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Original article

Association of serum carotenoids, retinol, and tocopherols with radiographic knee osteoarthritis: possible risk factors in rural Japanese inhabitants

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Abstract

Background. The consumption of antioxidant nutrients may influence the development and progression of osteoarthritis (OA). To determine the association between serum antioxidants and radiographic knee osteoarthritis, we undertook a cross-sectional investigation in a community-based study in Japan.

Methods. A total of 562 subjects (224 male, 338 female) ≥40 years of age were enrolled in the Comprehensive Health Examination Program (CHEP, Yakumo Study) from 2003 to 2005. Subjects were categorized to the OA group ($n = 140$) if either knee was graded as Kellgren-Lawrence (K-L) grade ≥2. The no-OA group was defined as showing radiographic findings of K-L 0 or 1 in either knee ($n = 422$). The serum levels of retinol, β-/γ-tocopherols, α-tocopherol, zeaxanthin/lutein, canthaxanthin, cryptoxanthin, lycopene, α-carotene, and β-carotene were measured by high-performance liquid chromatography. The values of these antioxidants were divided into tertiles, and a logistic regression analysis was performed to analyze the association between them and radiographic knee OA, adjusting for potential confounders.

Results. Logistic regression analysis showed that compared to the lowest tertile of β-/γ-tocopherols the adjusted odds ratio (OR) was 0.52 [95% confidence interval (CI) 0.29–0.93] in the highest tertile; it also indicated a linear trend across tertiles. Furthermore, the adjusted OR was significantly decreased only in the middle tertile of α-tocopherol (OR 0.51, 95% CI 0.29–0.90). We reevaluated any independent association for these tocopherols after adjustment by entering them into the model simultaneously. The significance of β-/γ-tocopherols was maintained. In contrast, no associations were found with any carotenoids or retinol.

Conclusions. High serum values of β-/γ-tocopherols were found to be significantly associated with a low OR for radiographic knee osteoarthritis. The decreasing risk with a high serum value of β-/γ-tocopherols may support the possible protective effects against knee OA.

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Introduction

In general, osteoarthritis (OA) is a disease attributable to regressive changes in the articular cartilage and other joint structures, as well as supervening destruction of and proliferative changes in cartilage and bone. Clinically, the condition is characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of local inflammation. Radiographic evidence of OA increases with age, from 27% in subjects < age 70 years to 44% in subjects ≥80 years.[1]

Vitamins and carotenoids are present in large quantities in green and yellow vegetables as well as fruits. Provitamin A (e.g., α-carotene, β-carotene, β-cryptoxanthin) are precursors of retinol. They exert a potent antioxidative effect and are speculated to possess anticancer and antiaging properties. Furthermore, tocopherols (vitamin E family) are known to be a potent antioxidant action.[2] Many epidemiological studies have reported that antioxidants have biological properties of antioxidant actions, immune enhancement, anticarcinogenesis, and decreased risk of cardiovascular disease, among others.[3–5] With regard to OA, biological mechanisms including genetic factors[6,7] and oxidative stress[8,9] are believed to be important.

Evidence has accumulated suggesting that the consumption of antioxidant nutrients may influence the development and progression of OA. Guinea pigs in experimentally induced OA of the knee maintained on high levels of vitamin C developed less severe OA on average than those maintained on minimal levels.[10] A diet supplemented with vitamins/selenium might be important in the prevention or therapy of mechanically induced OA.[11] Wang suggested a beneficial effect of vitamin C and E intake on knee cartilage or bone.[12] In the Framingham Osteoarthritis Cohort Study, dietary vitamin C, vitamin E, and β-carotene reduced the risk of progression of knee OA.[13] However, a Framingham study did not show clear evidence for a correlation between the incidence of OA and eating habits with respect to antioxidative vitamins. In addition, many previous studies in which nutrient values were calculated from dietary data provided by study participants in a questionnaire format, with few studies directly measuring serum values.[14,15]

We hypothesized that high serum values of antioxidant nutrients might be associated with a decreased risk of knee OA. We directly compared the serum values of several antioxidant nutrients in a group of patients with knee OA and one of subjects without OA. The present study was to determine the association between serum levels of carotenoids, retinol, and tocopherols and radiographic knee OA in a cross-sectional investigation.

Materials and methods

Setting

The Comprehensive Health Examination Program (CHEP, Yakumo Study) has been conducted in the town of Yakumo in a rural area of southern Hokkaido, Japan, every August since 1982. The total population of Yakumo is about 17 500, with persons ≥40 years of age accounting for about 12 000 (69%). This program comprises voluntary orthopedic and physical functional examinations for the inhabitants as well as internal medical examinations and psychological tests. Every year an announcement outlining the aims of the health screening program is mailed to the inhabitants, and their willingness to participate is confirmed by return mail. During the internal medical examinations, serum concentrations of various antioxidant nutrients were measured from 2003 to 2005. Subjects receiving health screening examinations during this period were focused on in this study. The total number of such subjects was 2337, amounting to a 19.5% participation rate (2337/12 000).

The inclusion criteria were defined as: (1) age ≥40 years; (2) subjects with blood samples including serum antioxidant levels; (3) subjects who underwent physical functional examinations and radiographic examinations of the knee; and (4) subjects who were informed about this study and consented to participate in it. Exclusion criteria were as follows: severe disability in walking and standing or dysfunction of the central or peripheral nervous systems.

The data regarding serum antioxidant concentrations were obtained from all subjects. To avoid repeat coding of the same initial diagnosis for all subjects during the study period, we analyzed the data from the year of the initial health screening. A total of 712 subjects had undergone health screening for a single year only, and 650 had undergone such screening in multiple years, but only their data from the initial year was used (Fig. 1). Three subjects were excluded from analysis because of missing serum antioxidant values. The total number of subjects analyzed was 1359. Of these 1359 study subjects, radiographic examinations of the bilateral knees were performed for 562 subjects (41%).

Finally, 562 subjects (224 men, 338 women) were investigated in this study. Age distributions were as follows: 9 (2 male, 7 female) at 40–49 years; 31 (4 male, 27 female) at 50–59 years; 327 (124 male, 203 female) at 60–69 years; 160 (80 male, 80 female) at 70–79 years; and 35 (14 male, 21 female) at ≥80 years.

Subjects were assigned to the OA group ($n = 140$) if either knee was graded as a Kellgren and Lawrence (K-L) grade of 2–4. The no-OA group was defined as showing radiographic findings of K-L 0 or 1 in either knee ($n = 422$). All participants gave written informed consent. The study protocol was approved by our institutional review board. We approved publication of this study.

Radiographic assessment

Radiographic examinations were performed on posteroanterior (PA) weight-bearing radiographs made with the knee in 45° of flexion, according to Rosenberg et al.[16] The radiological assessment was based on the Kellgren and Lawrence classification[17] based on the presence/absence of narrowing of the joint space, osteophyte formation, and osteosclerosis, with each knee joint being evaluated from grade 0 to grade 4. A knee was judged to be grade 0 when no findings of OA were evident, grade 1 when slight or unclear osteophyte formation was present, grade 2 when clear bone proliferative changes were noted in the absence of narrowing of the joint space, grade 3 when moderate narrowing of the joint space was present, and grade 4 when more pronounced narrowing of the joint space associated with osteosclerosis of the subchondral bone was found.

A single senior orthopedic surgeon (Y.H.), who was blinded to patient information, performed all the radiographic assessments. To evaluate the accuracy of the measurements, interobserver reproducibility was investigated in two orthopedic surgeons (Y.H., M.T.) using 50 radiographs of the knee. In addition, intraobserver reproducibility of one of the authors (Y.H.) was deter-

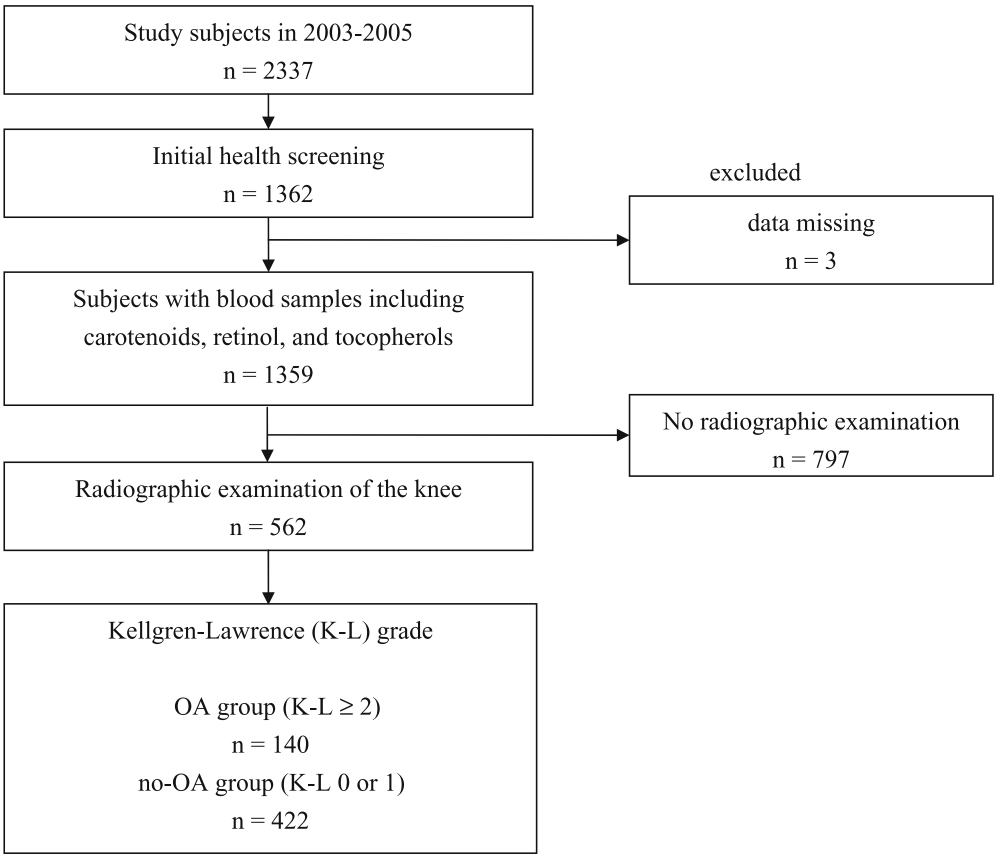

Fig. 1. Inclusion criteria for the Comprehensive Health Examination Program (CHEP) study

mined at monthly intervals. Interobserver and intraobserver reproducibility (kappa statistics) were 0.85 and 0.90, respectively.

Interview concerning selected characteristics

Physical characteristics were determined by experienced interviewers using a questionnaire and an interview at the time of the Comprehensive Health Examination Program. In addition to the basic characteristics of sex, age, and body mass index (BMI), history of knee injury sufficiently severe to impair ambulation, drinking history, smoking history, and physical exercise were determined. The BMI was calculated as weight (in kilograms) divided by the square of height (in meters). The responses on drinking or smoking history were obtained from the questionnaire divided into three levels: (1) current drinkers or smokers; (2) ex-drinkers or ex-smokers; (3) never drinkers or smokers. The responses on physical exercise were divided into four categories (no exercise, 1–2 h, 3–4 h, or ≥5 h/week).

Measurement of serum carotenoid, retinol, and tocopherol levels

All the samples were analyzed by trained staff blinded to case-control status. The serum values of retinol, carotenoids (including zeaxanthin/lutein, canthaxanthin, cryptoxanthin, lycopene, α-carotene, and β-carotene) and tocopherols (α- and β-/γ-tocopherols) were measured by high-performance liquid chromatography (HPLC).[18] Since the 1990s, for the HPLC analysis we have been using a method in which the peaks of lutein and zeaxanthin and β- and γ-tocopherol overlap at the same sites and cannot be separated.

Briefly, these components were extracted into 5.0 ml *n*-hexane from the mixture of 1.0 ml water, 1.0 ml ethanol, and 200 µl serum in a 15-ml brown spitz glass vial. A 3.0-ml volume of the *n*-hexane containing the extracted carotenoids in a brown glass vial was dried in a dark chamber. Dried carotenoids were dissolved in 100 µl ethanol. Aliquots (20 µl) of the dissolved material were injected into the HPLC (Waters, Milford, MA, USA). The operational conditions for the HPLC method were as follows: column, Nova Pak Cartridge C (4 pm, 8 × 10 cm; Waters); solvent, acetonitrile/dichloromethane/methanol 7:2:1 v/v (all HPLC grade, Katayama Chemical, Osaka, Japan); wavelength 436 nm (490E; Waters) for α-carotene, β-carotene, lycopene, cryptoxanthin, zeaxanthin, and canthaxanthin, 325 nm for retinol, 292 nm for tocopherol; flow rate 1.6 ml/mm (510; Waters). The ranges of repeatability and day-to-day variation (coefficients of variation) for the assays of carotenoids, retinol, and tocopherols were 4.6%–6.9%

and 6.3%–20.0%, respectively. Other biochemical analyses of the sampled sera including total serum cholesterol were performed using an autoanalyzer (JCA-RX20; Nihon Denshi, Tokyo, Japan) on the health examination day.

Statistical analysis

The SPSS statistical package (version 16.0) was used for the statistical analysis. Student's *t*-test was used to analyze age, BMI, and total serum cholesterol levels. We compared background characteristics between the OA and no-OA groups by the chi-squared test. Next, the analysis of covariance (ANCOVA) was used for comparisons of mean differences of serum carotenoid, retinol, and tocopherol levels between the OA and no-OA groups adjusted for age, sex, and BMI. Using a logistic regression analysis, with the presence/absence of knee OA as the objective variable and the serum values of the antioxidant nutrients divided into tertiles as the independent variables, crude odds ratios of the middle and highest tertiles were calculated for knee OA considering the lowest tertile of serum values as the referent category. In addition, adjusted odds ratios were calculated after adjusting for age, sex, BMI, total serum cholesterol level, history of knee injury, drinking habit, smoking history, and physical exercise.

As no externally determined criteria defining normal and abnormal serum antioxidant values are available, categorization was performed. According to categorization based on best fit in a multiple logistic regression model and with reference to previous studies, we adopted tertiles to clarify the relation between knee OA and antioxidants. The adopted independent variables are well recognized clinically as items showing true or potential OA–antioxidant association. Considering the problem of independent variable multicollinearity, we observed beforehand a correlation coefficient to confirm the presence/absence of any variables showing a marked linear relation. From the associations between antioxidants with smoking, drinking, and total serum cholesterol and those between knee OA with exercise duration and knee injury, we considered them to be necessary variables from a clinical standpoint and undertook a multiple logistic regression analysis.

Lipid concentrations in blood can confound the interpretation of antioxidant concentrations. The correlation between cholesterol and antioxidants is explained by the fact that antioxidants are carried by lipoproteins in blood.[19] Generally, status has been assessed by measuring antioxidant concentrations in blood with an adjustment for blood lipids. Because antioxidant nutrients in our study were fat-soluble, their values were corrected for each subject's total serum cholesterol value. We did not adjust for supplement use because we intended to assess overall bioavailability of the antioxidants using serum concentration as an indicator. To analyze a linear trend in across tertiles, we coded each tertile as 1, 2, or 3 and then treated it as a single variable using the logistic model. All P values were two-sided. A statistically significant difference was defined as $P < 0.05$.

Results

The overall prevalence of OA in this cohort was 24.9% (13.4% in men, 32.5% in women). Of the 140 with-OA subjects, 110 were women (78.6%). Various characteristics of the two groups are listed in Table 1. Compared with the no-OA group, the OA group had a higher mean age and higher BMI. The difference in a history of knee injury between the OA and no-OA groups was not statistically significant. The proportions of nonsmokers and nondrinkers were higher in the OA group than in the no-OA group. It was thought that the smoking and drinking habit prevalences were lower in women as compared than in men. Serum values of carotenoids, retinol, and tocopherols are expressed as mean values together with their standard deviation in Table 2. According to the serum level of β-/γ-tocopherols, the OA group was significantly different from the no-OA group. No significant differences were found between the OA and no-OA groups regarding the other antioxidants.

We analyzed the odds ratios of the risk of knee OA according to the serum values of each antioxidant nutrient (Table 3). Adjusted odds ratio of β-/γ-tocopherols in the highest tertile was significantly reduced to 0.52 (95% CI 0.29–0.93, $P = 0.028$) and indicated a linear trend in across tertiles (trend $P = 0.029$). As a result, the risk of knee OA as related to β-/γ-tocopherols was reduced by approximately 50% in the highest tertile. Furthermore, the adjusted odds ratio was significantly reduced to 0.51 (95% CI 0.29–0.90, $P = 0.021$) only in the middle tertile compared to the lowest tertile of serum values of α-tocopherol. The risk of knee OA in relation to α-tocopherol was U-shaped. The results of the present analysis indicated that serum values of tocopherol with different isoforms may exert a reciprocal influence. Considering that β-/ γ-tocopherols and α-tocopherol may confound each other, to clarify any independent association we reevaluated this issue after incorporating models at the same time (Table 4). In this analysis as well, the significance of β-/γ-tocopherols was maintained. α-Tocopherol (only the middle tertile), with $P = 0.055$, indicating marginal significance. No significant differences were found for adjusted odds ratios in the middle or highest tertile of any other carotenoids or retinol.

Table 1. Characteristics of patients with and without knee osteoarthritis

Characteristic	OA (n = 140)	no-OA (n = 422)	P
Age (years)	70.0 (7.2)	66.6 (6.8)	<0.001**
Female (no.)	110 (78.6%)	228 (54.0%)	<0.001**
Body mass index (kg/m^2)	25.3 (3.2)	23.8 (3.0)	<0.001**
Total serum cholesterol (mg/dl)	208.9 (33.1)	214.7 (33.3)	0.078
History of knee injury (no.)	8 (5.7%)	21 (5.0%)	0.774
Smoking (no.)			0.003**
Nonsmokers	109 (77.9%)	263 (62.3%)	
Ex-smokers	21 (15.0%)	100 (23.7%)	
Current smokers	10 (7.1%)	59 (14.0%)	
Alcohol drinking (no.)			0.045*
Nondrinkers	105 (75.0%)	275 (65.2%)	
Ex-drinkers	8 (5.7%)	20 (4.7%)	
Current drinkers	27 (19.3%)	127 (30.1%)	
Physical exercise/week (no.)			0.259
No exercise	85 (60.7%)	230 (54.6%)	
1–2 Hours	21 (15.0%)	80 (19.0%)	
3–4 hours	13 (9.3%)	59 (14.0%)	
≥5 Hours	21 (15.0%)	52 (12.4%)	

* $P < 0.05$, ** $P < 0.01$ significant difference
Values are shown as the mean (SD) for age, body mass index, and total serum cholesterol
In the Physical exercise category, data from one subject in the no-OA group were missing and so were excluded from the analysis

Table 2. Serum levels of carotenoids, retinol, and tocopherols in patients with and without knee osteoarthritis

Parameter (μmol/l)	OA (n = 140)	no-OA (n = 422)	P
Retinol	3.25 (0.93)	3.37 (1.15)	0.347
β-/γ-Tocopherols	2.21 (1.28)	2.43 (1.35)	0.009*
α-Tocopherol	27.75 (11.84)	26.29 (8.53)	0.749
Zeaxanthin/lutein	1.72 (1.07)	1.76 (1.15)	0.367
Canthaxanthin	0.019 (0.009)	0.021 (0.010)	0.061
Cryptoxanthin	0.35 (0.25)	0.33 (0.25)	0.800
Lycopene	0.54 (0.42)	0.51 (0.37)	0.758
α-Carotene	0.15 (0.16)	0.15 (0.15)	0.577
β-Carotene	1.03 (0.72)	0.99 (0.75)	0.656

Values are the mean (SD)
* $P < 0.01$ significant difference between means for the OA group and the no-OA group adjusted for age, sex, and body mass index by analysis of covariance

Discussion

The role of antioxidant nutrients such as vitamins and carotenoids in the development and progression of OA is a topic of considerable public and academic interest. Our results suggest that the high serum values of β-/γ-tocopherols decrease the odds ratio of the knee OA. The tocopherols are major components of vitamin E. Vitamin E with antioxidant activity is eight different molecules including α-, β-, γ-, and δ-tocopherols and the corresponding four tocotrienols.[2] Various biological activities, including the antioxidant activity of tocopherol, may play a role in the prevention of knee cartilage degeneration.[20] α-Tocopherol is one of the most potent antioxidant tocopherols. The β- and γ-tocopherols have less antioxidant activity than α-tocopherol. Recently, however, particularly γ-tocopherol has been demonstrated to exert important actions that differ from those of α-tocopherol.[21] It is estimated that γ-tocopherol has anti-inflammatory activity. γ-Tocopherol appears to be a more effective trap for lipophilic electrophiles, such as reactive nitrogen oxide species (RNOS), than is α-tocopherol.[22] Moreover, γ-tocopherol and its major water-soluble metabolite inhibit prostaglandin E_2 synthesis or cyclooxygenase-2 (COX-2) activity.[23] Chronic inflammation affects the progression of various degenerative disorders. γ-Tocopherol and its major water-soluble metabolite may inhibit the progression of OA by acting in a direction that suppresses inflammation.

A significant inverse relation between α-tocopherol levels and radiographic knee OA was observed only when comparing the second with the first tertile. We believe that the coincidental presence of extremely high values in the highest tertile in the OA cases may have

Table 3. Statistical evaluation of knee osteoarthritis and the serum carotenoid, retinol, and tocopherol levels

Factor	Category (μmol/l)	OA (n = 140)	no-OA (n = 422)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Retinol					
Lowest tertile		44	140	1.00	1.00
Middle tertile	2.81<	56	141	1.26 (0.80–2.00)	1.29 (0.76–2.21)
Highest tertile	3.68<	40	141	0.90 (0.55–1.47)	1.15 (0.65–2.03)
				Trend $P = 0.694$	Trend $P = 0.606$
β-/γ-Tocopherols					
Lowest tertile		54	138	1.00	1.00
Middle tertile	1.71<	52	143	0.93 (0.59–1.45)	0.77 (0.46–1.31)
Highest tertile	2.74<	34	141	0.62 (0.38–1.01)	0.52 (0.29–0.93)*
				Trend $P = 0.058$	Trend $P = 0.029$
α-Tocopherol					
Lowest tertile		54	141	1.00	1.00
Middle tertile	22.34<	35	142	0.64 (0.40–1.05)	0.51 (0.29–0.90)*
Highest tertile	28.57<	51	139	0.96 (0.61–1.50)	0.84 (0.51–1.56)
				Trend $P = 0.836$	Trend $P = 0.660$
Zeaxanthin/lutein					
Lowest tertile		40	140	1.00	1.00
Middle tertile	1.19<	55	141	1.37 (0.85–2.18)	1.52 (0.88–2.65)
Highest tertile	1.71<	45	141	1.12 (0.69–1.82)	1.09 (0.61–1.94)
				Trend $P = 0.672$	Trend $P = 0.821$
Canthaxanthin					
Lowest tertile		58	131	1.00	1.00
Middle tertile	0.016<	43	152	0.64 (0.40–1.01)	0.53 (0.31–1.01)
Highest tertile	0.024<	39	139	0.63 (0.40–1.02)	0.58 (0.33–1.01)
				Trend $P = 0.050$	Trend $P = 0.053$
Cryptoxanthin					
Lowest tertile		44	140	1.00	1.00
Middle tertile	0.21<	47	141	1.06 (0.66–1.70)	0.85 (0.48–1.50)
Highest tertile	0.36<	49	141	1.11 (0.69–1.77)	0.99 (0.55–1.78)
				Trend $P = 0.675$	Trend $P = 0.999$
Lycopene					
Lowest tertile		50	142	1.00	1.00
Middle tertile	0.31<	46	139	0.94 (0.59–1.49)	0.95 (0.56–1.63)
Highest tertile	0.58<	44	141	0.89 (0.56–1.41)	0.90 (0.52–1.57)
				Trend $P = 0.612$	Trend $P = 0.714$
α-Carotene					
Lowest tertile		45	139	1.00	1.00
Middle tertile	0.09<	49	142	1.07 (0.67–1.70)	1.13 (0.65–1.99)
Highest tertile	0.15<	46	141	1.01 (0.63–1.62)	1.02 (0.56–1.86)
				Trend $P = 0.976$	Trend $P = 0.958$
β-Carotene					
Lowest tertile		39	140	1.00	1.00
Middle tertile	0.56<	56	142	1.42 (0.88–2.27)	1.16 (0.66–2.06)
Highest tertile	1.14<	45	140	1.15 (0.71–1.88)	0.84 (0.45–1.56)
				Trend $P = 0.585$	Trend $P = 0.523$

Lowest tertile of each micronutrient is used as the reference for logistic regression analysis
Control subjects were not precisely divided into three equal groups because some controls had identical serum values
OR, odds ratio; CI, confidence interval
* $P < 0.05$ significant difference
[a] Adjusted for age, sex, body mass index, total serum cholesterol, history of knee injury, smoking, drinking, and physical exercise

attenuated a linear trend. Another possibility is that some antioxidant nutrients exhibit threshold for these serum concentrations decreasing the risk of knee OA. Too high serum concentrations of any antioxidant may not decrease the risk of OA. For instance, in the field of cancer studies, β-carotene has not only antioxidant activity but also prooxidant actions, especially at high concentrations and/or under high oxygen tension.[24]

A number of studies have been reported that the consumption of antioxidant nutrients such as tocopherols (vitamin E) prevent the development of knee OA.[11–13] On the other hand, some studies have been reported that consumption of antioxidant nutrients is not protective for the development and progression of OA.[25,26] The Framingham Study has suggested a reduction in the progression, but not the incidence, of

Table 4. Adjusted odds ratios with 95% confidence interval for risk of knee osteoarthritis in the multivariable models including serum α- and β/γ-tocopherol tertiles

Parameter	Adjusted OR[a] (95% CI)	P*
β-/γ-Tocopherols		
Lowest tertile	1.00	
Middle tertile	0.83 (0.49–1.40)	0.478
Highest tertile	0.53 (0.29–0.97)	0.040*
α-Tocopherol		
Lowest tertile	1.00	
Middle tertile	0.56 (0.31–1.01)	0.055
Highest tertile	1.07 (0.59–1.92)	0.832

Lowest tertile of each micronutrient is used as the reference for logistic regression analysis
[a]Adjusted for age, sex, body mass index, total serum cholesterol, history of knee injury, smoking, drinking, and physical exercise
* $P < 0.05$ significant difference

knee OA in subjects with a high dietary intake of vitamin E.[13] Brand et al. compared the intake of the placebo with vitamin E in knee OA. Increased pain over the course of the 6-month study was significantly less in the placebo group, but analysis of variance (ANOVA) indicated no significant difference between groups for change over time for the disease-specific outcome measure.[25] The data in the literature are miscellaneous, probably due to the fact that OA is a multifactorial disease.

One of the biological mechanisms implicated in the development of knee OA is damage to the articular cartilage induced by oxidative stress. The underlying mechanism has been attributed to reduced stabilization of connective tissue and loss of synovial fluid viscosity due to oxidation caused by depolymerization of hyaluronic acid.[27] Yudoh et al. had reported that the presence of oxidative stress induces telomere genomic instability, replicative senescence, and dysfunction of chondrocytes in OA cartilage, suggesting that oxidative stress, leading to chondrocyte senescence and cartilage aging, might be responsible for the development of OA.[9] In contrast, antioxidant nutrients act protectively against oxidative stress and thus play an important role in the cell.[28]

In the report of the Johnston County Osteoarthritis project[14] on various carotenoid serum values, the highest tertile of β-cryptoxanthin showed an odds ratio of 0.28 (95% CI 0.11–0.73), representing an approximately 70% reduction in the risk of knee OA. On the other hand, the odds ratios of trans-β-carotene and zeaxanthin were, respectively, 6.40 (95% CI 1.86–22.1) and 3.06 (95% CI 1.19–7.85) with an increased risk of knee OA. However, in the present study, no changes in the odds ratios of knee OA in these values were noted. We attribute these discordant results to racial differences in the target populations as well as differences in lifestyle, including dietary habits.[29] We could not evaluate racial differences because all of the subjects were Japanese with virtually the same lifestyle and culture. We perform our cohort study in the same region annually, but the serum antioxidant levels of this cohort were roughly similar to those found in other populations.[16]

Our study has several possible limitations. First, the prevalence of OA in women was 32.5%. Furthermore, women accounted for 110 of the 140 OA patients (78.6%), with the number of men with knee OA being small. OA of the knee is known to be generally more frequent in women. In epidemiological studies, the prevalence of knee OA in Japan has been reported to be 36.5% in women.[30] Thus, the fact that in the reported population women accounted for 79.9% of the cases with OA is not unreasonable, and it cannot be assumed that the proportion of men in our population was especially low. Second, the level of health consciousness in this group of long-term participants in a health-screening program is high, and bias may have been introduced by the fact that many of the subjects paid particular attention to nutritional concerns. Also, subjects with markedly impaired ambulation may be unable to undertake orthopedic evaluations or undergo various diagnostic imaging procedures. Third, OA develops and progresses over prolonged periods. In our study, which was cross-sectional in nature, we could not make any definitive conclusions regarding the role played by antioxidants in the development and progression of OA. We anticipate that future longitudinal studies will help to clarify this issue.

This is the first study to evaluate an association of serum antioxidant levels with knee OA in Japan. The strength of this study lies in the fact that the association with radiographic knee OA was determined by directly measuring and analyzing serum antioxidant levels in the subjects. The decreasing risk with a high serum value of β-/γ-tocopherols may support the hypothesis of possible protective effects against knee OA. To clarify whether high intake of tocopherol as a dietary supplement can reduce the risk of knee OA or, conversely, be deleterious, requires further investigation.

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