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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

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Abstract

Recent epidemiologic evidence has emerged to suggest that use of glucosamine and chondroitin supplements may be associated with reduced risk of colorectal cancer (CRC). We therefore evaluated the association between use of these non-vitamin, non-mineral supplements and risk of CRC in two prospective cohorts, the Nurses' Health Study and Health Professionals Follow-up Study. Regular use of glucosamine and chondroitin was first assessed in 2002 and participants were followed until 2010, over which time 672 CRC cases occurred. Cox proportional hazards regression was used to estimate relative risks (RRs) within each cohort, and results were pooled using a random effects meta-analysis. Associations were comparable across cohorts, with a RR of 0.79 (95% CI: 0.63–1.00) observed for any use of glucosamine and a RR of 0.77 (95% CI: 0.59–1.01) observed for any use of chondroitin. Use of glucosamine in the absence of chondroitin was not associated with risk of CRC, whereas use of glucosamine + chondroitin was significantly associated with risk (RR: 0.77; 95% CI: 0.58–0.999). The association between use of glucosamine + chondroitin and risk of CRC did not change markedly when accounting for change in exposure

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status over follow-up (RR: 0.75; 95% CI: 0.58–0.96), nor did the association significantly vary by sex, aspirin use, body mass index, or physical activity. The association was comparable for cancers of the colon and rectum. Results support a protective association between use of glucosamine and chondroitin and risk of CRC. Further study is needed to better understand the chemopreventive potential of these supplements.

Keywords

chemoprevention; chondroitin; colorectal cancer; epidemiology; glucosamine

INTRODUCTION

Glucosamine and chondroitin supplements are among the most popular specialty supplements in the United States, with a prevalence of use among older adults comparable to that of acetaminophen.[1] Generally taken for osteoarthritis, these non-vitamin, non-mineral supplements are often taken together in a single daily supplement. Although the effectiveness of these supplements on joint pain and function has been the subject of much debate,[2–7] recent evidence has emerged to suggest a potential beneficial effect on risk of colorectal cancer (CRC).[8, 9] In an exploratory analysis conducted within the VITamins and Lifestyle (VITAL) cohort, use of glucosamine and chondroitin supplements was observed to be associated with decreased risk of colorectal cancer (CRC),[9] with any use of glucosamine in the 10 years prior to baseline associated with a 27% reduced risk of CRC (hazard ratio [HR]: 0.73; 95% CI: 0.54–0.98) and any use of chondroitin associated with a 35% reduced risk (HR: 0.65; 95% CI: 0.45–0.93). A later, more in-depth analysis with extended follow-up revealed that persons using glucosamine + chondroitin on 4+ days/week for 3+ years had 45% lower CRC risk than non-users (HR: 0.55; 95% CI: 0.30–1.01).[8] A corroborating body of evidence from *in vitro*, animal, and human studies suggests that glucosamine and chondroitin have potential anti-inflammatory properties,[10–31] providing a plausible biologic mechanism by which these supplements may reduce risk of CRC.

Given the promising, albeit limited, evidence to suggest a potential chemopreventive effect, we therefore sought to examine the association between use of glucosamine and chondroitin and CRC in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS). Results were further examined for heterogeneity by sex, aspirin use, body mass index (BMI), physical activity, and anatomic subsite.

MATERIALS AND METHODS

Study Population

The NHS is an ongoing prospective cohort study established in 1976 when 121,700 registered nurses residing in 11 states completed and returned a self-administered questionnaire.[32] All nurses were female, married, and between the ages of 30 and 55. The HPFS is an on-going prospective cohort study of 51,529 US male health professionals who were between the ages of 40 and 75 at the time of baseline data collection (1986).[33] In both NHS and HPFS, study participants completed mailed questionnaires, which assessed

updated lifestyle and medical information every two years after baseline; follow-up questionnaires have been received from over 90% of study participants within each 2-year cycle. Study protocols were approved by the Institutional Review Boards of Brigham and Women's Hospital and the Harvard T. H. Chan School of Public Health (Boston, MA).

This study of glucosamine and chondroitin began in 2002 when use of these supplements was first assessed. A total of 93,507 women and 37,431 men answered the 2002 questionnaire, from whom we have excluded participants with any cancer diagnosed before 2002, except non-melanoma skin cancer (n=16,763 women, n=6,886 men). We also excluded those with history of conditions characterized by high levels of systemic inflammation, including rheumatoid arthritis (n=6,589 women, n=1,961 men) and ulcerative colitis/Crohn's disease (n=1,689 women, n=650 men). These exclusions resulted in a final sample size of 68,466 women and 27,934 men.

Exposure Assessment

After being queried about use of specific supplements in 2002, participants were then asked if "there are other supplements [taken] on a regular basis"; a list of supplements was provided, including glucosamine and chondroitin, from which participants could indicate regular use. From this information, participants were classified in terms of regular glucosamine use (yes vs. no) and regular chondroitin use (yes vs. no).

Given that these supplements are frequently combined into a single daily supplement, additional variables were created to better disentangle these exposures. Specifically, we created a 'glucosamine+chondroitin' variable, representing joint use of glucosamine and chondroitin. As approximately one-quarter of glucosamine users in this study reported use of glucosamine in the absence of chondroitin, we were also able to conduct sensitivity analyses for 'glucosamine alone,' defined by the use of glucosamine in the absence of chondroitin. However, we were unable to evaluate use of chondroitin alone, as nearly all (97–98% in HPFS and NHS, respectively) chondroitin users also reported use of glucosamine.

Given concern that participants' use of glucosamine and chondroitin may change over the course of follow-up,[34] a sensitivity analysis was conducted using a time-varying exposure variable, in which participants' use of "glucosamine+chondroitin" was updated over the course of follow-up. In the NHS, participants were asked to report regular use of "glucosamine/chondroitin" in the 2006 questionnaire, and in the HPFS, participants were asked to report on regular use of "glucosamine" and "chondroitin" separately in both the 2004 and 2006 questionnaire. This information enabled the creation of a time-varying "glucosamine+chondroitin" variable in which participants' exposure status was updated in 2004 and 2006 (HPFS) or 2006 (NHS). For example, if a non-user of "glucosamine +chondroitin" in 2002 indicated use in the 2006 questionnaire, they switched from "unexposed" to "exposed" at the time of 2006 questionnaire. In our study, 8.8% of the 59,631 "glucosamine+chondroitin" non-users at baseline (2002) indicated use in 2006, whereas 6.1% of the 8,835 "glucosamine+chondroitin" users at baseline (2002) discontinued use by the time of the 2006 questionnaire.

Outcome Ascertainment

A diagnosis of CRC was reported by study participants in each of the biennial questionnaires. Study participants provided permission for researchers to obtain medical records and pathologic reports on CRC, which were used to confirm a diagnosis of CRC and abstract information on stage, histology and location. Deaths were identified from state vital statistics records, the National Death Index, family report, and the postal system. For non-respondents who died of CRC, next of kin were contacted for permission to review medical records and pathology report. For deceased study participants with a known or suspected cancer for which the studies were unable to obtain medical records, the state tumor registries were contacted to confirm the cancer. The outcome of this study, colorectal carcinoma, was defined by the following International Classification of Disease (ICD)-9th Revision (ICD-9) codes: 153.0–153.4, 153.6–153.9, 154.0, and 154.1. In subsite-specific analysis, cases of colon cancer were defined by ICD-9 codes 153.0–153.4, 153.6–153.9, and rectal cancers were defined by ICD-9 codes 154.0 and 154.1. Rectosigmoid cancers were considered rectal cancers.

Statistical Analysis

Study participants were followed from the time of the 2002 questionnaire until the earliest date of the following: CRC diagnosis, death, or until end of follow-up (May 31, 2010 for NHS; January 31, 2010 for HPFS), whichever came first. The end of follow-up in both NHS and HPFS correspond to the most recent dates for which adjudicated outcome data are available. Cox proportional hazards regression models were used to calculate hazard ratios (HRs) as an estimate of the relative risks (RRs) and corresponding 95% Confidence Intervals (95% CIs), with age as the time-metric of analysis. Analyses were conducted separately within each cohort and effect estimates were pooled across cohorts using a random-effects meta-analysis.

Characteristics of the study population are provided in Table 1, according to use of glucosamine+chondroitin in 2002. Age-adjusted and multivariable-adjusted RRs for the association between glucosamine and chondroitin and risk of CRC are presented in Table 2. In multivariable-adjusted models, covariates were selected *a priori* based on their potential association with both the exposure and outcome of interest. Covariates include: age, race, smoking, adult BMI, family history of CRC, history of sigmoidoscopy/colonoscopy, arthritis, physical activity, aspirin use, use of non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs), multivitamin use, alcohol consumption, energy-adjusted total intake of calcium, vitamin D, folate, red meat, and processed meat. Multivariable-adjusted analyses in the NHS were further adjusted for postmenopausal hormone (PMH) use. Detailed information on the covariates and their categorizations are provided in the footnote of Table 2. All covariates for analyses pertaining to baseline exposure were taken from baseline (2002 questionnaire), with the exception of osteoarthritis; this variable was pulled from the 2000 questionnaire for the NHS analysis, as this information was not collected at the time of the 2002 questionnaire. In sensitivity analyses of time-varying glucosamine + chondroitin use, time-varying covariates were used, with covariates updated at each biennial questionnaire, through 2010.

We assessed whether the association between glucosamine+chondroitin use and CRC varied by sex by assessing the p-value for heterogeneity across cohorts. We further examined whether associations differed by factors associated with the hypothesized mechanism of action, including: use of aspirin (regular use: no vs yes), BMI (<25 vs 25+ kg/m^2), and physical activity (<15 metabolic equivalents of task [MET]-hours/week vs 15+ MET-hours/week). Strata-specific pooled estimates are presented in Table 3, along with p-values for heterogeneity across cohorts (termed 'p-heterogeneity within strata'). To address whether the association between glucosamine+chondroitin and CRC significantly varied by aspirin, BMI, and physical activity, we tested for heterogeneity across strata, and have termed the corresponding p-value the 'p-interaction.' Results were also examined by anatomic subsite (colon vs rectum).

A sensitivity analysis was conducted to address potential residual confounding by screening. To this end, we examined the association between use of glucosamine+chondroitin and risk of CRC among never-screened individuals, censoring participants at the date of first screening. Given the possibility that glucosamine/chondroitin users may switch to non-aspirin NSAIDs for management of osteoarthritis, we conducted an additional sensitivity analysis in which we updated the non-aspirin NSAID covariate so as to account for change in use after baseline.

Statistical analyses were conducted using SAS (Cary, NC; Version 9.2) and Stata (College Station, TX; Version 12).

RESULTS

Six hundred and seventy-two CRC cases occurred over the course of follow-up, 450 of which occurred among women and 222 of which occurred among men. Overall, 16.9% of the study population reported regular use of glucosamine, while 13.2% reported use of chondroitin, and 12.9% reported combined use of glucosamine+chondroitin. Users of glucosamine+chondroitin tended to engage in more health-seeking behaviors than non-users (see Table 1). For example, glucosamine+chondroitin users engaged in more physical activity than non-users and were more likely to report history of sigmoidoscopy/endoscopy and use of multivitamins. As glucosamine and chondroitin are primarily used for symptoms of osteoarthritis, users were more likely to report a history of arthritis and were more likely to report use of both aspirin and non-aspirin NSAIDs. For example, in the NHS, 24.2% of glucosamine+chondroitin users reported use of 6+ tablets/week of non-aspirin NSAIDS, as compared to 13.3% of non-users. Similarly, 78.1% of glucosamine+chondroitin users in the NHS reported PMH use, as compared to 70.1% of non-users. Lastly, glucosamine +chondroitin users consumed more calcium, folate, and vitamin D per calorie than non-users.

In an age-adjusted model (see Table 2), any glucosamine use was associated with a significant 30% reduced risk of CRC (RR: 0.70; 95% CI: 0.56, 0.88) as compared to non-use. This association attenuated modestly with multivariable adjustment (0.79; 95% CI: 0.63, 1.00). In age-adjusted models, any use of chondroitin was significantly associated with a 31% reduced risk of CRC (0.69; 95% CI: 0.53, 0.88), with a RR of 0.77 observed after

multivariable adjustment (95% CI: 0.59, 1.01). Glucosamine+chondroitin was significantly associated with a reduced risk of CRC in both age-adjusted analyses (0.68; 95% CI: 0.52, 0.88) and multivariable-adjusted analyses (0.77; 95% CI: 0.58, 0.999). When accounting for change in glucosamine+chondroitin use after baseline, results strengthened slightly, but remained materially unchanged (0.75; 95% CI: 0.58–0.96). Sensitivity analyses for use of 'glucosamine only' indicated no association between use of glucosamine only and risk of CRC (multivariable-adjusted pooled RR: 0.94; 95% CI: 0.62–1.42). No heterogeneity was observed across cohorts for any of the above-mentioned associations, and there was no evidence that the proportional hazards assumption was violated.

Results did not differ by sex, as indicated by the lack of heterogeneity across cohorts (Table 2). Associations were also examined after stratifying by aspirin use, BMI, and physical activity (Table 3). Results did not differ by aspirin use or physical activity, and although not statistically significant, a stronger association was observed among normal weight individuals (0.55; 95% CI: 0.34, 0.88) than overweight/obese individuals (0.91; 95% CI: 0.66, 1.27). Results were also comparable for cancers of the colon and rectum, with a RR of 0.76 (95% CI: 0.56, 1.02) observed for colon cancer, and a RR of 0.79 (95% CI: 0.43, 1.45) observed for rectal cancer. No significant heterogeneity was observed across cohorts for stratified estimates or subsite-specific estimates.

To rule out the possibility that results may be driven by differences in screening practices, we conducted a sensitivity analysis among the never screened population: in this analysis, we restricted the analysis to those with no history of endoscopy at baseline, and censored individuals at the date of first screen. In this sensitivity analysis, we found that use of glucosamine + chondroitin was associated with a statistically significant 42% reduced risk of CRC among the never-screened group (RR: 0.58; 95% CI: 0.38, 0.88).

Additional sensitivity analyses revealed that the association between glucosamine + chondroitin and CRC at baseline remained unchanged when accounting for change in NSAID use after baseline (results not shown), and the association did not change if additionally adjusted for duration of aspirin/non-aspirin NSAID use, fiber intake, or vitamin E intake.

DISCUSSION

In this analysis of two large prospective cohort studies, we observed an inverse association between use of glucosamine and chondroitin and risk of CRC, with no evidence of heterogeneity across cohorts. This finding supports a growing body of evidence that suggests that these supplements may have chemopreventive potential.

In the current study, use of glucosamine and chondroitin was associated with a reduced risk of CRC. Effect estimates for any use of glucosamine (HR: 0.79) and any use of chondroitin (HR: 0.77) align with findings of the VITAL study, in which any use of glucosamine was associated with an 27% reduced risk of CRC, while any use of chondroitin was associated with a 35% reduced risk.[9] Here, the association was strongest for use of combined glucosamine and chondroitin (RR: 0.77), with no association observed for use of

glucosamine only (RR: 0.94). This pattern of association, which has been observed in both studies of CRC[8] and inflammation,[29] suggests that it may be chondroitin or perhaps the combination of glucosamine + chondroitin driving the observed associations. Even so, such comparisons are exploratory in nature, given the relatively small number of persons reporting use of glucosamine alone.

In the earlier VITAL study, it was observed that the association between use of glucosamine and chondroitin and risk of CRC weakened with extended follow-up.[8] While the reasons underlying this pattern of association are unclear, it was hypothesized that such a pattern of association may emerge due to the increasing popularity of these supplements over the course of follow-up:[34] if the etiologically relevant time frame extends into follow-up, then failure to account for changing patterns of exposure over follow-up would be expected to attenuate observed associations as the duration of follow-up extends further from baseline, consistent with findings from the VITAL study.[8] In the current study, we were able to update exposure, and observed only a slight strengthening of association when accounting for change in use after baseline (2006 for NHS; 2004 and 2006 for HPFS), suggesting that change in exposure status, if comparable across the two populations, may not explain why the observed association weakened over follow-up in the VITAL study.

It is hypothesized that glucosamine and chondroitin may reduce risk of CRC through an anti-inflammatory mechanism. Evidence from *in vitro*, animal, and human studies indicates that these supplements have anti-inflammatory properties.[10–31] Specifically, *in vitro* models suggest that glucosamine and chondroitin inhibit the activation of nuclear factor kappa B (NFkB), a transcription factor central to the inflammatory cascade, by inhibiting the degradation of its inhibitory subunit, IkB, in a dose-dependent manner.[10, 11] Corroborating evidence has shown that glucosamine and chondroitin reduce factors downstream of NFkB, including: tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), cyclooxygenase-2 (COX-2), and prostaglandin E_2 (PGE_2).[10, 12–20] Further *in vitro* studies have demonstrated that glucosamine and chondroitin reduces inflammation in colonic cells.[15] A number of animal studies have demonstrated that administration of glucosamine/chondroitin is associated with reduced levels of inflammatory biomarkers downstream of NFkB activation.[21–25,26] Importantly, administration of glucosamine and chondroitin has been shown to have anti-inflammatory effects in the colon in animal models:[27, 28, 35] notably, in a recent study of mice with chemically-induced colitis, it was reported that glucosamine administration not only reduced markers of systemic inflammation, but that this was accompanied by a reduction in colonic NFkB mRNA expression.[35] These results, suggesting that glucosamine and chondroitin have biologic effect in the colon, add further plausibility to the observed epidemiologic association between use of glucosamine/chondroitin and risk of CRC. This laboratory evidence is supported by two human observational studies[29, 30] and a recent small pilot randomized controlled cross-over trial,[31] in which we observed glucosamine/chondroitin to be associated with reduced levels of the systemic inflammatory marker, CRP. Given that inflammation has been strongly implicated in the etiology of CRC,[36–40] this growing body of evidence offers a plausible biologic mechanism by which these supplements may reduce risk of CRC.

In our study, results were examined stratified by factors associated with inflammation. We observed that results did not markedly differ by sex, aspirin use, or physical activity, although it should be noted that power to detect subgroup-specific differences was limited. While no significant difference in association was observed by BMI (p-interaction: 0.09), the association between glucosamine+chondroitin use and risk of CRC was stronger among lean individuals (BMI <25 kg/m^2) than among overweight/obese individuals (BMI 25+ kg/m^2), with pooled RRs of 0.55 and 0.91 observed, respectively. It may be that the association is stronger among lean individuals, as lean individuals may be less likely to develop CRC through pathways independent of inflammation, such as hyperinsulinemia. Even so, this pattern of association is the opposite of what was observed in VITAL, in which the association between glucosamine/chondroitin was significantly stronger among overweight/obese individuals than normal weight individuals. In both of these studies, however, power to detect subgroup-specific differences was limited, and additional work is needed to understand the interplay between glucosamine/chondroitin use and obesity in relation to inflammation and risk of CRC.

This study has several important limitations. First, information on frequency and duration of glucosamine/chondroitin use was not collected in the NHS and HPFS, and we were therefore unable to evaluate how the association varies by frequency and duration of use. Due to the small number of persons using chondroitin alone, we were unable to evaluate use of ‘chondroitin alone’ in order to more fully extricate these exposures from one another. However, this is a limitation of any observational study, given that chondroitin is rarely used in the absence of glucosamine in the population. In this analysis and the prior VITAL analysis, we were under-powered to evaluate associations within strata of interest, and a larger population will be needed to better understand potential interaction. Additionally, given that use of glucosamine/chondroitin supplements is associated with use of NSAIDs, which have been shown to reduce risk of CRC,[40] results should be interpreted with caution. Even so, it should be noted that the effect estimates for the association between aspirin and CRC in these cohorts is comparable to the effect seen in RCTs,[40] indicating that we are measuring (and adjusting for) this important covariate well. Lastly, this study was conducted among a predominantly non-Hispanic white population, and it is unclear how these results would generalize to more diverse populations.

This study has several notable strengths. Importantly, results were obtained from two prospective studies that assessed use of glucosamine/chondroitin, along with detailed assessment on potential confounding factors, including use of aspirin, non-aspirin NSAID use, screening, and a variety of dietary and lifestyle factors associated with risk of CRC. In addition, the NHS and HPFS are comprised of health professionals, likely increasing the accuracy of self-reported health information. Further, in this analysis, we were able to conduct sensitivity analyses accounting for change in exposure status during follow-up, an important consideration, given that this study was conducted at a time in which these supplements shifted in popularity.[34] Lastly, we were able to conduct an additional sensitivity analysis to address concern about residual confounding by screening. In our analyses limited to never-screened individuals, we observed a strong inverse association between glucosamine + chondroitin and risk of CRC, suggesting that the observed association is not due to screening bias.

In conclusion, results of this study suggest a potential beneficial effect of glucosamine and chondroitin supplementation on risk of CRC, and further support the previously observed association between use of these supplements and risk of CRC in the VITAL study. Additional study is needed to better understand the association between use of glucosamine and chondroitin and risk of CRC, and the mechanisms by which these supplements may affect risk of CRC.

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ABBREVIATIONS

BMI	Body mass index
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
CRC	Colorectal cancer
HPFS	Health Professionals Follow-up Study
HR	Hazard ratio
ICD-9	International Classification of Disease-9th Revision
IL-6	Interleukin-6
IL-8	Interleukin-8
MET	Metabolic equivalents of task
MSM	Methylsulfonylmethane
NFkB	Nuclear factor kappa B
NHS	Nurses' Health Study
NSAID	Non-steroidal anti-inflammatory drugs
PGE-M	Prostaglandin E_2-Metabolite

PGE_2	Prostaglandin E_2
RR	Relative risk
TNF-α	Tumor necrosis factor-alpha
VITAL	VITamins And Lifestyle Study
95% CI	95% Confidence Interval

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BRIEF DESCRIPTION OF NOVELTY AND IMPACT OF PAPER

In this prospective study, we observed combined use of glucosamine and chondroitin supplements to be associated with a statistically significant 23% reduced risk of colorectal cancer (CRC). These results align with prior observations from the VITamins And Lifestyle (VITAL) study and may reflect the potential anti-inflammatory effect of these supplements. Given the need for safe, effective, and easily implemented CRC preventive strategies, these results merit further study.

Table 1

Baseline age-adjusted characteristics of participants by combined use of glucosamine and chondroitin in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)

	Nurses' Health Study		Health Professionals Follow-up Study	
	Glucosamine+Chondroitin No use (n=59,631)	**Glucosamine+Chondroitin Use (n=8,835)**	**Glucosamine+Chondroitin No Use (n=24,314)**	**Glucosamine+Chondroitin Use (n=3,620)**
Age, years	67.8 (7.1)	67.9 (6.7)	67.2 (8.6)	66.8 (8.0)
White, %	97.1	97.6	96.0	97.1
Body mass index[1], kg/m^2	25.3 (4.4)	25.8 (4.6)	25.9 (3.4)	26.0 (3.2)
Physical activity[2], MET-h/week	17.1 (16.2)	19.7 (18.0)	31.5 (25.8)	37.5 (28.1)
Family history of colorectal cancer, %	15.5	16.0	13.2	13.8
Aspirin use, tablets/wk				
- No use, %	64.6	55.8	63.1	53.6
- 1–5 tablets/week, %	11.2	13.5	11.8	14.8
- 6+ tablets/week, %	24.2	30.7	25.1	31.6
Other NSAID use, tablets/wk				
- No use, %	68.4	51.8	82.4	71.7
- 1–5 tablets/week, %	18.3	24.0	10.4	14.8
- 6+ tablets/week, %	13.3	24.2	7.2	13.5
History of sigmoidoscopy/colonoscopy	43.9	54.2	65.1	73.6
Past smoking, %	45.4	49.7	43.0	46.5
Current smoking, %	9.4	4.4	10.8	8.9
Multivitamin use, %	54.8	80.3	53.8	79.3
Alcohol[3], g/day	5.7 (8.4)	5.8 (8.2)	11.0 (13.0)	11.5 (12.6)
Total calcium intake[3], ug/d	1051 (374)	1228 (378)	951 (337)	1086 (383)
Total folate intake[3], ug/d	486 (186)	569 (190)	589 (228)	714 (251)
Total vitamin D[3], IU/d	378 (191)	465 (198)	432 (227)	534 (249)
Red meat[3], servings/wk	1.8 (1.1)	1.7 (1.0)	1.7 (1.3)	1.6 (1.2)
Processed meat[3], servings/wk	0.8 (1.0)	0.8 (0.9)	1.0(1.3)	1.0(1.1)
Other Arthritis[4], %	17.8	38.4	13.4	31.0
PMH use, %	70.1	78.1	NA	NA

ABBREVIATIONS: MET (metabolic equivalents of task); NSAID (non-steroidal anti-inflammatory drug); PMH (post-menopausal hormone)

Values are means (SD) or percentages and are standardized to the age distribution of the study population; table excludes users of glucosamine only or chondroitin only.

[1] Body mass index was calculated as weight in kilograms divided by the square of height in meters.

[2] Hours of metabolic equivalent of tasks (METs)

[3] Dietary intakes were estimated with a food frequency questionnaire in 2002.

[4] 2000 value used for the NHS.

Table 2

Multivariable[a] relative risks of colorectal cancer according to use of glucosamine and chondroitin in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) (2002–2010)

	Nurses' Health Study			Health Professionals Follow-up Study			Pooled			
	Cases	Cohort	RR (95% CI)	Cases	Cohort	RR (95% CI)	Cases	Cohort	RR (95% CI)	p-heterogeneity
BASELINE EXPOSURE										
Any glucosamine										
No	391	56,875	1.00 (Ref)	192	23,240	1.00 (Ref)	583	80,115	1.00 (Ref)	
Yes – age adjusted	59	11,591	0.70 (0.53, 0.92)	30	4,694	0.72 (0.49, 1.06)	89	16,285	0.70 (0.56, 0.88)	0.88
Yes - MV	59	11,591	0.82 (0.62, 1.09)	30	4,694	0.75 (0.50, 1.12)	89	16,285	0.79 (0.63, 1.00)	0.71
Any chondroitin										
No	407	59,499	1.00 (Ref)	198	24,202	1.00 (Ref)	605	83,701	1.00 (Ref)	
Yes – age adjusted	43	8,967	0.66 (0.48, 0.90)	24	3,732	0.74 (0.48, 1.13)	67	12,699	0.69 (0.53, 0.88)	0.69
Yes - MV	43	8,967	0.79 (0.57, 1.09)	24	3,732	0.75 (0.48, 1.17)	67	12,699	0.77 (0.59, 1.01)	0.88
Glucosamine + chondroitin										
No	407	59,631	1.00 (Ref)	200	24,314	1.00 (Ref)	607	83,945	1.00 (Ref)	
Yes – age adjusted	43	8,835	0.67 (0.49, 0.92)	22	3,620	0.69 (0.44, 1.07)	65	12,455	0.68 (0.52, 0.88)	0.93
Yes - MV	43	8,835	0.80 (0.58, 1.11)	22	3,620	0.70 (0.44, 1.11)	65	12,455	0.77 (0.58, 0.999)	0.65
TIME-VARYING EXPOSURE										
Glucosamine + chondroitin										
No	403	59,631	1.00 (Ref)	198	24,314	1.00 (Ref)	601	83,945	1.00 (Ref)	
Yes – age adjusted	47	8,835	0.65 (0.48, 0.89)	24	3,620	0.67 (0.44, 1.04)	71	12,455	0.66 (0.52, 0.85)	0.90
Yes - MV	47	8,835	0.77 (0.56, 1.05)	24	3,620	0.70 (0.45, 1.10)	71	12,455	0.75 (0.58, 0.96)	0.75

ABBREVIATIONS: MV (multivariable); RR (relative risk); 95% CI (95% confidence interval)

[a]Multivariable relative risks were adjusted for age (in month), race (white vs. not), smoking (0, 1–10, or > 10 pack-years), BMI (< 25, 25 –< 27.5, 27.5 –< 30, ≥ 30 kg/m^2), history of colorectal cancer in a parent or sibling (yes or no), history of sigmoidoscopy/endoscopy (yes or no), physical activity (< 3, 3-< 27, 27+ MET-hrs/wk), aspirin use (non-user, 1, 2–3, 4–5, 6+ days/week), other NSAID use (non-user, 1, 2–3, 4–5, 6+ days/week), multivitamin use (yes or no), alcohol consumption (0 –< 5, 5 –< 10, 10 –< 15, or ≥ 15 g/d), energy-adjusted total intake of calcium, vitamin D, folate, red meat, processed meat (all in tertiles), other arthritis (yes or no), and postmenopausal hormone use (never/pre-menopausal, past, current; women only). Of note, for the analyses using baseline exposure, the covariates assessed in 2002 were used. For the analyses on time-varying exposure, we accounted for changes in covariates assessed from 2002 to 2010.

Table 3

Multivariable[a] relative risks of colorectal cancer according to baseline use of glucosamine and chondroitin in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) (2002–2010), by aspirin use, BMI, and physical activity

	Glucosamine+Chondroitin No Use			Glucosamine+Chondroitin Use			p-heterogeneity within strata[b]	p-interaction[c]
	Cohort N	Case N	Pooled RR (95% CI)[a]	Cohort N	Case N	Pooled RR (95% CI)[a]		
ASPIRIN[d]								
No	38,580	305	1.00 (Ref)	5,681	30	0.72 (0.49, 1.07)	0.81	0.67
Yes	44,982	295	1.00 (Ref)	6,715	34	0.81 (0.56, 1.17)	0.52	
BMI[e]								
Normal Weight (<25 kg/m^2)	41,922	303	1.00 (Ref)	5,154	27	0.55 (0.34, 0.88)	0.99	0.09
Overweight/Obese (25+ kg/m^2)	41,570	297	1.00 (Ref)	7,233	37	0.91 (0.66, 1.27)	0.44	
PHYSICAL ACTIVITY[f]								
Low (<15 MET-hours/wk)	43,460	289	1.00 (Ref)	5,979	19	0.78 (0.51, 1.20)	0.41	0.89
High (15+ MET-hours/wk)	40,102	311	1.00 (Ref)	6,417	45	0.75 (0.52, 1.07)	0.46	

ABBREVIATIONS: BMI (body mass index); MET (metabolic equivalents of task); RR (relative risk); 95% CI (95% confidence interval)

[a]Multivariable relative risks were adjusted for age (in month), race (white vs. not), smoking (0, 1–10, or > 10 pack-years), BMI (< 25, 25 –< 27.5, 27.5 –< 30, ≥ 30 kg/m^2), history of colorectal cancer in a parent or sibling (yes or no), history of sigmoidoscopy/endoscopy (yes or no), physical activity (< 3, 3-< 27, 27+ MET-hrs/wk), aspirin use (non-user, 1, 2–3, 4–5, 6+ days/week), other NSAID use (non-user, 1, 2–3, 4–5, 6+ days/week), multivitamin use (yes or no), alcohol consumption (0 –< 5, 5 –< 10, 10 –< 15, or ≥ 15 g/d), energy-adjusted total intake of calcium, vitamin D, folate, red meat, processed meat (all in tertiles), other arthritis (yes or no), and postmenopausal hormone use (never/pre-menopausal, past, current, women only).

[b]p-heterogeneity within strata represents the strata-specific p-values for heterogeneity across the NHS and HPFS. For example, the p-heterogeneity=0.81 for 'no aspirin use' indicates that the association between glucosamine+chondroitin and risk of CRC among non-users of aspirin was comparable in the NHS and the HPFS.

[c]p-interaction indicates whether the pooled association varies by a given stratifying factor. For example, the p-interaction=0.67 for aspirin represents that the pooled association between glucosamine +chondroitin and risk of CRC does not significantly vary by aspirin use

[d]442 persons have been excluded from aspirin-stratified analyses due to missing data for aspirin

[e]521 persons have been excluded from BMI-stratified analyses due to missing data for BMI

[f]442 persons have been excluded from physical activity-stratified analyses due to missing data for physical activity