Tocopherols are lipid-soluble antioxidants that may protect against some conditions of aging. The authors examined associations between radiographic knee osteoarthritis and serum levels of α-, δ-, and γ-tocopherol and the α:γ-tocopherol ratio in African-American and White adults from the Johnston County Osteoarthritis Project (North Carolina, 1991–1997). Two hundred cases with radiographic knee osteoarthritis (Kellgren-Lawrence grades ≥2) and 200 controls (Kellgren-Lawrence grade 0) were randomly selected and matched by age, ethnicity, and sex. Serum tocopherol levels were measured by high performance liquid chromatography. Conditional logistic regression was used to estimate associations between radiographic knee osteoarthritis and tertiles of each tocopherol measure, independent of confounders. Persons in the highest tertile of the α:γ-tocopherol ratio had half the odds of radiographic knee osteoarthritis as those in the lowest tertile (adjusted odds ratio = 0.5, 95% confidence interval: 0.2, 1.2). This inverse association occurred in all ethnic and sex subgroups, significantly in African Americans and men. Radiographic knee osteoarthritis was inversely associated with serum α-tocopherol in African Americans and men, positively associated with serum γ-tocopherol in men, and unassociated with serum δ-tocopherol. Associations between radiographic knee osteoarthritis and tocopherol isoforms are complex and may vary by ethnicity and sex.

Abbreviation: HPLC, high performance liquid chromatography.

Osteoarthritis is the most common form of arthritis, affecting approximately 21 million adults in the United States (1). Symptomatic osteoarthritis of the knee and hip is the leading cause of disability, work disability, and diminished quality of life among persons aged 65 years or older and is responsible for a large proportion of costs associated with joint replacement surgery and other direct and indirect health-care costs (2–8). Identifying potentially modifiable risk factors or protective factors for osteoarthritis would have a significant public health impact.

Dietary factors are receiving renewed attention in this regard in humans and in animal models of osteoarthritis (9–17). More than two decades ago, Schwartz and others (18–21) observed that high doses of vitamin C could limit the progression of surgically induced osteoarthritis in guinea pigs, and Black et al. (22) demonstrated that incubation of...
rabbit articular cartilage with α-tocopherol preserved cartilage load-carrying capacity and viability. More recently, Kurz et al. (17) reported that a diet containing vitamins E, C, A, B6, and B2 and selenium increased the expression of antioxidant enzymes and decreased the incidence of osteoarthritis in STR/1N mice.

Micronutrients might mediate the osteoarthritis process by blocking oxidative damage. Nitric oxide and reactive oxygen species inhibit collagen and proteoglycan synthesis, activate matrix metalloproteinases, increase the susceptibility of cartilage to injury by other oxidants, and induce apoptosis (23–26). Tocopherols are the most potent lipid-soluble antioxidants in blood, breaking free-radical chain reactions of lipid peroxidation (27). The antioxidant roles and serum levels of the different tocopherol isomers are highly interdependent and may be complementary in function (28, 29). There is some evidence suggesting that isomer concentrations relative to each other may be important in preventing specific types of oxidative damage (30–33), with γ-tocopherol possibly being more important than α-tocopherol in removing nitrogen oxides and other electrophilic mutagens (28–30).

Tocopherols may also mediate the osteoarthritis process by interfering with inflammation, which is increasingly recognized as important in osteoarthritis (23, 24). Here, too, recent evidence has suggested that γ-tocopherol and its metabolite may be more potent than α-tocopherol in inhibiting cyclooxygenase 2 in lipopolysaccharide-stimulated macrophages and in interleukin-1β-treated human epithelial cells (30). In this study, α- and γ-tocopherols demonstrated similar inhibition of inducible nitric oxide synthase and total reactive oxygen intermediates, and importantly, the anti-inflammatory effects were independent of the antioxidant effects (30).

Epidemiologic studies examining the role of antioxidants, specifically tocopherols, in human osteoarthritis are few (10, 14). In the Framingham Osteoarthritis Study, McAlindon et al. (10) reported that intakes of vitamin C, β-carotene, and possibly vitamin E were associated with a reduced risk of progression of radiographic knee osteoarthritis but were not protective against incident disease. Several methodologically limited clinical trials have suggested that vitamin E supplementation might be superior to placebo and equal in effectiveness to antiinflammatory medication in relieving osteoarthritis symptoms, but other studies have failed to show an effect (13, 15, 16, 34, 35).

To our knowledge, no studies have examined biomarkers of individual tocopherols in relation to radiographic knee osteoarthritis. Measurement of tocopherols in plasma or serum can provide estimates of dietary tocopherol intake, but a plasma or serum micronutrient measure may more accurately reflect a biologically active internal dose, avoiding the difficulties inherent in the measurement of dietary intake and differences in absorption and metabolism (36, 37). The ratio of serum α-tocopherol to γ-tocopherol, which represents the amount of circulating α-tocopherol and the degree to which its binding protein is saturated (31), has been proposed as a better reflector of vitamin E intake and tocopherol status in the body, partly because the ratio can be elevated by modest levels of supplementation that do not raise plasma α-tocopherol levels (38, 39) and because it obviates the need for lipid correction (31, 38).

No study of vitamin E and osteoarthritis has included African Americans, despite reports that African Americans have lower dietary intakes and/or biologic levels of tocopherols than do Whites (31, 40–44). The Framingham Study’s observation concerning vitamin E and progression of radiographic knee osteoarthritis was restricted to men; no further studies of possible sex differences in the effects of tocopherols on osteoarthritis have been conducted. We examined cross-sectional associations between radiographic knee osteoarthritis and serum biomarkers of α-, δ-, and γ-tocopherol and the α:γ-tocopherol ratio in the Johnston County Osteoarthritis Project, a community-based, biennial study of osteoarthritis carried out in North Carolina. We hypothesized that these tocopherols would be associated with lower odds of radiographic knee osteoarthritis. Our second goal was to explore potential ethnic and sex differences in the relations between radiographic knee osteoarthritis and these tocopherol measures.

MATERIALS AND METHODS

Study population

The Johnston County Osteoarthritis Project is an ongoing population-based cohort study of knee and hip osteoarthritis among African Americans and Whites in a rural North Carolina county. The baseline examination was carried out between 1991 and 1997; we used those data for this study. The sampling methods and study protocol have been described elsewhere (45). Briefly, participants were recruited through probability sampling of streets, with oversampling of African Americans. All civilian, noninstitutionalized, African American or White adults aged 45 years or older who were physically and mentally capable of completing two home interviews and a clinic visit were eligible. When this nested sample was chosen, the response rate was 66 percent; there were no differences between respondents and nonrespondents with regard to age, ethnicity, sex, or presence of knee pain (45). This study was approved by the institutional review boards of the University of North Carolina School of Medicine and the Centers for Disease Control and Prevention. Written informed consent was obtained from all participants by trained interviewers before the first home interview.

Sample selection

Radiographic knee osteoarthritis was defined from weight-bearing bilateral anteroposterior radiographs of the knee, according to the Kellgren-Lawrence grading scheme: 0 = no osteoarthritis; 1 = questionable osteoarthritis; 2 = mild osteoarthritis; 3 = moderate osteoarthritis; and 4 = severe osteoarthritis (46). One hundred cases from each ethnic group were randomly selected from those participants having Kellgren-Lawrence grade 2 or higher osteoarthritis in either knee. Two hundred controls were randomly selected from those participants with Kellgren-Lawrence grade 0 in

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both knees, matched to cases by age (± 1 year), sex, and ethnicity.

Measures

Questionnaires. Each participant completed two interviewer-administered home questionnaires. Relevant variables for this study included demographic factors, educational attainment, and current smoking and alcohol consumption status.

Radiographs. Knee radiographs were read for Kellgren-Lawrence grade by a single bone and joint radiologist (J. B. R.) without knowledge of participant clinical status, using the standard atlas (46). Interrater reliability (assessed by another trained radiologist) and intrarater reliability were high (intrarater reliability: weighted \( \kappa = 0.859 \); intrarater reliability: weighted \( \kappa = 0.886 \)), as described previously (45).

Clinical procedures. Height was measured in centimeters and weight in kilograms. Five milliliters of nonfasting blood was collected by venipuncture. The samples were immediately spun, and 1-mL aliquots of serum were withdrawn into opaque cryotubes without preservatives for serum total cholesterol determination by modified Trindar reaction (47) and frozen storage at –86°C.

Nutrient analyses. Assessment of serum concentrations of \( \alpha-, \delta-, \) and \( \gamma- \) tocopherol levels by high performance liquid chromatography (HPLC) was performed at Craft Technologies, Inc. in Wilson, North Carolina. For serum extraction and HPLC methods, a modification of the procedures described by Nomura et al. (48) was used. Briefly, after thawing, 150-µL aliquots of serum were diluted with 150 µL of water and deproteinized by vortexing with 300 µL 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 dissolved with vortexing in 35 µL of ethyl acetate, diluted with 100 µL of mobile phase, and ultrasonically agitated for 15 seconds prior to placement in the autosampler. A 15-µL volume was injected.

The HPLC system consisted of a computer data system, a solvent degasser, a Spherisorb ODS2 column (Keystone Scientific, Bellefonte, Pennsylvania) (3 µm, 4.6 × 150 mm with titanium frits), a guard column containing the same stationary phase, an autosampler maintaining samples at 20°C and the HPLC column at 29°C, and a programmable fluorescence detector for measurement of tocol and tocopherols at 296 nm excitation/340 nm emission. The separation was performed isocratically using a mobile phase of 83 percent acetonitrile:13 percent dioxane:4 percent methanol containing 150 mM ammonium acetate:0.1 percent triethylamine at a flow rate of 1.5 mL/minute.

Quality control. In-house quality control samples were analyzed at the beginning, at the end, and at 24-sample intervals. Linear calibration curves consisted of multiple concentrations of analytes spanning the physiologic levels of serum tocopherols. Serum quantitation was performed by internal standard calibration using peak area ratios. The relative standard deviation of analytes in quality control samples ranged from 3 percent to 10 percent.

The repeatability of the laboratory protocol for each tocopherol was evaluated by examining scatterplots of assay results for 40 “phantom” samples plotted against their 40 duplicates and calculating Pearson correlation coefficients. These coefficients ranged from 0.91 to 0.95.

Statistical analyses

Samples with nondetectable levels of any tocopherol were assigned a value of one half of the detection limit for that tocopherol. Geometric mean values for serum levels of \( \alpha-, \delta-, \) and \( \gamma- \) tocopherol and the \( \alpha-\gamma- \) tocopherol ratio were compared across ethnic and sex subgroups with a \( t \) statistic. \( P \) values of 0.05 or lower were considered indicative of significant differences between subgroups. Geometric means were then similarly compared across case and control groups, for the overall sample and for ethnic and sex subgroups.

Odds ratios and 95 percent confidence intervals for each osteoarthritis-tocopherol association were estimated by multivariable conditional logistic regression using tertiles of tocopherol values, with the lowest tertile being used as the referent. We tested for trend by including one term for the tocopherol in which the median value of controls in each tertile was assigned to participants in that tertile; \( p \) values of 0.10 or lower indicated a trend.

Potential confounders of a priori interest included education (high school graduation or not), current smoking (yes or no), current alcohol consumption (yes or no), body mass index (weight (kg)/height (m)\(^2\), coded as a continuous variable), and serum total cholesterol level (coded as a quadratic spline term (49)). Confounding of each osteoarthritis-tocopherol relation by each covariate was defined by a 5 percent or greater change in odds ratios for the tocopherol effect when that covariate was removed from the model.

We previously observed associations between serum carotenoid levels and radiographic knee osteoarthritis (14). Accordingly, we checked each osteoarthritis-tocopherol association for potential confounding by individual tertiles of lutein, zeaxanthin, \( \alpha- \) and \( \beta- \) cryptoxanthin, \( \beta- \) and \( \gamma- \) lycopene, \( \alpha- \) carotene, \( \alpha- \) and \( \beta- \) carotene, and the other tocopherols. Of these, the tocopherols, \( \alpha- \) carotene, \( \beta- \) carotene, \( \alpha- \) and \( \beta- \) carotene, and lutein changed at least one tocopherol estimate by more than 5 percent and defined our “set” of potential nutrient confounders.

Effect modification by ethnicity and sex was assessed in stratified analyses and in overall models that included interaction terms, with \( p \) values of 0.10 or lower indicating significant group-specific differences in osteoarthritis-tocopherol effects.

RESULTS

Table 1 shows selected characteristics of the sample, which was two thirds female, with ages ranging from 45 years to 92 years and a mean of 62.4 years (standard deviation, 10.3). Cases had a significantly higher mean body mass index than controls and were less likely to have completed high school.
Table 2 shows geometric means and corresponding 95 percent confidence intervals for serum tocopherol measures in the overall sample and in ethnic and sex subgroups. African Americans had statistically significantly lower geometric mean values than Whites for α-tocopherol (p = 0.0001), δ-tocopherol (p = 0.01), and the α:γ-tocopherol ratio (p = 0.001). Geometric mean values for tocopherols did not vary significantly by sex.

Table 3 demonstrates associations between geometric mean levels of serum tocopherols and radiographic knee osteoarthritis. Persons with radiographic knee osteoarthritis had lower geometric mean values for the α:γ-tocopherol ratio and higher geometric mean values for δ- and γ-tocopherol than did controls. Geometric mean α-tocopherol values were not significantly associated with radiographic knee osteoarthritis. These observations were seen in each ethnic and sex subgroup (data not shown).

Associations between tocopherol tertiles and radiographic knee osteoarthritis are shown in table 4. Persons in the highest tertile of the α:γ-tocopherol ratio were about half as likely as those in the lowest tertile to have radiographic knee osteoarthritis, with a significant monotonic trend across tertiles and an ethnic group interaction. This inverse association occurred in all subgroups but was statistically significant in African Americans and men, with trends being observed across tertiles (table 5).

Tertiles of α-tocopherol were inversely associated with radiographic knee osteoarthritis in men, with a statistically significant sex interaction and a monotonic trend across tertiles being seen (table 5). Those in the highest tertile of δ-tocopherol or γ-tocopherol were more than twice as likely to have radiographic knee osteoarthritis as those in the lowest tertile, but after adjustment, neither association was statistically significant. However, a statistically significant sex interaction between γ-tocopherol and radiographic knee osteoarthritis was noted, with a significant direct association and a monotonic trend across tertiles being observed in men only (p = 0.01).

**DISCUSSION**

Our results suggest a complex interplay among these tocopherol isoforms and potential ethnic and sex differences in the associations between these tocopherols and radiographic knee osteoarthritis. Contrary to our hypotheses, α-tocopherol was not protective against radiographic knee osteoarthritis overall, and δ- and γ-tocopherol were instead associated with increased odds of radiographic knee osteoarthritis. The α:γ-tocopherol ratio was the biomarker that was

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**Table 1. Selected characteristics of participants in a case-control study of serum tocopherol levels and knee osteoarthritis, North Carolina, 1991–1997**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall sample (n = 400)</th>
<th>Cases* (n = 200)</th>
<th>Controls (n = 200)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational level of less than high school graduation (%)</td>
<td>45.7</td>
<td>52.0</td>
<td>39.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>18.8</td>
<td>15.5</td>
<td>22.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Current drinker of alcoholic beverages (%)</td>
<td>20.3</td>
<td>18.1</td>
<td>22.6</td>
<td>0.28</td>
</tr>
<tr>
<td>Mean body mass index†</td>
<td>29.5 (6.7)‡</td>
<td>32.0 (7.2)</td>
<td>27.1 (5.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean serum total cholesterol level (mg/dl)</td>
<td>218.8 (45.3)</td>
<td>220.9 (46.6)</td>
<td>216.6 (44.0)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* 68.5% of cases (n = 137) had Kellgren-Lawrence grade 2 radiographic knee osteoarthritis; 23% (n = 46) had Kellgren-Lawrence grade 3 osteoarthritis; and 8.5% (n = 17) had Kellgren-Lawrence grade 4 osteoarthritis.
† Weight (kg)/height (m)².
‡ Numbers in parentheses, standard deviation.

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**Table 2. Geometric mean values for serum tocopherol measures in the overall sample and by ethnic group and sex, North Carolina, 1991–1997**

<table>
<thead>
<tr>
<th>Tocopherol measure*</th>
<th>Overall sample (n = 400)</th>
<th>Ethnic group</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 95% CI†</td>
<td>Mean 95% CI†</td>
<td>Mean 95% CI†</td>
</tr>
<tr>
<td>α:γ-tocopherol ratio</td>
<td>3.87 3.61, 4.16</td>
<td>3.45 3.17, 3.75</td>
<td>4.35 3.89, 4.87</td>
</tr>
<tr>
<td>α-tocopherol (mg/ml)</td>
<td>11.11 10.69, 11.53</td>
<td>10.04 9.58, 10.51</td>
<td>12.29 11.61, 13.01</td>
</tr>
<tr>
<td>γ-tocopherol (mg/ml)</td>
<td>2.87 2.71, 3.03</td>
<td>2.91 2.71, 3.12</td>
<td>2.82 2.60, 3.07</td>
</tr>
<tr>
<td>δ-tocopherol (mg/ml)</td>
<td>0.143 0.134, 0.152</td>
<td>0.132 0.120, 0.144</td>
<td>0.155 0.141, 0.169</td>
</tr>
</tbody>
</table>

* Values below the detection limit: none for α-tocopherol, 60 for δ-tocopherol, and two for γ-tocopherol.
† CI, confidence interval.
most consistently inversely associated with radiographic knee osteoarthritis. The relative importance of these two tocopherol isoforms in vivo is currently the subject of considerable debate (30, 32, 50–53).

The major sources of tocopherols in food are fats and oils (33, 38, 54, 55). Average servings of most foods contain 1 percent or less of the Recommended Dietary Allowance of 15 mg/day of α-tocopherol, which suggests that many people may have a diet that is suboptimal in levels of this nutrient (56, 57). Higher intake can be achieved through the use of vitamin E supplements, which contain mainly natural and synthetic forms of α-tocopherol (31, 33, 54, 55, 58).

Although γ-tocopherol is the principal tocopherol in the typical US diet, the preferential reincorporation of α-tocopherol into low density lipoproteins results in higher concentrations of α-tocopherol in plasma and most human tissues (27, 33, 58). Supplementation with α-tocopherol leads to saturated plasma levels of α-tocopherol and depression of plasma levels of γ-tocopherol (31, 33, 38, 54, 55, 58), suggesting that intestinal uptake and plasma transport make more efficient use of α-tocopherol than the other isoforms. In addition, the tocopherol transfer protein prefers α-tocopherol to the other vitamin E forms and uses all α-tocopherol for antioxidative activity before using the other isoforms (31, 57). Even though in vitro studies have shown that γ-tocopherol may be a more potent antioxidant and anti-

<table>
<thead>
<tr>
<th>Tocopherol measure</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>α:γ-tocopherol ratio*</td>
<td>3.41</td>
<td>3.12, 3.73</td>
</tr>
<tr>
<td>α-tocopherol (µg/ml)</td>
<td>10.89</td>
<td>10.37, 11.44</td>
</tr>
<tr>
<td>γ-tocopherol (µg/ml)*</td>
<td>3.19</td>
<td>2.99, 3.41</td>
</tr>
<tr>
<td>δ-tocopherol (µg/ml)*</td>
<td>0.160</td>
<td>0.147, 0.175</td>
</tr>
</tbody>
</table>

* p ≤ 0.004 in t test comparing the natural logarithms of serum levels between cases and controls.
† CI, confidence interval.

<table>
<thead>
<tr>
<th>Tocopherol measure</th>
<th>Lower cutpoint of tertile</th>
<th>Unadjusted OR†</th>
<th>95% CI†</th>
<th>Adjusted‡ OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>α:γ-tocopherol ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower tertile</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td>0.4, 1.7</td>
<td></td>
</tr>
<tr>
<td>Middle tertile</td>
<td>2.67</td>
<td>0.8</td>
<td>0.5, 1.3</td>
<td>0.8</td>
<td>0.4, 1.7</td>
</tr>
<tr>
<td>Upper tertile</td>
<td>4.14</td>
<td>0.4</td>
<td>0.2, 0.7</td>
<td>0.5</td>
<td>0.2, 1.2*</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower tertile</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td>0.7, 3.4</td>
<td></td>
</tr>
<tr>
<td>Middle tertile</td>
<td>9.47 µg/ml</td>
<td>1.2</td>
<td>0.7, 1.9</td>
<td>1.5</td>
<td>0.7, 3.4</td>
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<tr>
<td>Upper tertile</td>
<td>12.38 µg/ml</td>
<td>0.7</td>
<td>0.5, 1.2</td>
<td>1.0</td>
<td>0.4, 2.1</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower tertile</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Middle tertile</td>
<td>2.49 µg/ml</td>
<td>1.6</td>
<td>1.0, 2.6</td>
<td>0.7</td>
<td>0.4, 1.6</td>
</tr>
<tr>
<td>Upper tertile</td>
<td>3.77 µg/ml</td>
<td>2.2</td>
<td>1.3, 3.7</td>
<td>1.0</td>
<td>0.4, 2.5</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower tertile</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Middle tertile</td>
<td>0.116 µg/ml</td>
<td>1.8</td>
<td>1.1, 2.9</td>
<td>1.4</td>
<td>0.7, 2.8</td>
</tr>
<tr>
<td>Upper tertile</td>
<td>0.200 µg/ml</td>
<td>2.4</td>
<td>1.4, 4.0</td>
<td>1.9</td>
<td>0.8, 4.6</td>
</tr>
</tbody>
</table>

* p ≤ 0.10 in trend test.
† OR, odds ratio; CI, confidence interval.
‡ Adjusted for body mass index, education, serum total cholesterol level, and a set of nutrient confounders, including the tocopherols, α-carotene, β-carotene, cis-β-carotene, and lutein.
inflammatory agent than \( \alpha \)-tocopherol in some situations (28–30, 59), it may not be the more important isoform in vivo as long as adequate \( \alpha \)-tocopherol is available (50). Since we observed a direct rather than an inverse relation between \( \delta \) - and \( \gamma \)-tocopherol levels and radiographic knee osteoarthritis, one might conjecture that persons with high \( \gamma \)-tocopherol levels (and perhaps high \( \delta \)-tocopherol levels as well) and a high level of oxidative stress in our study may have exhausted their \( \alpha \)-tocopherol in an unsuccessful attempt to curtail the osteoarthritis process.

Observational studies have suggested that vitamin E supplementation may be protective against several chronic diseases whose etiologies involve oxidative stress, such as cancer, dementia, and heart disease (33, 60–62), but randomized clinical trials have often failed to replicate these results (63, 64). Recent clinical and in-vitro studies have suggested that \( \gamma \)-tocopherol may be more important than \( \alpha \)-tocopherol in protection from cardiovascular disease and other diseases (32, 53, 59), and this may be the reason why studies of \( \alpha \)-tocopherol supplementation (as opposed to dietary food sources of vitamin E richer in \( \gamma \)-tocopherol) may have had disparate results (65–69). Our results might appear to be contrary to the findings of these studies. However, not all clinical studies of \( \alpha \) - and \( \gamma \)-tocopherols and their ratio have found high \( \gamma \)-tocopherol levels to be beneficial (31, 33, 52, 70, 71). Ingles et al. (31) found that a high \( \alpha \)-\( \gamma \)-tocopherol ratio was associated with lower odds of large colonic adenomas, and other investigators have reported either no association or a direct association between \( \gamma \)-tocopherol and colon cancer (33), invasive cervical cancer (71), lung cancer (70), and coronary heart disease death or nonfatal myocardial infarction (52).

Do the few studies of vitamin E and osteoarthritis in the literature help us to explain our findings? In the Framingham Osteoarthritis Study, dietary vitamin E intake was associated with lower odds of radiographic knee osteoarthritis progression in men only (10). Some of our observations were also most notable in, or restricted to, men, including an inverse association between radiographic knee osteoarthritis and serum \( \alpha \)-tocopherol and a positive association between \( \gamma \)-tocopherol and this outcome. (The latter result should be interpreted cautiously because of the extremely wide confidence interval.) Although our study cannot be compared directly with the Framingham Study because of differences in design, study population, assessment of tocopherol status, and analysis, both studies suggest possible sex differences in the relations between vitamin E and radiographic knee osteoarthritis. Whether these sex differences are the result of hormonal differences associated with osteoarthritis metabolic processes or sex differences in tocopherol metabolism or exposure to other potentially important factors, such as use of lipid-modifying medications, deserves further study.
All clinical trials of vitamin E supplementation in osteoarthritis have utilized α-tocopherol, and results have been mixed at best (13, 15, 16, 34, 35). Those trials showing a salutary effect of vitamin E on osteoarthritis symptoms were typically small and/or short-term and had various methodological problems (13, 34). In the first placebo-controlled structure modification trial of vitamin E in knee osteoarthritis, Wluaka et al. (15) and Brand et al. (16) reported no effect of vitamin E supplementation on symptoms at 6 months or 2 years or on cartilage volume, measured by magnetic resonance imaging, at 2 years. Interestingly, women were slightly more likely to be randomized to vitamin E therapy in this trial (15). Whether men might be more likely to respond to vitamin E therapy in osteoarthritis is not known. If such a sex difference in the effectiveness of vitamin E in knee osteoarthritis exists, this might have contributed to the null result.

Furthermore, all trials of vitamin E in osteoarthritis have been done in Caucasian study populations. In our study, we observed ethnic differences in the associations between radiographic knee osteoarthritis and α-tocopherol and the α: γ-tocopherol ratio, with strong inverse associations being noted in African Americans. As was previously reported by others (31, 40–44), African Americans in our study had lower geometric mean levels of α-tocopherol and δ-tocopherol and α:γ-tocopherol ratio. In addition, African Americans may take supplements less frequently than Whites (31, 41, 72). Whether African Americans with osteoarthritis may respond more favorably than Whites to supplements or other interventions aimed at improving tocopherol status has not been examined.

There are several other possible explanations as to why our hypothesized inverse associations between serum tocopherols and radiographic knee osteoarthritis were not observed (14). First, measurement of tocopherol status was done contemporaneously with the assessment of osteoarthritis status rather than before osteoarthritis onset, making it impossible to determine temporality. Dietary intake may change as a result of pain and osteoarthritis-associated disability. For example, persons taking medications for osteoarthritis may be more likely to take vitamin supplements than those who do not take such medications (72). However, it is unlikely that those with increased levels of γ-tocopherol and osteoarthritis in our study were taking vitamin E supplements, because supplementation increases serum α-tocopherol level and decreases serum γ-tocopherol level (31, 38, 54, 55, 58).

Second, we used a single serum measure of tocopherol status. However, diurnal and day-to-day variations in α-tocopherol levels are generally low (73). Although data are somewhat conflicting (74), several studies have shown no significant seasonal variation in α-tocopherol levels (75–77). One study showed small differences in serum α- and γ-tocopherol levels measured 15 years apart, and rank-order correlations were consistent and high (75). For these reasons, we do not think that our choice of tocopherol assessment had a serious adverse impact on our ability to observe associations between tocopherols and radiographic knee osteoarthritis. Indeed, most other studies of serum biomarkers of tocopherol status have used single measures (31, 33, 52, 70, 71).

However, we do not know how well serum tocopherol measurements reflect tocopherol levels in the joint. In a study of inflammatory joint disease, α-tocopherol levels in knee synovial fluid were significantly lower than in the same patient’s serum, although mean serum α-tocopherol levels in patients did not differ significantly from those in controls (78). This suggests that α-tocopherol may be locally consumed at the site of oxidative and/or inflammatory activity. We did not have access to synovial fluid in our study, so we cannot determine whether and in what direction the tocopherol levels in the joint may have varied from those in the serum. It is intriguing to speculate that larger differences in synovial fluid tocopherol levels might have existed between cases and controls than were observed in serum. In future studies, investigators should also consider the most representative measure of antioxidant burden and total antioxidant capacity specific to the site of action.

It is not known whether tocopherol effects vary by radiographic severity. The fact that vitamin E intake protected against knee osteoarthritis progression but not incidence in the Framingham Study (10) suggests that this might be so. Our study had 85 percent power to detect an odds ratio of 0.4 using tertiles and 200 pairs. Consequently, we were not able to examine differences between grade 2 or mild radiographic knee osteoarthritis (137 cases) and grades 3 and 4 or moderate/severe radiographic knee osteoarthritis (63 cases). In addition, persons with grade 1 or “questionable” osteoarthritis (26 percent of participants in the Johnston County Osteoarthritis Project, from which this sample was selected) were excluded from the study. This precludes our evaluation of the possible transition to definite radiographic knee osteoarthritis. In future longitudinal studies, investigators should evaluate whether tocopherols act differently at different stages of the osteoarthritis process.

Finally, we also cannot exclude possible confounding by vitamin C, which was protective against progression of knee osteoarthritis in the Framingham cohort (10), or by selenium. Vitamin C, a potent antioxidant in vivo, may additionally protect against osteoarthritis by stimulating increased collagen and proteoglycan synthesis (19, 79, 80). Although food sources of vitamin C are quite different from those of tocopherols, intake of vitamin C has been positively associated with α-tocopherol levels in blood and inversely associated with γ-tocopherol levels (81, 82). Furthermore, synergism between antioxidant systems operating in the lipid and aqueous phase of the cell may be important, because after α-tocopherol acts to break the lipid-peroxidation free-radical chain reaction, the newly formed α-tocoferoxyl can react with vitamin C to regenerate α-tocopherol (27).

The results of this study are important. As the number of people affected by osteoarthritis increases and the impact of osteoarthritis-associated disability grows (3, 4, 6), identification of potentially modifiable risk factors and preventive factors will become increasingly relevant. Preventive measures might be aimed at persons at high risk of osteoarthritis incidence and progression, such as the obese and persons with prior joint injury or a positive family history.
(14). Dietary intervention may be particularly relevant among those with inadequate nutrition, such as older persons and African Americans (31, 40–44, 83, 84). The results of this study, data from animal models, and preliminary human epidemiologic studies (10, 14, 17–19, 21, 80) suggest that further study of the effects of tocopherols on the incidence and progression of osteoarthritis, particularly in ethnic and sex subgroups, is warranted.

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