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VITAMIN SUPPLEMENTS AND RADIOGRAPHIC KNEE OSTEOARTHRITIS: THE CLEARWATER OSTEOARTHRITIS STUDY

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ABSTRACT

Objective: To evaluate the association between vitamin supplements and the incidence and progression of radiographic knee osteoarthritis (OA). **Study Design:** Prospective cohort. **Methods:** Men and women aged 40 years and older ($N = 2239$) participating in the community-based Clearwater Osteoarthritis Study (1988–current) with biennial, sequential radiographs. The Lawrence & Kellgren ordinal scale was used to determine evidence of the study outcome, radiographic knee OA, and progression of radiographic knee OA. The study exposure was baseline history of self-reported vitamin supplement usage. **Results:** Individuals without baseline knee OA who self-reported vitamin supplement usage were 12% less likely to develop knee OA than were those individuals who self-reported no vitamin supplement usage (RR = 0.88; 95% C.I. 0.86–0.89). Those with baseline knee OA who self-reported vitamin supplement usage were 7% less likely to experience knee OA progression than were those individuals who self-reported no vitamin supplement usage (RR = 0.93; 95% C.I. 0.87–0.99). **Conclusion:** After consideration of BMI, gender, age, history of knee trauma, exercise and vitamin supplement usage prior to study baseline, these findings suggest that vitamin supplement usage may play a protective role in knee OA. Vitamin supplements may be a cost-effective strategy to reduce the incidence

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and/or slow the progression of knee OA. As many approved OA drugs are accompanied by adverse side effects, the role of vitamin supplements in the development and progression of knee OA warrants further investigation.

Keywords: Osteoarthritis; Knee; Vitamin; Epidemiology; Incidence; Progression.

INTRODUCTION

Osteoarthritis (OA), the most common joint disease, is a condition of synovial joints characterized by focal cartilage loss and the accompanying reparative bone response. OA affects more than 21-million Americans, and is a leading cause of disability in the USA.[23] It frequently affects the middle-aged and older population, involving various sites such as the neck, lower back, knees, hips, and fingers. Individuals with symptomatic knee OA who experience pain and loss of function comprise approximately 10% of populations over 65 years of age.[7]

While the role that mechanical trauma can play in the development or progression of OA is fairly self-evident and well documented,[4,6,9,26] researchers have long considered the potential role of systemic factors. The health of articular cartilage and bone is dependent upon the regular provision of nutrients, and it has been suggested that diets deficient in nutrients may lead to arthropathy.[20] A study in 2007 examined the effect of dietary antioxidants on knee structure in a cohort of healthy, middle-aged subjects with no clinical knee osteoarthritis. Their findings suggested the beneficial effect of fruit consumption and vitamin C intake as they were associated with a reduction in bone size and the number of bone-marrow lesions, both of which are important in the pathogenesis of knee osteoarthritis.[24] In their study, Wang *et al.* noted that ''*. . . all studies to date have been carried out in patients with OA. No studies have examined the effect of antioxidants on knee cartilage and bone in healthy subjects, prior to symptomatic disease*.'' Our current investigation followed a group of subjects who were free of radiographic knee OA (RKOA) to quantify the role of vitamin-supplement usage and incident RKOA. Additionally, among a group of subjects with RKOA, we examined the relationship between vitamin-supplement usage and RKOA progression.

METHODS

The two-fold objective of our study was to evaluate the association between vitamin-supplement usage and (1) the subsequent development of RKOA among those free of RKOA at study baseline; and (2) the subsequent progression of RKOA among those with RKOA at study baseline. Our hypotheses were: (1) Among those *free* of RKOA at baseline, the likelihood of developing RKOA from those who report vitamin-supplement usage is less than the likelihood of developing RKOA from those who report no vitamin-supplement usage; and (2) Among those *with* baseline RKOA, the likelihood of RKOA progression among those who report vitamin-supplement usage is less than the likelihood of RKOA progression among those who report no vitamin-supplement usage. The study exposure was vitamin-supplement usage, defined as the cumulative number of years of self-reported usage since study enrollment. The two study outcomes were RKOA incidence and RKOA progression.

In 1988, The Arthritis Research Institute of America (ARIA), located in Clearwater, Florida, initiated The Clearwater Osteoarthritis Study (COS). The COS is an on-going community-based, prospective cohort study designed to identify the

major risk factors for the development of OA, to differentiate risk factors for localized and generalized primary OA, as well as to identify risk factors for the progression of OA. This 25-year longitudinal study follows individuals who are 40 years of age and older, collecting demographic, historical, clinical, and radiological data. To date, more than 3700 enrollees have been recruited and examined. ARIA is located appropriately in Pinellas County in Florida, drawing upon a population with a large percentage of residents 65 years and older (22.5%).[23] The study sample of this older community was comprised of volunteer participants who were recruited by various methods. These included: invitational letters, television and radio announcements, newspaper articles publicizing the COS study, articles posted in community organizations' bulletins, as well as seminars held at community clubs and organizations. In efforts to include younger subjects who are more likely to be free of OA, concerted recruitment efforts were used to encourage participation by employees of the Pinellas County School System, the City of Clearwater, and Pinellas County, Inc.

At initial contact with participants, a description of study procedures was given, followed by a screening questionnaire, detailing inclusion and exclusion criteria. After eligibility was determined and the informed consent was obtained, participants were asked to complete the *COS History Questionnaire*. This instrument collects detailed information such as demographics, family history, childhood history, adult habits and behavior, adult diet and work history, leisure/sports activities, and injury history.

At the initial and all subsequent ARIA appointments, a physical exam was conducted, including X-rays. Study participants were re-evaluated biennially, updating both the history questionnaire and the clinical exam information. Using standardized exposure techniques, a licensed X-ray technician took four films, including anterior weight-bearing knee radiographs. The films were then interpreted by a board-certified radiologist. A case was defined when radiographic structural evidence of disease was found. Each knee radiograph was graded 0 to 4 for OA by the ordinal criteria of Kellgren & Lawrence: 0, absent; 1, questionable osteophytes and no joint-space narrowing; 2, definite osteophytes with possible joint-space narrowing; 3, definite joint-space narrowing with moderate multiple osteophytes and some sclerosis; 4, severe joint-space narrowing with the presence of cysts, osteophytes and sclerosis.[14] Subjects whose X-rays were interpreted as grades 0 or 1 were considered disease-free for knee OA; subjects whose knee radiographs were interpreted as grades 2, 3, or 4 were classified as cases.

Our study outcomes were defined as follows. An *incident* case was a subject, initially free of RKOA in both knees at baseline, who developed RKOA in either or both knees. Study subjects with either uni-lateral or bi-lateral baseline RKOA were classified for the outcome *progression* if either of their RKOA knees experienced an increase of one Kellgren & Lawrence grade, or more, during the follow-up period. The following study subjects were excluded from enrollment: individuals with self-reported rheumatoid arthritis or variants (lupus erythematosus, ankylosing spondylitis, etc.); gout; disabling neuralgic disease; those confined to a wheelchair; and lastly, those who were mentally incompetent. The study radiologist was blinded to information about the individual study participants. As part of quality control of the study, every tenth subject's assembled films were independently interpreted by a non-affiliated radiologist blinded to the results of the first reading. The inter-observer variability of X-ray interpretations was calculated using the kappa coefficient, measuring the amount of agreement that is above random chance.[15]

The *COS History Questionnaire* contains a module querying study participants' vitamin history usage. For the current study, the following question was asked: ''*Have you ever taken vitamins on a regular basis (three times a week or more)?*'' If the subjects answered yes, then they were questioned ''*Age started?*'' and ''*Age stopped?*'' Our study exposure was defined as the cumulative number of years of self-reported vitamin-supplement usage since study enrollment. The following factors were considered as potential confounders: baseline body mass index (BMI) (weight/height^2), gender, age, history of knee injury, exercise status, and total years of vitamin-supplement usage prior to study baseline. Baseline BMI, age, and prior years of vitamin-supplement usage were kept as continuous variables in the analyses. History of knee trauma was a self-reported yes/no variable. The questionnaire asked ''*Have you ever been told by a doctor that you have a cracked, fractured, broken or dislocated bone?*'' If the subject answered yes, they were asked to select from a list of body sites (e.g. knee) to indicate where they sustained an injury. Subjects who self-reported that they exercised for at least 20 minutes for three (or more) times per week were categorized as exercisers.

Data Analysis

Putative confounders were identified and included in the final adjusted analyses. As the study participants had been observed for unequal lengths of time and some study subjects were censored, proportional hazards (Cox's) regression was employed[3,5] to quantify the relationship between vitamin-supplement usage and RKOA, while simultaneously controlling for the influence of exogenous factors. The period of observation was the interval between the time of study entry and either: (a) the development of RKOA; (b) study withdrawal; or (c) censoring. Technically, our null hypothesis was that the survival curves for those who do, and do not, report vitamin-supplement usage are identical. The exponentiated beta coefficients were used to calculate the hazard ratio (risk ratio). Statistical Analysis Software (SAS), Version 9.13[21] was employed, specifically PROC PHREG, for the computer analysis of these data. It should be noted that the results reported for the unadjusted analyses also used Cox's regression, with vitamin usage as the only independent variable in the model. All risk ratios reported within were hazard ratios. Separate power calculations were conducted to determine the sufficiency of sample size for each of the two analyses (RKOA incidence and RKOA progression).[13] Each of the two analyses independently had over 80% power to detect a 20%, or greater, difference in outcome (incidence or progression) by vitamin-supplement usage, if indeed a difference existed (two-tailed; $\alpha = 0.05$).

RESULTS

Inter-reader reliability by a second radiologist reflected 93% agreement ($\kappa = 0.85$). Among the 2022 study participants free of RKOA at baseline, 754 (37%) developed RKOA during the observation period. Among the 217 study participants with RKOA at baseline, 96 (44%) experienced RKOA progression during the observation period. The mean period of observation among those with and without baseline RKOA was 7.4 (SD = 5.1) and 5.6 (SD = 4.1) years, respectively. Several factors associated with RKOA and vitamin-supplement usage were investigated to identify potential confounders. A factor was considered a confounder if it was associated with the outcome (RKOA incidence/RKOA progression) and was differentially distributed by vitamin-supplement usage status. Baseline BMI, gender, age, history of knee injury, and exercise status were considered as potential confounders.

Table 1 Study Sample Baseline Characteristics by Radiographic Knee Osteoarthritis (RKOA) Status.

	Incidence Analysis ($N = 2022$)		Progression Analysis ($N = 217$)	
	N	%*	*N*	%*
Female	1410	69.7	133	61.3
Knee trauma (Yes)	944	46.7	106	48.9
Exercise status (Yes)	794	44.5	74	39.8
	Mean	SD	Mean	SD
Body mass index	25.4	4.4	27.9	5.5
Age	60.5	10.3	65.9	9.6
Total years of vitamin-supplement usage prior to study baseline	15.7	16.3	12.7	16.0

Table 2 Incident Radiographic Knee Osteoarthritis and Vitamin-Supplement Usage.

Factors Associated with Incident RKOA	Risk Ratio	95% CI
Vitamin usage (unadjusted)	0.89†	0.88–0.90
Vitamin usage, BMI	0.89†	0.88–0.91
Vitamin usage, BMI, gender	0.89†	0.88–0.91
Vitamin usage, BMI, gender, age	0.89†	0.88–0.91
Vitamin usage, BMI, gender, age, knee trauma	0.89†	0.88–0.91
Vitamin usage, BMI, gender, age, knee trauma, exercise	0.90†	0.88–0.91
Vitamin usage, BMI, gender, age, knee trauma, exercise, and vitamin-supplement usage prior to study baseline	0.88†	0.86–0.89

† = *p*-value < 0.0001; CI = Confidence interval.

Table 3 Radiographic Knee Osteoarthritis Progression and Vitamin-Supplement Usage.

Factors Associated with RKOA Progression	Risk Ratio	95% CI
Vitamin usage (unadjusted)	0.88†	0.84–0.93
Vitamin usage, BMI	0.89†	0.84–0.94
Vitamin usage, BMI, gender	0.89†	0.84–0.94
Vitamin usage, BMI, gender, age	0.92††	0.87–0.97
Vitamin usage, BMI, gender, age, knee trauma	0.92††	0.87–0.97
Vitamin usage, BMI, gender, age, knee trauma, exercise	0.93††	0.88–0.99
Vitamin usage, BMI, gender, age, knee trauma, exercise, and vitamin-supplement usage prior to study baseline	0.93††	0.87–0.99

† = *p*-value < 0.0001; †† = *p*-value < 0.05; CI = Confidence interval.

Table 1 summarizes study sample characteristics for the RKOA incidence and progression analyses.

The unadjusted risk ratio, generated using Cox's regression with vitamin-supplement usage as the only independent variable, quantified the association between vitamin-supplement usage and incident RKOA. These data indicated that those individuals reporting vitamin-supplement usage are 11% less likely to develop RKOA than are those individuals who reported no vitamin-supplement usage (risk ratio = 0.89; 95% C.I. 0.88–0.90) (Table 2). Adjusted analyses simultaneously considered the possible effects of baseline BMI, gender, age, knee trauma history, exercise status, and total years of vitamin-supplement usage prior to study baseline. The final adjusted association between vitamin-supplement usage and incident RKOA indicated that those individuals reporting vitamin-supplement usage are 12% less likely to develop RKOA than are those individuals who reported no vitamin-supplement usage (risk ratio = 0.88; 95% C.I. 0.86–0.89) (Table 2).

Among those subjects *with* baseline RKOA, the unadjusted risk ratio quantified the association between vitamin-supplement usage and the subsequent progression of RKOA. These data indicated that those individuals reporting vitamin-supplement usage are over 12% less likely to experience RKOA progression than are those individuals who reported no vitamin-supplement usage (risk ratio = 0.88; 95% C.I. 0.84–0.93) (Table 3). Adjusted analyses

simultaneously considered the same set of potential confounders as noted for the incidence analyses. The final adjusted association between vitamin-supplement usage and RKOA progression indicated that those individuals reporting vitamin-supplement usage are 7% less likely to experience RKOA progression than are those individuals reporting no vitamin-supplement usage (risk ratio = 0.93; 95% C.I. 0.87–0.99) (Table 3).

DISCUSSION

The results of this epidemiological investigation indicate that those individuals with a history of self-reported vitamin-supplement usage are less likely to develop RKOA than are those individuals without a history of vitamin-supplement usage. In a similar manner, our findings suggest that those with RKOA who reported vitamin-supplement usage were less likely to experience worsening of their RKOA than those who reported no vitamin-supplement usage. While only a clinical trial can establish a causal relationship, the current prospective cohort study enabled investigators to address four important criteria for causality[10]:

(1) The strength of the association indicates that those who have a history of vitamin-supplement usage are 12% and 7% less likely to develop incident RKOA or experience progression of RKOA, respectively. After eliminating the influence of numerous potential confounding factors, statistical significance was maintained for both incident RKOA ($p < 0.0001$) and RKOA progression ($p < 0.02$).

(2) These findings are consistent with related epidemiological studies, noting a heightened risk for RKOA progression among those with relatively low levels of vitamin D. McAlindon and colleagues[17] noted that low intake and low serum levels of vitamin D each seemed to be associated with an increased risk for progression of osteoarthritis of the knee. In a similar manner, a study in 1999 indicated that low serum levels of vitamin D may be associated with incident changes of radiographic hip OA characterized by joint-space narrowing.[16] In a 2006 study, Neogi *et al.* reported data which supported the hypothesis of an association between low plasma levels of vitamin K and increased prevalence of hand and knee OA.[19] Animal-model studies have previously demonstrated a relationship between nutrition and OA. A 1981 experiment induced OA in guinea pigs which were treated before surgery with either 150 mg/day or 2.4 mg/day of vitamin C.[22] Guinea pigs treated with the higher dose of vitamin C showed consistently less severe joint damage than animals on the lower dose of vitamin C. Meacock and colleagues published related findings in 1990. They produced OA in guinea pigs using a medial meniscectomy and added ascorbic acid to the drinking water of one experimental group. The other group had feed that contained less ascorbic acid.[18] The authors suggested that the higher levels of ascorbic acid appeared to have some protective effect ($p = 0.008$) on the development of spontaneous lesions.

(3) The prospective cohort design was able to clearly establish the temporal relationship between vitamin-supplement usage and the subsequent development, or progression, of RKOA. The major strength of this study was the collection of serial radiographs for all participants beginning at study entry, allowing us to determine pre-existing RKOA disease status among the study subjects. Few epidemiological studies have been able to examine this relationship prospectively in such detail.

(4) This investigation examines a risk factor for RKOA that has biological plausibility. Reactive oxygen species (ROS) include oxygen ions, free radicals and peroxides, both

inorganic and organic. ROSs are natural by-products of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress, ROS levels can increase dramatically, resulting in significant damage to cell structures. Reactive oxygen species are implicated in cellular activities related to a variety of inflammatory responses. Evidence suggests that cells within joints produce ROS, and that oxidative damage is physiologically important. In laboratory studies, human chondrocytes have been found to be potent sources of ROS.[11,12] Humans have a multi-layered antioxidant defense system[8] including a number of small-molecule antioxidants that play an important part, particularly in the extra cellular space, where antioxidant enzymes are sparse. These include the micronutrients ascorbate (vitamin C), tocopherol (vitamin E), and carotene (a vitamin A precursor).[2] Osteoarthritis is characterized by the loss of cartilage and concurrent changes in subchondral bone, and there is evidence that subchondral bone has a major influence on the development of OA and its worsening.[25] This scientific evidence, in light of the aforementioned role of ROS, suggests a plausible association between vitamin-supplement usage and the incidence and progression of osteoarthritis. In their 1997 review, Ameye and Chee noted, ''*Because the mechanisms of cartilage degradation in OA are multifactorial and some nutritional compounds usually contain multiple active compounds that target multiple pathways, nutrition could provide an alternative to pharmacological interventions whose often monomodal mode of action may explain their partial lack of clinical efficacy in OA*.''[1]

Other factors may be involved. Those who choose to take a daily vitamin-supplement may have a higher interest in personal health issues than others. They may live generally healthier lives including exercise and better diets.

Differences by selected characteristics between those subjects who were lost to follow-up and those who were not lost were examined (Tables 4 and 5). Overall, it can be seen that the two groups, both the lost and the retained, had similar baseline characteristics. As a result, we would not expect such losses to affect the results seen.

Table 4 Evaluation of Losses to Follow-Up by Baseline Characteristics Vitamin-Supplement Usage and Incident Radiographic Knee Osteoarthritis.

	Lost ($n = 438$)	**Retained** ($n = 1584$)
	%	%
Vitamin-supplement usage (yes)	58	52
Female	68	70
Knee trauma (Yes)	46	47
Exercise status (Yes)	49	43
	Mean	Mean
Body mass index	25.6	25.4
Age	62	61
Vitamin-supplement usage (years) prior to study baseline	12.2	10.6

Table 5 Evaluation of Losses to Follow-Up by Baseline Characteristics Vitamin-Supplement Usage and Radiographic Knee Osteoarthritis Progression.

	Lost ($n = 66$) %	**Retained** ($n = 151$) %
Knee OA score = 2	59	73
Knee OA score = 3	41	28
Vitamin-supplement usage (yes)	68	54
Female	61	62
Knee trauma (Yes)	47	50
Exercise status (Yes)	40	40
	Mean	Mean
Body mass index	28.5	27.6
Age	69	65
Vitamin-supplement usage (years) prior to study baseline	18.7	10.0

The fact that many people continue to routinely take a simple multiple vitamin-supplement warrants investigations such as the current study. Long-term clinical trials would be required to definitively determine which vitamins in such supplements provide the causal mechanisms, if any. As we have noted, many vitamin trials have been disappointing and such a trial focused specifically on OA at any site may be several years away or may never be conducted. The difficulties are obvious. In the meantime, it likely behooves those taking such supplements to continue to do so. When taken in low doses, which are the composition of most supplements, harm seems unlikely and the gain, as well as a potentially reduced risk for radiographic knee osteoarthritis, may well be an important and low-cost benefit.

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