able to continue taking their normal drugs. It is unclear if the lack of association is due to the choice of supplement formulation (glucosamine alone), form (glucosamine hydrochloride), study design (e.g., rheumatoid arthritis patients often take strong anti-inflammatories, and continued use of these drugs would likely conceal differences between groups), or other factors.

Despite a growing body of evidence to suggest that use of glucosamine/chondroitin is associated with reduced inflammation, it is unclear what characteristics of exposure may be driving the observed association. Research has also demonstrated an inverse association with risk of colorectal cancer and lung cancer[36–40]; as inflammation is thought to play a role in the development of these cancers,[41–46] this may offer a plausible biologic mechanism by which these supplements may reduce risk. Better understanding the characteristics of exposure associated with inflammation is critical to moving this body of research forward. The authors, therefore, evaluated the association between glucosamine and chondroitin use in relation to CRP, by supplement form, formulation, and dose in the National Health and Nutrition Examination Survey (NHANES).

Methods

Study population

NHANES is a nationally representative cross-sectional survey of persons living in the United States.[47] This analysis used data from cycles for which relevant data were available (1999–2000 to 2009–2010). Information was collected during an in-home interview, with further data collection, physical examination, and blood collection performed at Mobile Examination Centers.

This analysis includes persons ≥25 years of age who completed both the in-home interview and blood collection ($n = 26{,}253$); pregnant women were excluded ($n = 1398$). The authors further excluded high CRP outliers (top 2% of age-gender-body mass index [BMI] groups) to exclude acutely ill persons ($n = 542$), as well as those with unreliable diet data (as determined by NHANES; $n = 870$), those missing information on supplement use ($n = 67$), and those missing covariates ($n = 1459$), resulting in a final $n = 21{,}917$.

Exposure

The NHANES interview includes a series of questions related to use of dietary supplements, from which supplement form, formulation, and dose was assessed.[48] Participants indicating use of supplements in the 30 days prior were asked to show the interviewer the bottles of each supplement used. When containers were not seen, participants were asked to recall each product taken. This information was linked to a supplement database, which includes detailed information on ingredients and dose.

Use (yes vs. no) of glucosamine and chondroitin was defined as use in the 30 days prior. The authors used this information to determine whether a person used glucosamine (yes vs. no) and chondroitin (yes vs. no), and further examined associations for supplement formulation (categorized as use of both glucosamine+chondroitin, glucosamine alone, or neither, with neither as the reference).

A separate analysis, restricted to users, was conducted to calculate a *p*-value for the difference between glucosamine+chondroitin versus glucosamine alone. Use of chondroitin alone cannot be studied, given the very small number of people taking chondroitin in the absence of glucosamine. Consequently, the association for chondroitin (yes vs. no) is essentially the same as glucosamine+chondroitin. However, the authors have maintained the presentation of overall chondroitin use so as to facilitate ease of comparison with prior studies before evaluating associations by formulation, form, and dose.

In analyses of supplement form, use of glucosamine was defined as glucosamine sulfate or glucosamine hydrochloride. The authors excluded persons for whom supplement form was not known ($n = 155$) and those who reported use of glucosamine sulfate and glucosamine hydrochloride ($n = 25$). Again, a three-level categorical exposure was used, with no

use as the reference group. In a separate model limited to glucosamine users, the authors compared users of glucosamine sulfate with users of glucosamine hydrochloride to assess the statistical significance of glucosamine form.

For dose, the authors used information on the mg of glucosamine/chondroitin contained per pill and the number of pills taken per day to get the average mg/day consumed, with users categorized as follows: glucosamine (<800 mg/day, 800 to <1200 mg/day, 1200+ mg/day), chondroitin (<500 mg/day, 500 to <1000 mg/day, 1000+ mg/day). These analyses were limited to users. In exploratory analyses of duration, categories defined as follows: glucosamine (≤1 year, >1 to ≤4 years, >4 years), chondroitin (≤1 year, >1 to ≤3 years, >3 years), with the *p*-trend calculated among users for whom this information was available.

Outcome

CRP, an acute-phase protein synthesized as a result of inflammation, was assessed at the time of interview. CRP has been associated with both risk of CRC in meta-analyses of prospective studies[49] and has been associated with use of glucosamine/chondroitin in prior studies.[29,30] In NHANES, serum high-sensitivity CRP (hsCRP) was measured using latex-enhanced nephelometry.[50]

Statistical analysis

To normalize the right-skewed distribution of hsCRP, values were log-transformed using the natural logarithm. Linear regression was used to model the association between exposure and log-transformed hsCRP. Minimally adjusted models include gender and age; fully adjusted models additionally include race/ethnicity, education, smoking, BMI, physical activity, prescription nonsteroidal anti-inflammatory drug (NSAID) use, any prescription steroid use, vitamin E use, alcohol intake, coffee intake, fiber intake, saturated fat intake, dietary omega-3 intake, omega-3 supplement use, dietary omega-6 intake, statin use, cancer, diabetes, heart disease, arthritis, and survey cycle. Detailed covariate information is provided in table footnotes.

Given that information on use of any aspirin or nonaspirin NSAID use was only available for a subset of cycles (1999–2004), the authors conducted a sensitivity analysis restricted to these cycles and found that their inclusion did not meaningfully change effect estimates; therefore, these variables have not been included in the final models.

Results are presented as the exponentiated beta-coefficients, representing the ratio of geometric mean hsCRP concentrations among persons in the category of interest to those in the reference category.[30] As NHANES is a stratified complex multistage probability-based survey that oversamples certain population subgroups, all participants have been assigned analytic weights to account for unequal sampling probability and nonresponse.

All analyses are stratified by time (early years: 1999–2004 and later years: 2005–2010), given significant effect modification by calendar time. To further shed light on associations and understand variation by time, the authors have explored interaction of glucosamine+chondroitin use and CRP by the following factors: age, gender, BMI, health status, and physical activity.

Data were collected by the National Center for Health Statistics. As de-identified data are publicly available for download,[47] the Memorial Sloan Kettering Institutional Review Board determined that this did not constitute human subjects research. Analyses were conducted using Stata 15 (StataCorp, College Station, TX).

Results

As shown in Table 1, glucosamine+chondroitin users are more likely than nonusers to be older, non-Hispanic white, and highly educated. These patterns do not vary over calendar time. Distributions of additional variables are shown in Supplementary Table S1. Most distributions were comparable by time, with some exceptions. For example, in the early years (1999–2004), glucosamine+chondroitin users and nonusers were comparable with regard to health status, with 18% of glucosamine+chondroitin users reporting poor/fair health status, as compared with 17.3% of nonusers. However, in later years (2005–2010), glucosamine+chondroitin users were less likely to report fair/poor health status than nonusers (10.1% vs. 17.9%, respectively). A similar pattern was observed for diabetes.

The association between glucosamine and CRP varied by calendar time (*p*-interaction = 0.04), with a statistically significant inverse association between glucosamine and CRP in early years (ratio = 0.87; 95% confidence interval [CI] = 0.79–0.96), and no association in later years (ratio = 1.09; 95% CI = 0.94–1.28) (Table 2). A comparable pattern was observed for chondroitin (*p*-interaction = 0.01), again with a significant inverse association in early years (ratio = 0.83; 95% CI = 0.72–0.95) and a nonsignificant positive association in later years (ratio = 1.16; 95% CI = 0.99–1.35).

When examined by supplement formulation, the authors observed a significant inverse association for use of glucosamine+chondroitin as compared with use of neither in early years (ratio = 0.82; 95% CI = 0.72–0.95); no association was observed for use of glucosamine alone (ratio = 0.96; 95% CI = 0.80–1.14); however, the difference between combined use and use of glucosamine alone did not reach statistical significance (*p*-value = 0.12). Conversely, a positive association was observed for combined use of glucosamine+chondroitin in the later years (ratio = 1.16; 95% CI = 1.00–1.35), again with no association observed for glucosamine alone (ratio = 0.95; 95% CI = 0.74–1.22). Again, the direct comparison of glucosamine+chondroitin as compared with glucosamine alone in later years was not statistically significant (*p*-value = 0.08). The interaction across years was significant (*p*-interaction = 0.03).

In early years, there was a statistically significant inverse association for glucosamine sulfate and CRP (ratio = 0.78; 95% CI = 0.64–0.95) and a nonsignificant inverse association was observed between glucosamine hydrochloride and CRP (ratio = 0.86; 95% CI = 0.72–1.05); the difference by supplement form was not significant (*p*-value = 0.21). No significant associations were observed by supplement form in later years (*p*-value = 0.38). A *p*-interaction = 0.05 was observed for the interaction between supplement form and calendar time.

When examining associations by dose, no statistically significant associations were observed in early years or in later years, nor did the association significantly vary by time. Furthermore, no significant associations were observed in exploratory analyses of duration in early or later years.

Table 1. Distribution of Participant Characteristics, by Glucosamine+Chondroitin Use and Survey Cycle (1999–2004 and 2005–2010)

	1999–2004				2005–2010			
Variable	*Overall* N *(Weighted %)*[a]	*No use of glucosamine or chondroitin* N *(Weighted %)*	*Any use of glucosamine only* N *(Weighted %)*	*Combined use of glucosamine+ chondroitin* N *(Weighted %)*	*Overall* N *(Weighted %)*[b]	*No use of glucosamine or chondroitin* N *(Weighted %)*	*Any use of glucosamine only* N *(Weighted %)*	*Combined use of glucosamine+ chondroitin* N *(Weighted %)*
Overall	9931 (100)	9540 (95.4)	125 (1.6)	264 (3.0)	11,986 (100)	11,413 (94.6)	158 (1.5)	405 (3.9)
Age (years)								
25 to <40	2549 (31.8)	2529 (32.9)	10 (11.4)	10 (6.7)	3150 (29.4)	3112 (30.7)	12 (7.5)	25 (6.5)
40 to <50	1939 (23.2)	1902 (23.7)	18 (17.0)	18 (10.9)	2297 (22.3)	2237 (22.8)	22 (10.6)	36 (13.8)
50 to <60	1492 (18.7)	1423 (18.3)	22 (26.3)	47 (28.3)	2059 (21.2)	1948 (20.7)	31 (33.5)	80 (28.2)
60 to <70	1817 (13.5)	1695 (12.8)	38 (29.6)	84 (29.1)	2154 (14.3)	1992 (13.5)	50 (30.6)	108 (27.6)
70 to <80	1305 (8.9)	1221 (8.5)	19 (10.9)	64 (18.6)	1499 (8.5)	1366 (8.1)	30 (12.6)	101 (16.3)
80+	829 (3.9)	770 (3.8)	18 (4.7)	41 (6.3)	827 (4.4)	758 (4.2)	13 (5.3)	55 (7.7)
Sex								
Male	5034 (49.2)	4845 (49.3)	56 (44.0)	131 (49.3)	6009 (48.3)	5718 (48.5)	72 (40.9)	213 (46.9)
Female	4897 (50.8)	4695 (50.7)	69 (56.0)	133 (50.7)	5977 (51.7)	5695 (51.5)	86 (59.1)	192 (53.1)
Race/ethnicity								
Non-Hispanic white	5262 (75.0)	4952 (74.3)	99 (91.7)	210 (89.4)	6130 (73.5)	5721 (72.7)	106 (87.2)	296 (88.0)
Non-Hispanic black	1762 (9.6)	1741 (9.9)	7 (2.6)	14 (2.1)	2270 (10.2)	2226 (10.6)	9 (2.1)	35 (2.8)
Mexican American	2183 (6.3)	2141 (6.5)	13 (2.1)	28 (1.9)	2111 (7.3)	2058 (7.6)	22 (4.9)	31 (2.2)
Other	724 (9.1)	706 (9.3)	6 (3.6)	12 (6.6)	1475 (9.0)	1408 (9.1)	21 (5.7)	43 (7.1)
Education								
Less than high school	3139 (19.3)	3079 (19.8)	19 (6.9)	39 (11.1)	3396 (17.8)	3301 (18.3)	31 (9.9)	64 (9.1)
High school grad/GED or equivalent	2355 (25.6)	2263 (25.8)	30 (21.4)	62 (22.3)	2846 (24.2)	2724 (24.3)	35 (29.8)	86 (20.6)
Some college or AA degree	2487 (29.0)	2370 (28.9)	34 (31.1)	83 (30.7)	3211 (29.7)	3038 (29.6)	44 (27.9)	122 (31.9)
College grad or above	1950 (26.1)	1828 (25.5)	42 (40.6)	80 (35.9)	2533 (28.3)	2350 (27.8)	48 (32.3)	133 (38.5)
<25 (Underweight/ normal)	2970 (32.8)	2865 (33.1)	29 (24.7)	75 (29.6)	3219 (29.5)	3081 (29.7)	37 (29.8)	98 (24.0)
25 to <30 (Overweight)	3697 (35.8)	3551 (35.8)	46 (35.0)	100 (34.9)	4211 (34.5)	3992 (34.2)	70 (40.0)	146 (38.0)
30 to <35 (Obese)	1966 (18.8)	1882 (18.7)	27 (23.0)	57 (21.9)	2596 (20.5)	2474 (20.6)	31 (16.5)	90 (21.7)
35+ (Severely obese)	1298 (12.6)	1242 (12.5)	23 (17.2)	32 (13.5)	1960 (15.5)	1866 (15.5)	20 (13.7)	71 (16.3)
Smoking								
Never	4889 (49.1)	4690 (48.9)	70 (56.7)	129 (51.0)	6171 (51.4)	5869 (51.4)	90 (51.2)	206 (51.4)
Former	2915 (27.8)	2756 (27.4)	42 (33.2)	116 (39.9)	3281 (26.7)	3057 (25.9)	52 (37.3)	168 (40.0)
Current: low (≤15 cigarettes/day)	1262 (11.9)	1246 (12.3)	7 (5.3)	8 (3.1)	1590 (12.6)	1564 (13.0)	5 (3.1)	21 (5.7)
Current: high (>15 cigarettes/day)	865 (11.2)	848 (11.5)	6 (4.8)	11 (6.0)	944 (9.4)	923 (9.7)	11 (8.4)	10 (2.8)

AA, associate degree; GED, general educational development.

Table 2. Association Between Glucosamine and Chondroitin Supplement Use, and C-Reactive Protein, by Form, Formulation, and Dose

	1999–2004					*2005–2010*					
		Age and gender adjusted[a]		*Multivariable adjusted*[b]			*Age and gender adjusted*[a]		*Multivariable adjusted*[b]		
Variable	N *(Wgt %)*	*Ratio*	*95% CI*	*Ratio*	*95% CI*	N *(Wgt %)*	*Ratio*	*95% CI*	*Ratio*	*95% CI*	p-*interaction*
Overall use variables											
Glucosamine											
No use	9542 (95.4)	1	REF	1	REF	11,423 (94.6)	1	REF	1	REF	0.04
Use	389 (4.6)	0.84	0.73–0.96	0.87	0.79–0.96	563 (5.4)	0.98	0.83–1.16	1.09	0.94–1.28	
Chondroitin											
No use	9665 (97.0)	REF	REF	REF	REF	11,571 (96.0)	1.00	REF	1	REF	0.01
Use	266 (3.0)	0.78	0.67–0.90	0.83	0.72–0.95	415 (4.0)	1.05	0.87–1.27	1.16	0.99–1.35	
Use, by supplement formulation											
Glucosamine+chondroitin											
Use of neither	9540 (95.4)	1	REF	1	REF	11,413 (94.6)	1	REF	1	REF	0.03
Use of glucosamine alone	125 (1.6)	0.96	0.75–1.23	0.96	0.80–1.14	158 (1.5)	0.83	0.61–1.13	0.95	0.74–1.22	
Use of both	264 (3.0)	0.78	0.67–0.90	0.82	0.72–0.95	405 (3.9)	1.05	0.87–1.26	1.16	1.00–1.35	
Both versus glucosamine alone[c]			*p*: 0.05		*p*: 0.12			*p*: 0.18		*p*: 0.08	
Use, by supplement form											
Glucosamine											
No use	9540 (96.6)	1	REF	1	REF	11,413 (95.2)	1	REF	1	REF	0.05
Use of glucosamine sulfate	131 (1.6)	0.76	0.57–1.02	0.78	0.64–0.95	92 (1.0)	1.10	0.73–1.66	1.20	0.91–1.57	
Use of glucosamine HCl	154 (1.8)	0.88	0.68–1.12	0.87	0.72–1.05	395 (3.8)	0.99	0.81–1.19	1.09	0.89–1.32	
Glucosamine HCl versus glucosamine sulfate[d]			*p*: 0.54		*p*: 0.21			*p*: 0.56		*p*: 0.38	
Use, by supplement dose (among users)											
Glucosamine dose (mg)											
Use, <800	231 (59.9)	1	REF	1	REF	271 (48.7)	1	REF	1	REF	0.55
Use, 800 to <1200	68 (22.1)	0.86	0.56–1.33	1.08	0.78–1.48	92 (15.1)	0.83	0.55–1.24	0.93	0.70–1.24	
Use, 1200+	73 (18.1)	0.85	0.61–1.20	1.01	0.79–1.26	197 (36.2)	1.13	0.88–1.45	1.13	0.93–1.37	
			p-trend: 0.29		*p*-trend: 0.88			*p*-trend: 0.37		*p*-trend: 0.24	
Chondroitin dose (mg)											
Use, <500	147 (55.2)	1	REF	1	REF	163 (41.4)	1	REF	1	REF	0.63
Use, 500 to <1000	63 (3.07)	0.90	0.64–1.28	1.08	0.85–1.39	115 (27.3)	0.96	0.67–1.38	0.87	0.63–1.20	
Use, 1000+	40 (14.1)	1.28	0.94–1.74	1.27	0.95–1.69	122 (31.3)	0.99	0.70–1.41	0.95	0.71–1.28	
			p-trend: 0.42		*p*-trend: 0.11			*p*-trend: 0.95		*p*-trend: 0.71	

(*continued*)

TABLE 2. (CONTINUED)

	1999–2004					2005–2010					
		Age and gender adjusted[a]		Multivariable adjusted[b]			Age and gender adjusted[a]		Multivariable adjusted[b]		
Variable	N *(Wgt %)*	*Ratio*	*95% CI*	*Ratio*	*95% CI*	N *(Wgt %)*	*Ratio*	*95% CI*	*Ratio*	*95% CI*	p-*interaction*
Use, by duration (among users)											
Glucosamine (years)											
≤1 year	188 (47.8)	1	REF	1	REF	206 (32.8)	1	REF	1	REF	0.04
>1 to ≤4 years	129 (34.3)	1.02	0.71–1.48	1.16	0.88–1.53	152 (27.9)	0.88	0.58–1.33	0.91	0.64–1.28	
>4 years	71 (18.0)	0.78	0.57–1.06	0.92	0.69–1.23	205 (39.4)	0.79	0.54–1.16	0.89	0.62–1.26	
		p-trend: 0.26		*p*-trend: 0.92			*p*-trend: 0.22		*p*-Trend: 0.49		
Chondroitin (years)											
≤1 year	123 (46.7)	1	REF	1	REF	142 (31.7)	1	REF	1	REF	0.32
>1 to ≤3 years	80 (30.2)	1.06	0.68–1.66	1.34	0.92–1.82	94 (22.4)	0.84	0.54–1.31	0.84	0.57–1.22	
>4 years	61 (23.1)	0.83	0.59–1.15	1.00	0.72–1.40	169 (45.9)	0.77	1.08–2.08	0.78	0.57–1.06	
		p-trend: 0.39		*p*-trend: 0.77			*p*-trend: 0.16		*p*-trend: 0.12		

[a]Adjusted for age (25–39, 40–49, 50–59, 60–69, 70–79, and 80+) and gender.

[b]Adjusted for gender, age (25–39, 40–49, 50–59, 60–69, 70–79, and 80+), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and other), education (less than high school, high school grad or equivalent, some college or AA degree, and college grad or above), smoking (never, former, ≤15 cigarettes/day if current, and >15 cigarettes/day if current), BMI (underweight/normal, overweight, obese, and severely obese), physical activity (none, any moderate, and any vigorous), any prescription NSAID use, any prescription steroid use, any non-MVMM vitamin E use, alcohol intake (<1 drink/month, ≥1 drink/month to <4 drinks/week, ≥4 drinks/week to <2 drinks/day, and ≥2 times/day), coffee intake (quartiles), fiber intake (quartiles), saturated fat intake (quartiles), dietary omega-3 intake (quartiles), any non-MVMM omega-3 supplement use, dietary omega-6 intake (quartiles), statin use, cancer, diabetes, heart disease, arthritis, and survey cycle.

[c]*p*-Value estimated directly comparing users of glucosamine alone with users of glucosamine+chondroitin, in a model restricted to users.

[d]*p*-Value estimated directly comparing users of glucosamine sulfate with users of glucosamine hydrochloride, in a model restricted to users.

BMI, body mass index; MVMM, multivitamin, multimineral; NSAID, nonsteroidal anti-inflammatory drug.

No significant interaction was observed between glucosamine+chondroitin and age, gender, BMI, health status, or physical activity within either time period (Supplementary Tables S2 and S3).

Discussion

In this nationally representative survey, the authors observed no clear overall pattern of association between glucosamine and chondroitin and CRP, by supplement formulation, form, and dose—in large part, due to unexpected strong effect modification by time. Specifically, they observed a significant inverse association for glucosamine/chondroitin in early years, and an unexpected null association in later years.

Although the geometric mean of CRP did not significantly differ when directly comparing users of glucosamine+chondroitin to users of glucosamine alone, it should be noted that as compared with nonuse significant associations were observed for use of glucosamine+chondroitin (inverse association with CRP in early years, positive association in later years), whereas no significant associations were observed for use of glucosamine alone. Associations with CRP did not significantly differ when directly comparing supplements by form (glucosamine sulfate vs. hydrochloride), although a significant inverse association was only observed for glucosamine sulfate in the early years. No significant trends were observed by dose, nor in exploratory analyses of duration.

In this study, the authors observed an inverse association between use of glucosamine and CRP in the early years, with a null association in later years. A comparable pattern of association was observed for chondroitin use. Although the inverse association in early years aligned with expectation and the prior literature,[29–31,34] it is unclear why the association changed over time.

It is conceivable that this pattern may be driven by changing characteristics of exposure over time. In this study, the authors have evaluated patterns by supplement formulation, form, and dose, but it is possible that there are other unmeasured characteristics of exposure, such as supplement quality, which have changed over time. It is also possible the factors associated with use have changed over time in a way not captured by covariate adjustment, resulting in the observed pattern of association. For example, if there is an unmeasured healthy behavior more strongly associated with use in the early years, such a variable could potentially explain the pattern of results if also associated with CRP. However, the authors have carefully adjusted for many healthy behaviors, and it is hard to imagine a variable that could explain this pattern of results.

It is also possible that there may be an effect modifier that changed in prevalence over time, which could explain observed pattern of association; however, there was no evidence of effect modification for the factors examined. Although this pattern of results does raise the potential of bias or chance, it should be noted that the prior literature does largely support an inverse association with inflammation, including two small trials (which should not be subject to concerns of residual confounding).[29–31,34,35]

When evaluating whether the association varied by supplement formulation, the authors observed no significant difference in the geometric mean CRP for users of glucosamine+chondroitin as compared with users of glucosamine alone. This held in both early and later years. However, it should be noted that the significant associations were only observed for glucosamine+chondroitin (inverse in early years, positive in later years), with no significant associations observed for glucosamine alone. Approximately 70% of glucosamine in the U.S. population is taken in conjunction with chondroitin and nearly all chondroitin is taken with glucosamine (a reflection of the supplement products available on the market). Thus, the sample size is smaller for glucosamine alone and the authors cannot rule out modest effect estimates pertaining to use of glucosamine alone.

However, this pattern in the early years is consistent with what has been observed elsewhere: in exploratory analyses conducted in studies of inflammation, oxidative stress, and CRC, the association has been consistently stronger for glucosamine+chondroitin than for glucosamine alone.[29,36,37,51] These analyses suggest that the biologic effect may be driven by chondroitin or the combination of glucosamine+chondroitin, rather than glucosamine alone; however, prior exploratory analyses of glucosamine alone were quite underpowered, with wide CIs limiting the interpretation of the null association for glucosamine alone.

Even so, it is unclear why the association for glucosamine/chondroitin would change direction over time. It is possible that this pattern reflects some change in the glucosamine+chondroitin supplements available (or the chondroitin component). It is also possible that the association between glucosamine+chondroitin and CRP could be differentially confounded in early and late years. Further research in a large study will be needed to better understand this question, preferably one with recent data to reflect current patterns of use.

Associations also did not significantly differ by supplement form (glucosamine sulfate vs. hydrochloride). However, the significant inverse association in the early years was only observed for glucosamine sulfate, with no significant association observed for glucosamine hydrochloride. Although trials have largely used glucosamine hydrochloride as the experimental agent,[3,31,34,35] glucosamine sulfate accounts for $\sim$30% of use in the population, and it was unknown if the association with inflammation differs by supplement form. In this study, there is some suggestion that the early inverse association is reflected by glucosamine sulfate, although a nonsignificant inverse association was also observed for glucosamine hydrochloride in the early years.

Although prior studies have not directly compared glucosamine hydrochloride and sulfate, prior trials of inflammation have used glucosamine hydrochloride and have observed significant reductions in inflammation, unlike this study. No significant associations were observed in later years. Again, an explanation for why this pattern was observed over time remains unclear. It is possible that there is a change to the quality of supplements over time or some other change to an unmeasured characteristic of these supplements or their users. Although the authors have been extensive with covariates included, it is possible that there is an unknown/unmeasured factor differentially confounding results over time, given changes in prevalence and/or changes in the strength of association with either the exposure or outcome. The impact of chance also cannot be ruled out.

The authors also evaluated whether the relationship between glucosamine/chondroitin and CRP strengthened with increasing dose. Evidence of a ''dose–response'' relationship would lend credibility to a true biologic effect, while

also informing the dose needed to observe an association with inflammation. In this study, no such significant trend was observed, raising a question about whether the inverse association in early years might reflect bias or chance, rather than a true causal association. A similar pattern was observed in exploratory analyses of duration. Given the lack of prior study on these specific questions, they should be evaluated in a contemporary large study.

This study leverages rich data on supplement use in a large nationally representative population of U.S. adults. NHANES has extensive covariate data, enabling a well-controlled analysis. This is the first study to evaluate whether the association between glucosamine and chondroitin and inflammation varies by supplement form and dose, and offers a large sample size to evaluate whether the association varies by supplement formulation—a question only previously evaluated in small exploratory analyses.

Given strong effect modification by time, all results had to be stratified by time, cutting power to evaluate associations of interest, also precluding defining exposure by regular use, so as to not further reduce power. However, most glucosamine/chondroitin users take the supplements regularly.[2] In exploratory analyses, duration was assessed for the particular supplement used, and may not reflect total duration of use of a given ingredient; thus, these duration-specific analyses should be interpreted as exploratory. Although supplement use was assessed in the prior 30 days (reflecting exposure before CRP measurement), it is always possible that use in this period may not reflect use in the etiologically relevant timeframe. Finally, although the authors have taken extensive effort in covariate adjustment, it is conceivable that there is some residual confounding, and if the degree of confounding varies over time, this could give rise to the unexpected change in association over time.

In conclusion, in this nationally representative study, the authors observed no clear pattern of association between glucosamine and chondroitin use and CRP, largely due to the unexpected variation over calendar time. Although inverse associations in early years support prior study, null and/or positive associations in later years suggest that further research is needed to better understand why these associations changed over time, and if they reflect a true change in exposure, bias, or chance.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This study is supported by the National Institutes of Health (P30 CA008748 and R03 CA212983). X.Z. is supported by NIH K07 CA188126, R21CA238651, and American Cancer Society Research Scholar Grant (RSG NEC-130476). Y.C. is supported by NIH K07CA218377.

Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3

References

1. Qato DM, Alexander GC, Conti RM, et al. Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. JAMA 2008;300:2867–2878.
2. Kantor ED, Rehm CD, Du M, et al. Trends in dietary supplement use among US adults from 1999–2012. JAMA 2016;316:1464–1474.
3. Clegg DO, Reda DJ, Harris CL, et al. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. N Engl J Med 2006;354:795–808.
4. Singh JA, Noorbaloochi S, MacDonald R, Maxwell LJ. Chondroitin for osteoarthritis. Cochrane Database Syst Rev 2015;1:CD005614.
5. Zhang W, Moskowitz RW, Nuki G, et al. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthr Cartil 2008;16:137–162.
6. Jordan KM, Arden NK, Doherty M, et al. EULAR Recommendations 2003: An evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). Ann Rheum Dis 2003;62:1145–1155.
7. McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: A systematic quality assessment and meta-analysis. JAMA 2000;283:1469–1475.
8. Bruyere O, Reginster JY. Glucosamine and chondroitin sulfate as therapeutic agents for knee and hip osteoarthritis. Drugs Aging 2007;24:573–580.
9. Roman-Blas JA, Castaneda S, Sanchez-Pernaute O, et al. Combined treatment with chondroitin sulfate and glucosamine sulfate shows no superiority over placebo for reduction of joint pain and functional impairment in patients with knee osteoarthritis: A Six-Month Multicenter, Randomized, Double-Blind, Placebo-Controlled Clinical Trial. Arthritis Rheum 2017;69:77–85.
10. Largo R, Alvarez-Soria MA, Diez-Ortego I, et al. Glucosamine inhibits IL-1beta-induced NFkappaB activation in human osteoarthritic chondrocytes. Osteoarthr Cartil 2003; 11:290–298.
11. Xu CX, Jin H, Chung YS, et al. Chondroitin sulfate extracted from the Styela clava tunic suppresses TNF-alpha-induced expression of inflammatory factors, VCAM-1 and iNOS by blocking Akt/NF-kappaB signal in JB6 cells. Cancer Lett 2008;264:93–100.
12. Sakai S, Sugawara T, Kishi T, et al. Effect of glucosamine and related compounds on the degranulation of mast cells and ear swelling induced by dinitrofluorobenzene in mice. Life Sci 2010;86:337–343.
13. Iovu M, Dumais G, du Souich P. Anti-inflammatory activity of chondroitin sulfate. Osteoarthr Cartil 2008;16 Suppl 3:S14–18.
14. Wu YL, Kou YR, Ou HL, et al. Glucosamine regulation of LPS-mediated inflammation in human bronchial epithelial cells. Eur J Pharmacol 2010;635:219–226.
15. Yomogida S, Hua J, Sakamoto K, Nagaoka I. Glucosamine suppresses interleukin-8 production and ICAM-1 expression by TNF-alpha-stimulated human colonic epithelial HT-29 cells. Int J Mol Med 2008;22:205–211.
16. Neil KM, Orth MW, Coussens PM, et al. Effects of glucosamine and chondroitin sulfate on mediators of osteoarthritis in cultured equine chondrocytes stimulated by use of

recombinant equine interleukin-1beta. Am J Vet Res 2005; 66:1861–1869.
17. Hong H, Park YK, Choi MS, et al. Differential down-regulation of COX-2 and MMP-13 in human skin fibroblasts by glucosamine-hydrochloride. J Dermatol Sci 2009; 56:43–50.
18. Nakamura H, Shibakawa A, Tanaka M, et al. Effects of glucosamine hydrochloride on the production of prostaglandin E2, nitric oxide and metalloproteases by chondrocytes and synoviocytes in osteoarthritis. Clin Exp Rheumatol 2004;22:293–299.
19. Rajapakse N, Kim MM, Mendis E, Kim SK. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 in lipopolysaccharide-stimulated RAW264.7 cells by carboxybutyrylated glucosamine takes place via down-regulation of mitogen-activated protein kinase-mediated nuclear factor-kappaB signaling. Immunology 2008;123:348–357.
20. Gouze JN, Bordji K, Gulberti S, et al. Interleukin-1beta down-regulates the expression of glucuronosyltransferase I, a key enzyme priming glycosaminoglycan biosynthesis: Influence of glucosamine on interleukin-1beta-mediated effects in rat chondrocytes. Arthritis Rheum 2001;44:351–360.
21. Largo R, Martinez-Calatrava MJ, Sanchez-Pernaute O, et al. Effect of a high dose of glucosamine on systemic and tissue inflammation in an experimental model of atherosclerosis aggravated by chronic arthritis. Am J Physiol Heart Circ Physiol 2009;297:H268–276.
22. Azuma K, Osaki T, Wakuda T, et al. Suppressive effects of N-acetyl-D-glucosamine on rheumatoid arthritis mouse models. Inflammation 2012;35:1462–1465.
23. Campo GM, Avenoso A, Campo S, et al. Efficacy of treatment with glycosaminoglycans on experimental collagen-induced arthritis in rats. Arthritis Res Ther 2003;5:R122–131.
24. Hua J, Suguro S, Hirano S, et al. Preventive actions of a high dose of glucosamine on adjuvant arthritis in rats. Inflamm Res 2005;54:127–132.
25. Arafa NM, Hamuda HM, Melek ST, Darwish SK. The effectiveness of Echinacea extract or composite glucosamine, chondroitin and methyl sulfonyl methane supplements on acute and chronic rheumatoid arthritis rat model. Toxicol Indus Health 2013;29:187–201.
26. Chou MM, Vergnolle N, McDougall JJ, et al. Effects of chondroitin and glucosamine sulfate in a dietary bar formulation on inflammation, interleukin-1beta, matrix metalloprotease-9, and cartilage damage in arthritis. Exp Biol Med 2005;230:255–262.
27. Hori Y, Hoshino J, Yamazaki C, et al. Effects of chondroitin sulfate on colitis induced by dextran sulfate sodium in rats. Jap J Pharmacol 2001;85:155–160.
28. Yomogida S, Kojima Y, Tsutsumi-Ishii Y, et al. Glucosamine, a naturally occurring amino monosaccharide, suppresses dextran sulfate sodium-induced colitis in rats. Int J Mol Med 2008;22:317–323.
29. Kantor ED, Lampe JW, Navarro SL, et al. Associations between glucosamine and chondroitin supplement use and biomarkers of systemic inflammation. J Altern Complement Med 2014;20:479–485.
30. Kantor ED, Lampe JW, Vaughan TL, et al. Association between use of specialty dietary supplements and C-reactive protein concentrations. Am J Epidemiol 2012;176:1002–1013.
31. Navarro SL, White E, Kantor ED, et al. Randomized trial of glucosamine and chondroitin supplementation on inflammation and oxidative stress biomarkers and plasma proteomics profiles in healthy humans. PLoS One 2015;10: e0117534.
32. Bak YK, Lampe JW, Sung MK. Effects of dietary supplementation of glucosamine sulfate on intestinal inflammation in a mouse model of experimental colitis. J Gastroenterol Hepatol 2014;29:957–963.
33. Li Q, Withoff S, Verma IM. Inflammation-associated cancer: NF-kappaB is the lynchpin. Trends Immunol 2005;26: 318–325.
34. Nakamura H NK. Effects of glucosamine/chondroitin supplement on osteoarthritis: Involvement of PGE2 and YKL-40. J Rheumatism Joint Surg 2002;21:175–184.
35. Nakamura H, Masuko K, Yudoh K, et al. Effects of glucosamine administration on patients with rheumatoid arthritis. Rheumatol Int 2007;27:213–218.
36. Kantor ED, Lampe JW, Peters U, et al. Use of glucosamine and chondroitin supplements and risk of colorectal cancer. Cancer Causes Control 2013;24:1137–1146.
37. Kantor ED, Zhang X, Wu K, et al. Use of glucosamine and chondroitin supplements in relation to risk of colorectal cancer: Results from the Nurses' Health Study and Health Professionals follow-up study. Int J Cancer 2016;139: 1949–1957.
38. Satia JA, Littman A, Slatore CG, et al. Associations of herbal and specialty supplements with lung and colorectal cancer risk in the VITamins and Lifestyle study. Cancer Epidemiol Biomarkers Prev 2009;18:1419–1428.
39. Brasky TM, Lampe JW, Slatore CG, White E. Use of glucosamine and chondroitin and lung cancer risk in the VITamins And Lifestyle (VITAL) cohort. Cancer Causes Control 2011;22:1333–1342.
40. Kantor ED, Newton CC, Giovannucci EL, et al. Glucosamine use and risk of colorectal cancer: Results from the Cancer Prevention Study II Nutrition Cohort. Cancer Causes Control 2018;29:389–397.
41. Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. JAMA 2004;291:585–590.
42. Cai Q, Gao YT, Chow WH, et al. Prospective study of urinary prostaglandin E2 metabolite and colorectal cancer risk. J Clin Oncol 2006;24:5010–5016.
43. Wang S, Liu Z, Wang L, Zhang X. NF-kappaB signaling pathway, inflammation and colorectal cancer. Cell Mol Immunol 2009;6:327–334.
44. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: A common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin 2006;56:69–83.
45. Rothwell PM, Wilson M, Elwin CE, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet 2010;376: 1741–1750.
46. Lee JM, Yanagawa J, Peebles KA, et al. Inflammation in lung carcinogenesis: New targets for lung cancer chemoprevention and treatment. Crit Rev Oncol Hematol 2008; 66:208–217.
47. National Center for Health Statistics (2005) NHANES 2003–2004 Public Data General Release File Documentation. Hyattsville, MD: National Center for Health Statistics. Online document at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/general_data_release_doc_03-04.pdf, accessed June 3, 2020.
48. National Center for Health Statistics. National Health and Nutrition Examination Survey 1999–2016 data documen-

tation, codebook, and frequencies: Dietary supplement database—Ingredient information. Online document at: http://wwwn.cdc.gov/Nchs/Nhanes/1999-2000/DSII.htm, accessed June 3, 2020.
49. Zhou B, Shu B, Yang J, et al. C-reactive protein, interleukin-6 and the risk of colorectal cancer: A meta-analysis. Cancer Causes Control 2014;25:1397–1405.
50. National Center for Health Statistics. National Health and Nutrition Examination Survey. 2003–2004 Data Documentation, Codebook, and Frequencies. C-Reactive Protein (CRP), Bone Alkaline Phosphatase (BAP), and Parathyroid Hormone (PTH) (L11_C) Hyattsville, MD: National Center for Health Statistics; 2006. Online document at: www.cdc.gov/nchs/nhanes/nhanes2003-2004/L11_C.htm, accessed June 2, 2020.
51. Kantor ED, Ulrich CM, Owen RW, et al. Specialty supplement use and biologic measures of oxidative stress and DNA damage. Cancer Epidemiol Biomarkers Prev 2013; 22:2312–2322.

Address correspondence to:
Elizabeth D. Kantor, PhD, MPH
Department of Epidemiology and Biostatistics
Memorial Sloan Kettering Cancer Center
485 Lexington Avenue, 2nd Floor
New York, NY 10017
USA

E-mail: kantore@mskcc.org