# Serum carotenoids and radiographic knee osteoarthritis: the Johnston County Osteoarthritis Project

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## Abstract

*Objective:* Antioxidant intake has been associated with less progression of radiographic knee osteoarthritis (OA), but studies of carotenoid biomarkers and OA have not been done. We examined associations between serum concentrations of nine naturally occurring carotenoids and radiographic knee OA.

*Design:* The study design was matched case–control. Sera were analysed by high-performance liquid chromatography for nine carotenoids: lutein, zeaxanthin, α- and β-cryptoxanthin, *trans*- and *cis*-lycopene, α-carotene, and *trans*- and *cis*-β-carotene. Conditional logistic regression was used to estimate the association between tertiles of each carotenoid and radiographic knee OA, independent of body mass index, education, serum cholesterol, and the other carotenoids.

*Setting:* Johnston County, North Carolina, United States of America.

*Subjects:* Two-hundred cases with radiographic knee OA (Kellgren–Lawrence grades ≥2) and 200 controls (Kellgren–Lawrence grade=0) were randomly selected from the Johnston County Osteoarthritis Project, and were matched on age, gender and race.

*Results:* Participants with serum levels of lutein or β-cryptoxanthin in the highest tertile were approximately 70% less likely to have knee OA than controls {odds ratio (OR) [95% confidence interval (CI)] = 0.28 [0.11, 0.73] and 0.36 [0.14, 0.95], respectively}. Those in the highest tertile of *trans*-β-carotene (OR = 6.40 [1.86, 22.1]) and zeaxanthin (OR = 3.06 [1.19, 7.85]) were more likely to have knee OA.

*Conclusions:* While certain carotenoids may protect against knee OA, others may increase the odds of knee OA. Further study of carotenoids and knee OA are warranted before clinical recommendations about these substances and knee OA can be made.

Osteoarthritis (OA) is the most common chronic medical condition in people aged 65 years and older, affecting approximately 85% of adults aged 75–79 years[1]. The disease is characterised by loss of cartilage in joints such as knees or hips, and is commonly associated with pain, restricted mobility and functional decline. OA is often regarded as a condition that occurs as part of the natural ageing process. But in fact, the changes in the joint that occur in OA are quite different from those of normal ageing, and there seem to be many factors contributing to variation in the occurrence and extent of the OA process[2].

Dietary factors have been shown to modulate the expression and severity of OA in animal models, in which high doses of vitamin C prevented progression of surgically induced OA in guinea pigs[3]. One postulated mechanism by which certain micronutrients mediate OA incidence and progression is through their antioxidant properties[4]. Reactive oxygen species are generated by

cells within joints and can cause oxidative damage to various macromolecules. For example, free radicals react with hyaluronic acid, which forms the central axis of proteoglycans in cartilage and maintains the viscosity of synovial fluid within joints. The reaction results in depolymerisation of the macromolecule, leading to destabilisation of connective tissue and loss of synovial fluid viscosity[5]. Similar oxidative reactions with lipids, proteins and DNA may contribute to OA development and progression.

The human body has an extensive line of antioxidant defences in which enzymes and micronutrients quench reactive oxygen species by deoxygenation, thereby rendering these free species harmless[6]. Antioxidant enzymes such as superoxide dismutase are the primary defences in the intracellular space, while micronutrients play a more important role in the intercellular space where antioxidant enzymes are sparse[7]. Micronutrients with known antioxidant properties include α-tocopherol and carotenoids, although the antioxidant activity of these compounds *in vivo* is unproven[8,9]. The relative antioxidant potentials of isomer configurations of these micronutrients, including β- and α-carotene, or the differences between *cis* and *trans* forms of micronutrients remain unclear[8,9].

The relationship between OA and dietary antioxidants in humans has not received extensive study to date. The Framingham Osteoarthritis Study examined the effect of antioxidant micronutrient intake on the incidence and progression of radiographic knee OA[10]. High interim dietary intakes of vitamin C or β-carotene were found to be associated with 60–70% decreased risk of cartilage loss over an 8- to 10-year follow-up period. A 30% reduction in OA progression was also seen for persons who consumed diets rich in vitamin E. Contrary to the findings for OA progression, OA incidence did not appear to be affected by reported dietary intakes of β-carotene, vitamin C or vitamin E. These data indicate that the OA process might be modified by external factors such as micronutrient intake.

An ongoing study of OA, the Johnston County Osteoarthritis Project, offers another opportunity to study the OA–micronutrient relationship. This project is a community-based longitudinal study of knee and hip OA in a rural North Carolina county. We examined associations between carotenoids and OA by performing a case–control study of serum levels of nine carotenoids and prevalent radiographic knee OA within this population. The serum micronutrient measure circumvents difficulties in measurement of dietary intake as well as inter-individual differences in absorption and metabolism, and may be more useful as a biological marker of internal dose[11]. We measured nine carotenoids in serum, with distinction between different isomer configurations and *cis* and *trans* forms.

An association between serum antioxidants and OA might suggest preventive measures for persons who have a suspected predisposition to OA, such as obese persons, those suffering joint injury, or persons with a family history of OA. In addition, intervention in the early stages of OA by dietary modification or micronutrient supplementation might offer a feasible means of preventing progression into severe disease stages. Dietary intervention may be particularly relevant for preventing OA in older persons, who are susceptible to inadequate nutrition[12,13].

## Subjects and methods

### Study population

The Johnston County Osteoarthritis Project is an ongoing community-based cohort study of knee and hip OA in African-Americans and Caucasians in a rural North Carolina county. The sampling methods and study protocol have been described in detail elsewhere[14]. Briefly, participants were recruited by probability sampling of streets of Johnston County, North Carolina, with deliberate oversampling of African-Americans. All civilian, non-institutionalised, African-American or Caucasian adults aged 45 years and older who were physically and mentally capable of completing the study protocol, including two home interviews and a clinic visit, were eligible. The estimated response rate at the time this nested sample was chosen was 66%, with no differences according to age, gender, race, or presence of knee pain between respondents and non-respondents[14]. All analyses in this paper used data obtained from the baseline evaluation of study participants.

### Ethics

All procedures in this study were approved by the Institutional Review Boards of the University of North Carolina Schools of Medicine and Public Health.

### Sample selection

Radiographic knee OA was defined from weight-bearing bilateral anteroposterior radiographs of the knee, according to the Kellgren and Lawrence (K–L) grading scheme: $0 = $ no OA; $1 = $ questionable OA; $2 = $ mild OA; $3 = $ moderate OA; and $4 = $ severe OA[15]. Cases were randomly selected from those participants having K–L grade 2, 3 or 4 in either knee, stratifying on race prior to random selection so that 100 African-American and 100 Caucasian subjects were selected. Controls were randomly selected from those participants with K–L grade 0 in both knees, and were matched to cases by age (±1 year), gender and race.

### Measures

#### Questionnaires

Each subject completed two interviewer-administered questionnaires in his/her home, the first upon recruitment

and the second during a subsequent home visit. Information of interest for this study included demographics, current alcohol consumption and current smoking status.

### Radiographs

Knee radiographs were read blindly by a single bone and joint radiologist (JBR) for K–L grade and for individual radiographic features, using standard atlases[15,16]. Inter- and intra-rater reliabilities for the radiologist were high, as reported previously[14].

### Clinical procedures

Height was measured in centimetres and weight in kilograms. Five cubic centimetres of non-fasting blood were collected by venipuncture. Following aspiration, the samples were immediately spun down and 1 cm³ aliquots of serum were drawn off into opaque cryotubes without preservatives, for total serum cholesterol determination by modified Trindar reaction[17] and subsequent frozen storage at $-86$°C.

### Nutrient analyses

Banked serum samples for the 400 participants were packed in dry ice and shipped to Craft Technologies, Inc. in Wilson, North Carolina for assessment of concentrations of lipid-soluble carotenoids in serum. The panel of carotenoids analysed included lutein, zeaxanthin, α- and β-cryptoxanthin, trans- and cis-lycopene, α-carotene, and trans- and cis-β-carotene.

The compounds were identified and quantified using high-performance liquid chromatography (HPLC). For serum extraction and HPLC methods, a modification of the procedures described by Nomura et al.[18] was used. Briefly, after thawing, 150 μl aliquots of serum were diluted with 150 μl of water and deproteinated by vortexing with 300 ml of ethanol containing tocol as an internal standard and butylated hydroxytoluene as an antioxidant. The samples were extracted twice with 1 ml of hexane; the combined supernatant was evaporated under nitrogen. The residue was then dissolved with vortexing in 35 μl of ethyl acetate, diluted with 100 μl of mobile phase, and agitated ultrasonically for 15 s prior to placement in the autosampler. A 15 μl volume was injected.

The HPLC system consisted of a computer data system, a solvent degasser, an autosampler maintaining samples at 20°C, a Spherisorb ODS2 column (3 μm, 4.6 mm × 150 mm with titanium frits), a guard column containing the same stationary phase, a column heater at 29°C, a programmable ultraviolet/visible detector to measure retinol at 325 nm and carotenoids at 450 nm, and a programmable fluorescence detector to measure tocol and tocopherols at 296 nm excitation/340 nm emission. The separation was performed isocratically using a mobile phase of 83% acetonitrile/13% dioxane/ 4% methanol containing 150 mM ammonium acetate and 0.1% triethylamine at a flow rate of 1.5 ml min⁻¹.

### Quality control

In-house quality control (QC) samples were analysed at the beginning, end, and at 24 sample intervals. Linear calibration curves were prepared consisting of multiple concentrations of analytes which spanned the physiological levels of the analysed carotenoids in serum. The calibrants included lutein, zeaxanthin, α- and β-cryptoxanthin, lycopene, α- and β-carotene. Serum quantification was performed by internal standard calibration using peak area ratios. The relative standard deviation of analytes in QC samples ranged from 3 to 10%.

Ten per cent of all samples ($n = 40$) were submitted to the laboratory as duplicate 'phantom samples', labelled with a new identification number. The repeatability of the laboratory protocol was evaluated for each carotenoid by examination of scatter plots of assay results for the 40 phantom samples plotted against their 40 duplicate samples, and calculation of Pearson correlation coefficients. Pearson correlation coefficients ranged between 0.83 and 0.99. Except for α-cryptoxanthin ($r = 0.83$), the correlation coefficients were all greater than 0.90, and only these were included in statistical analyses.

## Statistical analyses

Analyses were done on a microcomputer using SAS, version 6.12 (Cary, NC). Mean serum carotenoid levels were compared across case and control groups by calculating a $t$-statistic with its $P$-value, after log-transformation of the skewed data produced a more symmetrical distribution. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for each OA–carotenoid association were then estimated using unadjusted and multivariable conditional logistic regression models, respectively, comparing cases and controls across carotenoid tertiles, using the lowest tertile as a referent category. Potential confounders of *a priori* interest included education (<high school graduate/≥high school graduate), current smoking (yes/no), current alcohol consumption (yes/no), body mass index (BMI in kg m⁻²) and total serum cholesterol (mg dl⁻¹). These factors have been shown to be associated with levels of lipid-soluble nutrients in serum[19], and are either known or suspected to be independent factors associated with knee OA[20]. Each covariate was assessed for its importance as a confounder of each OA–carotenoid relationship by the percentage change in the odds ratios for the carotenoid effect when that covariate was removed from the model. Changes in risk estimates of greater than 5% between nested models were considered to indicate confounding of the carotenoid effect by that covariate.

Because a number of the carotenoids studied often occur together in foods, the serum levels of one are likely

**Table 1** Selected characteristics of subjects

| | Cases* (*n* = 200) | Controls (*n* = 200) | |
| --- | --- | --- | --- |
| Characteristic | Mean (SD) or % | Mean (SD) or % | OR (95% CI) |
| Education less than high school graduate | 52.0% | 39.0% | 1.80 (1.15–2.81) |
| Currently smokes | 14.5% | 20.5% | 0.72 (0.39–1.32) |
| Currently drinks alcohol | 17.0% | 21.0% | 0.70 (0.40–1.22) |
| Body mass index (BMI) (kg m$^{-2}$) | 32.00 (7.19) | 27.05 (4.99) | 1.17 (1.11–1.22) |
| Total serum cholesterol (mmol l$^{-1}$) | 5.72 (1.21) | 5.61 (1.14) | 1.00 (0.99–1.01) |

* 68.5% (137) of cases had Kellgren–Lawrence radiographic grade 2; 23% (46) had Kellgren–Lawrence grade 3; and 8.5% (17) had Kellgren–Lawrence grade 4.

to be associated with those of another. For this reason, confounding of each OA–carotenoid association by other carotenoids is possible. We therefore further examined each OA–carotenoid association in one multivariable model which controlled for the effects of the covariates and all other carotenoids simultaneously. All carotenoids were entered into the model as tertile categories, using the lower tertile as a referent group. Confounding by covariates was reassessed for this model, using the same criteria previously described. Potential collinearity of the micronutrient variables was assessed with regression diagnostics, similar to those used for linear regression[21]. Models were considered acceptable with no serious collinearity between predictors if the following criteria were met: variance inflation factor values did not exceed 10.0, and condition index values in a principal components analysis of the predictors were each less than 30.0. None of the models violated these criteria.

In order to assess a linear trend, we analysed each carotenoid as a continuous variable consisting of the original measured serum concentrations in a multivariable conditional logistic regression model including the covariates and all other carotenoid tertiles. Odds ratios and 95% confidence intervals were calculated to reflect the OA–carotenoid association corresponding to an increase in serum level as seen from the 10th to the 90th carotenoid percentile. *P*-values less than 0.20 were considered to indicate a linear trend.

## Results

The distribution of selected characteristics in the study population is shown in Table 1. Matching ensured equal distributions of age, gender and race between the cases and controls. In total, 200 African-Americans and 200 Caucasians were selected, with two-thirds female. Ages of subjects ranged from 45 to 92 years, with a mean [standard deviation (SD)] of 62.4 (10.3) years. Compared with controls, cases were less likely to have completed high school (*P* = 0.01) and had a higher mean BMI (*P* = 0.0001), but were otherwise similar.

Geometric means of serum carotenoid concentrations by case or control status are presented in Table 2, listed in decreasing order. Mean serum levels of *trans*- and *cis*-lycopene, lutein, α-carotene and β-cryptoxanthin were slightly lower among cases than controls.

The associations between serum carotenoid tertiles and radiographic knee OA are presented in Table 3. Unadjusted analyses of individual carotenoids revealed inverse associations between radiographic knee OA and *cis*-lycopene, lutein and β-cryptoxanthin, in which subjects in the highest tertiles of these carotenoids were approximately 35–45% less likely to have radiographic knee OA. After adjustment for body mass index, education and total serum cholesterol, the inverse associations remained but were diluted (data not shown). Current smoking and alcohol consumption did not confound the association

**Table 2** Geometric means of serum carotenoid concentrations (μmol l$^{-1}$) by radiographic knee osteoarthritis

| | Cases | Controls | |
| --- | --- | --- | --- |
| Carotenoid | GM* (95% CI) | GM (95% CI) | *P*-value† |
| *Trans*-β-carotene | 0.257 (0.227, 0.294) | 0.272 (0.242, 0.304) | 0.56 |
| *Trans*-lycopene | 0.216 (0.194, 0.238) | 0.233 (0.212, 0.257) | 0.26 |
| *Cis*-lycopene | 0.201 (0.183, 0.220) | 0.216 (0.197, 0.238) | 0.24 |
| Lutein | 0.176 (0.162, 0.190) | 0.195 (0.183, 0.209) | 0.04 |
| β-Cryptoxanthin | 0.096 (0.087, 0.105) | 0.112 (0.101, 0.121) | 0.02 |
| Zeaxanthin | 0.054 (0.051, 0.058) | 0.056 (0.053, 0.062) | 0.54 |
| α-Carotene | 0.045 (0.041, 0.048) | 0.050 (0.045, 0.056) | 0.13 |
| *Cis*-β-carotene | 0.045 (0.041, 0.048) | 0.041 (0.037, 0.045) | 0.96 |

* GM = geometric mean.
† Matched *t*-test based on log-transformed values.

**Table 3** Associations between serum carotenoids and radiographic knee osteoarthritis

| Carotenoids | Tertile lower cutpoints ($\mu$mol l$^{-1}$) | Unadjusted OR (95% CI) | Adjusted OR (95% CI)* | Adjusted OR (95% CI) comparing the 90th to the 10th percentile* | P-value for linear trend |
|---|---|---|---|---|---|
| *Trans*-β-carotene | | | | | |
| Lower tertile | | 1.00 | 1.00 | 1.78 (0.77–4.15) | 0.18 |
| Middle tertile | 0.181 | 0.84 (0.51–1.39) | 1.83 (0.81–4.14) | | |
| Upper tertile | 0.376 | 0.98 (0.57–1.69) | 6.40 (1.86–22.1) | | |
| *Trans*-lycopene | | | | | |
| Lower tertile | | 1.00 | 1.00 | 1.29 (0.36–4.62) | 0.69 |
| Middle tertile | 0.183 | 1.13 (0.68–1.89) | 1.84 (0.53–6.36) | | |
| Upper tertile | 0.320 | 0.68 (0.40–1.17) | 1.28 (0.30–5.51) | | |
| *Cis*-lycopene | | | | | |
| Lower tertile | | 1.00 | 1.00 | 0.57 (0.17–1.90) | 0.36 |
| Middle tertile | 0.170 | 0.74 (0.45–1.21) | 0.54 (0.16–1.84) | | |
| Upper tertile | 0.289 | 0.62 (0.36–1.07) | 0.61 (0.12–2.95) | | |
| Lutein | | | | | |
| Lower tertile | | 1.00 | 1.00 | 0.48 (0.24–0.97) | 0.04 |
| Middle tertile | 0.151 | 0.66 (0.42–1.05) | 0.60 (0.28–1.27) | | |
| Upper tertile | 0.229 | 0.55 (0.33–0.92) | 0.28 (0.11–0.73) | | |
| β-Cryptoxanthin | | | | | |
| Lower tertile | | 1.00 | 1.00 | 0.59 (0.30–1.13) | 0.11 |
| Middle tertile | 0.074 | 0.65 (0.40–1.06) | 0.60 (0.28–1.28) | | |
| Upper tertile | 0.128 | 0.55 (0.64–0.91) | 0.36 (0.14–0.95) | | |
| Zeaxanthin | | | | | |
| Lower tertile | | 1.00 | 1.00 | 1.22 (0.54–2.74) | 0.63 |
| Middle tertile | 0.047 | 0.98 (0.62–1.57) | 2.28 (1.06–4.94) | | |
| Upper tertile | 0.070 | 1.02 (0.63–1.63) | 3.06 (1.19–7.85) | | |
| α-Carotene | | | | | |
| Lower tertile | | 1.00 | 1.00 | 1.04 (0.70–1.53) | 0.86 |
| Middle tertile | 0.032 | 1.04 (0.64–1.71) | 1.06 (0.50–2.23) | | |
| Upper tertile | 0.058 | 0.87 (0.52–1.43) | 1.47 (0.63–3.46) | | |
| *Cis*-β-carotene | | | | | |
| Lower tertile | | 1.00 | 1.00 | 0.70 (0.25–1.92) | 0.48 |
| Middle tertile | 0.028 | 0.96 (0.59–1.55) | 1.06 (0.48–2.36) | | |
| Upper tertile | 0.052 | 0.95 (0.56–1.62) | 0.66 (0.22–1.97) | | |

\* Adjusted models included BMI, education, total serum cholesterol, all carotenoids.

with OA for any of the carotenoids and were removed from the models.

In the multivariable model simultaneously including covariates and all carotenoid tertiles, inverse associations between knee OA and lutein and β-cryptoxanthin were strengthened, such that those with serum levels in the upper tertiles of these carotenoids were approximately 70% less likely to have radiographic knee OA. In addition, those in the highest tertile of *trans*-β-carotene and zeaxanthin were more likely to have knee OA.

In the models with individual carotenoids as continuous variables, the inverse associations of lutein and β-cryptoxanthin with knee OA appeared to follow a linear trend, while a positive linear trend was indicated for *trans*-β-carotene, consistent with the results noted with these carotenoids defined as tertiles.

## Discussion

This is the first study to evaluate serum levels of carotenoids and knee OA. Except for β-carotene, none of the compounds examined in this study has been previously evaluated for involvement in the OA disease process. We hypothesised that serum concentrations of antioxidant carotenoids would be inversely associated with radiographic knee OA, because of the potential for these compounds to quench oxygen free radical species which may be involved in joint damage. Our data indicated that serum levels of lutein and β-cryptoxanthin were inversely associated with radiographic knee OA, while cases were more likely than controls to have higher serum levels of *trans*-β-carotene and zeaxanthin. No consistent association between knee OA and the other carotenoids was seen.

Lutein has been the subject of considerable research as an antioxidant, particularly in the context of age-related macular degeneration, in which a low density of macular pigment is thought to be a risk factor[22]. Lutein has been shown to increase the level of macular pigment and has also been shown to protect liver cells from oxidative damage and inhibit mammary tumour growth in mice[22–28]. The cryptoxanthins, including β-cryptoxanthin, have also been the focus of research in light of their potential antioxidant properties[26–28]. However, the effects of lutein or β-cryptoxanthin upon cartilage or other joint components have not been evaluated.

Although our findings of positive associations between knee OA and β-carotene and zeaxanthin differ from our

hypothesis, these associations are not biologically implausible, given the current lack of extensive knowledge concerning the environment within the joint. It is known that carotenoids can have both antioxidant and pro-oxidant properties, depending on the oxygen concentration of the subcellular environment[8]. Depending on the oxygen pressure and bioavailability of compounds within the joint, a carotenoid such as β-carotene could potentially promote rather than prevent lipid peroxidation. The fact that those receiving β-carotene supplementation in clinical trials had an increased incidence of lung cancer[29,30] illustrates the complexity of biological activity of this carotenoid *in vivo*.

Our findings in reference to β-carotene differ from those of the Framingham cohort, in which low intake of β-carotene was associated with knee OA progression, but not incidence[10]. Differences between the two studies are likely the result of differing study designs, exposure measures and adjustment for confounders. First, the β-carotene–knee OA association was examined longitudinally within the Framingham cohort, utilising interim intake of this antioxidant as assessed by food-frequency questionnaire. Our cross-sectional study, lacking detailed dietary data, examined OA prevalence and used serum β-carotene as an exposure measure. Although there are clear advantages to each nutritional exposure measure, the use of serum levels avoids dietary recall error and varying amounts of carotenoids in foods between regions, seasons and cooking practices[9]. The serum measure also accounts for differences in inter-individual absorption and metabolism and may be more reflective of a biologically active internal dose[11]. In addition, our study did not control for potential confounding by vitamin C, which was inversely associated with knee OA progression in the Framingham study[10].

There are several possible explanations why other hypothesised inverse associations with knee OA in our study may have been obscured if they do in fact exist. First, the cross-sectional nature of the data presents difficulty in determining whether observed associations are indicative of disease prevention or are rather a consequence of disease. Complex relationships exist between relevant variables, in that health factors and behaviours potentially leading to OA (nutrient intake, BMI) may also change as a result of the onset of pain and functional loss associated with OA. Data from the south-eastern United States show that persons who take medications for OA are more likely to take vitamin supplements than those who do not report taking such medications[31]. Such behavioural changes in dietary or supplemental nutrient intake, had they occurred in our study participants, could potentially explain the positive associations observed for *trans*-β-carotene, in which subjects with serum concentrations in the upper tertile were approximately six times as likely to have knee OA. Although we did not have detailed data on dietary or

supplemental nutrient intake in our study, our analyses done among subjects reporting knee pain did not support such an explanation. Despite the limited inference possible from the cross-sectional study design, it should be noted that similar use of cross-sectional measures of serum micronutrients has proved informative in studies of cardiovascular disease, in which prospective studies have later corroborated cross-sectional results[32,33].

Second, the measure of carotenoids in serum may be reflective of, but not necessarily specific to, carotenoid levels at the site of action. There is evidence that cells within joints, such as chondrocytes in cartilage, are active producers of reactive oxygen species, suggesting that combative antioxidant activity may be important within the joints themselves. One study indicated that α-tocopherol levels in the synovial fluid of patients with inflammatory joint disease were significantly lower than the level in matched serum of the same patient[34], suggesting that the consumption of α-tocopherol may be localised at the site of oxidative activity. Although we cannot determine whether our serum measures reflect the carotenoid concentrations in the joint, we can thus conjecture that larger differences in levels might have existed within knee joints than in serum.

Third, as above, potentially uncontrolled confounding in our study, in particular by vitamin C, could influence the associations we noted (or failed to observe) between carotenoids and knee OA. Fruit and vegetables containing carotenoids usually also contain high concentrations of vitamin C, resulting in a strong correlation between these compounds in serum. Vitamin C is a potent antioxidant *in vivo*, and may additionally protect against OA by other mechanisms such as stimulating increased collagen and proteoglycan synthesis[7,35]. Because we did not have data on serum vitamin C levels in our study, the effect of this micronutrient on knee OA, and on the relationships between carotenoids and knee OA, could not be assessed.

In addition, our cases were defined on the basis of having at least one knee with Kellgren–Lawrence radiographic grade 2. This defines OA by the presence of a definite osteophyte but does not require joint space narrowing. Unfortunately, we did not have a sufficient number of informative pairs with Kellgren–Lawrence grades 3 or 4 in this matched sample to allow separate analysis according to a stricter OA definition requiring definite joint space narrowing, or to test whether there was a significant difference in the relationships with carotenoids between grades 2 and grades 3 and 4. Similarly, analysis according to the individual radiographic features of osteophytes and joint space narrowing was precluded, because insufficient numbers in the more severe categories made meaningful comparisons impossible.

Despite its limitations, our study had several significant strengths, particularly in its ethnically diverse,

community-based sample and biological exposure measure. Our data provide some evidence that circulating levels of certain carotenoids are inversely associated with prevalent radiographic knee OA, implying a potentially protective effect for these compounds. However, the complex relationships between serum carotenoid levels and the OA disease process remain to be elucidated and should be examined prospectively. Future studies should also consider the most representative measure of antioxidant activity specific to the site of action. Given the significant impact of OA and the promising but small body of knowledge about its relationship to nutritional factors, the effect of antioxidants on the incidence and progression of OA is worthy of further study.

## Acknowledgements

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