Conclusions:
0.42; 95% CI: 0.22, 0.84).
but 3 other forms of natural tocopherols (α, γ, and δ) exist in nature.
Objective: We aimed to investigate plasma concentrations of the natural tocopherols and the tocopherol oxidation markers α-tocopherolquinone (αTQ) and 5-nitro-γ-tocopherol (5NGT) in relation to cognitive function in the elderly.
Design: Baseline plasma tocopherols and their oxidation markers were measured in 761 elderly Italian subjects from a population-based cohort assessed in 1999–2000 for mild cognitive impairment (MCI) and dementia. In 2003–2004, information about cognitive status was collected for 615 of the 666 subjects without baseline cognitive impairment. Tocopherols and oxidation markers were analyzed as plasma absolute values divided by serum total cholesterol because lipids affect their blood availability. Analyses were adjusted for sociodemographic, genetic, lifestyle, and medical confounders.
Results: Compared with the corresponding lowest tertile, the risk of prevalent dementia was higher for the highest tertile of δ-tocopherol÷cholesterol [odds ratio (OR): 3.87; 95% CI: 1.46, 10.27] and αTQ÷cholesterol (4.02; 1.45, 11.14), but the risk of incident dementia was not directly associated with plasma vitamin E metabolites. A U-shaped association, with lower risk for intermediate tertiles, was found for prevalent MCI with 5NGT÷cholesterol (0.39; 0.17, 0.91) and for incident dementia with γ-tocopherol÷cholesterol (hazard ratio: 0.42; 95% CI: 0.22, 0.84).
Conclusions: Plasma concentrations of some non-α-tocopherol forms of vitamin E are associated with cognitive impairment in elderly people. However, the associations depend on concurrent cholesterol concentration and need further investigation.

INTRODUCTION
The potential for intervention through dietary supplementation is spurring research about the possible beneficial effects of vitamin E for human cognition (1). Vitamin E is a generic term for a large number of lipid-soluble metabolites sharing the ability to prevent the propagation of free radical reactions as chain-breaking antioxidants (2). The most abundant form of vitamin E in the human body, the one with the highest biological activity and the one that is most extensively investigated, is α-tocopherol, but 3 other forms of natural tocopherols (β, γ, and δ) have also been characterized (2, 3).

Several lines of evidence support a role for oxidative stress in the pathogenesis of both Alzheimer disease (AD) (4) and atherosclerosis (5). Vascular damage, in turn, may directly cause vascular dementia (VD), and it frequently coexists and interacts with the neuropathological features of AD (6). However, data about the role of vitamin E in cognitive decline in older age are still inconclusive. Studies on cognitive function and dietary tocopherols provided inconsistent results (7–13). Also inconsistent are the results from studies of cognitive function and blood tocopherols (13–20). However, apart from a single exception (15), α-tocopherol was the only measured form of blood vitamin E, but dietary intake studies suggest that other tocopherols may be important for cognitive function (11).

In the present study, we investigated whether plasma concentrations of the 4 known forms of tocopherol were associated with baseline cognitive function and 4-y risk of dementia in a population-based cohort of elderly Italian subjects. In addition, as markers of the corresponding tocopherol’s antioxidant activity, we measured the plasma concentrations of α-tocopherolquinone (αTQ), the major oxidation product of α-tocopherol (2), and of 5-nitro-γ-tocopherol (5NGT), the major nitration product of γ-tocopherol (3). No previous data are available about the association of these tocopherol oxidation markers with cognitive function in the elderly.

SUBJECTS AND METHODS
Study population
The data are from the Conselice Study of Brain Ageing (CSBA), a population-based study of elderly Italian persons that

1 From the Department of Internal Medicine, Cardioangiology, and Hepatology, University Hospital Sant’Orsola-Malpighi, University of Bologna, Bologna, Italy (GR, PF, AL, NP, and ER); the Institute of Gerontology and Geriatrics, Perugia University Hospital, Perugia, Italy (FM, RC, and PM); and the Department of Medicine, McMaster University, Hamilton, ON, Canada (CP).
2 Supported by grants from the Italian Ministry of University and Scientific Research (basic-oriented research funds). The 5-nitro-γ-tocopherol used for the HPLC analyses was a gift from Kenneth Hensley (Oklahoma Medical Research Foundation, Oklahoma City, OK).
3 Reprints not available. Address correspondence to G Ravaglia, Department of Internal Medicine, Cardioangiology, and Hepatology, University Hospital S Orsola-Malpighi, Via Mazzarelli, 9–40138 Bologna, Italy. E-mail: ravaglia@med.unibo.it.
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aimed to investigate epidemiology and risk factors for cognitive impairment. The CSBA design and methods were described in detail elsewhere (21). Briefly, in 1999 and 2000, 1016 (75%) of the 1353 persons ≥65 y old residing in the Italian municipality of Conselice (Ravenna province, Emilia Romagna region) participated in the prevalence study. Follow-up assessment was conducted in 2003 and 2004. Conselice has been a wealthy, industrialized city since the 150s, but many of the older inhabitants were raised and lived most of their youth in a rural environment.

Written informed consent was obtained from all participants. The study was approved by the Institutional Review Board of the Department of Internal Medicine, Cardioangiology, and Hepatology of the University of Bologna.

Case findings

A 2-phase procedure was used at baseline, including a cognitive screening phase and an extensive clinical assessment of those positive at cognitive screening to confirm a diagnosis of mild cognitive impairment (MCI) or dementia. The cognitive screening phase included 1) a standardized personal interview for collection of data on sociodemographic characteristics, lifestyle, medical history (including review of medical records), functional status [basic (22) and instrumental (23) activities of daily living]; evaluation of depressive symptoms by using the Geriatric Depression Scale (24); and cognitive screening using the Italian version of the Mini-Mental State Examination [MMSE (25)], for which standardized age- and education-specific coefficients are available (26); 2) a standardized medical and neurologic examination; and 3) a routine biochemical blood and urine analysis along with genotyping for APOE e4 allele (27).

Subjects with MMSE scores <24 were considered positive at cognitive screening and underwent the Mental Deterioration Battery (28), a neuropsychological instrument validated for clinical and epidemiologic use in rural and poorly educated Italian subjects. The Mental Deterioration Battery includes tests for memory (immediate and delayed recall of Rey’s 15 words), language (sentence construction), frontal function (phonologic word fluency), abstract reasoning (Raven’s 47 progressive colored matrices), and visuospatial abilities (freehand copying of drawings and copying of drawings with landmarks). Memory was further tested by using the prose memory test (29). All included tests are provided with standardized thresholds for the definition of impairment in the corresponding cognitive domain (score ≤ 1.5 SD from the mean for a reference adult Italian population-based cohort), and age- and education-specific coefficients to be applied to the subject’s raw score before comparison with the corresponding threshold.

Subjects with MMSE scores <10 did not undergo further neuropsychological testing. When recent neuroradiologic data were not available, the subject was scheduled for a noncontrast computed tomography brain scan. Standardized information about the functional and mental status of subjects who were positive at cognitive screening was also obtained from a collateral informant (a relative or any other person with a reliable knowledge of the subject, including the name of his or her medical practitioner). Dementia was diagnosed with the DSM-IV clinical criteria (30), AD with the National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer’s Disease and Related Disorders Association criteria (31), and VD with the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l’Enseignement en Neurosciences criteria (32).

The definition of MCI evolved after the CSBA started and was not part of the original study design, but current international MCI consensus criteria (33) were retrospectively adapted to diagnose cases. An MCI diagnosis was given to all subjects scoring <24 on the MMSE who 1) had age- and education-adjusted scores ≤1.5 SD below the reference threshold on any of the tests used for neuropsychological evaluation; 2) were able to independently perform basic and instrumental activities of daily living (dependency due to physical impairment was not considered); and 3) did not meet clinical criteria for dementia.

In 2003 and 2004, identification and differential diagnosis of dementia incident cases among the 761 survivors who agreed to undergo another examination followed the same 2-phase procedure used to identify prevalent cases. In addition, standardized information about cognitive status at follow-up was sought from several sources (eg, the subjects themselves, relatives, general practitioners, and death certificates) for subjects who died before (n = 152) or refused to undergo (n = 103) the second examination.

Subjects affected by major sensory-motor deficits or any psychiatric condition other than dementia deemed to hamper a reliable cognitive assessment, and for whom we could not ascertain whether there had actually been a decline from a previous higher level of functioning, were diagnosed as cognitively unclassifiable and excluded from the present analysis (n = 19 at baseline and n = 4 at follow-up).

Laboratory tests

Venous blood samples were taken after an overnight fast and no more than a week after the medical interview. Samples were put on ice and centrifuged at 4 °C. Tocopherols and their derivatives were measured by using reverse-phase HPLC on plasma aliquots that were stored at −80 °C until they were analyzed. Aliquots of 200 μL were mixed and extracted 3 times with a 1:2 ratio of ethanol to exane, concentrated to dryness with high-purity nitrogen gas, and reconstituted in 300 μL mobile phase. Tocol; β-tocopherol (Matreya DBA, Milan, Italy); α-, γ-, and δ-tocopherol (Sigma Aldrich, Milan, Italy); αTQ (Research Org, Rome, Italy); and 5NGT were diluted in mobile phase before injection as internal standards. After filtration, analyte separation was conducted at room temperature on a Discovery C18 column (25 cm × 4.6 mm, 5-μm diameter; Sigma Aldrich) using an HPLC Coularray system (ESA, Chelmsford, MA). Mobile phase [30 mmol lithium acetate/L, 83% HPLC grade acetoni trile, 12% HPLC grade methanol, and 0.2% HPLC grade acetic acid (pH 6.5)] was delivered at 1 mL/min. Serum total cholesterol was measured on fresh serum by enzymatic assay (Roche Diagnostics, Monza, Italy) on a Hitachi 917 System Autoanalyzer (Boehringer Mannheim, Mannheim, Germany).

Covariates

Covariates were defined by using data collected at baseline. Educational status was categorized as 3 y versus ≥4 y of formal education (the first achievable educational degree at the time the CSBA participants went to school). Smoking status was categorized as never, former, and current smokers. Subjects were classified as having a sedentary lifestyle if they performed moderate
physical activity for <4 h/wk. Diagnoses of cardiovascular disease (ie, myocardial infarction, angina, peripheral vascular disease, and congestive heart failure) and stroke were based on medical history as provided by the patients and as confirmed by their general practitioner and information from previous medical records. Body mass index (BMI; in kg/m²) was calculated.

The participants’ dietary intakes were not measured, but the standardized baseline interview provided information about the average weekly servings of 9 food categories: meat, milk, cheese, fish, fruit, vegetables, legumes, cereals, and olive oil. Wine consumption was recorded as the number of drinks per day. Therefore, by following an approach used in previous studies that suggested a beneficial effect of a Mediterranean diet on dementia risk (34), a Mediterranean diet score was created by assigning a value of 0 or 1 to each of the available food categories. For categories presumed to be beneficial (ie, fruit, vegetables, legumes, cereals, fish, and olive oil), a score of 1 was assigned when consumption was at or above the median (fruit and vegetables: ≥7 servings/wk; legumes: ≥1 servings/wk; cereals: ≥14 servings/wk; fish: ≥1 servings/wk; olive oil: ≥7 servings/wk). For categories presumed to be detrimental (eg, meat, milk, and cheese), a value of 1 was assigned when consumption was below the median (meat: <3 servings/wk; milk: <7 servings/wk; cheese: <2 servings/wk). For wine intake, moderate consumption (>0 but <2.5 drinks/d) was considered a beneficial feature of the Mediterranean diet and assigned a score of 1, whereas lower or higher consumption was assigned a score of 0. A Mediterranean diet score was generated for each participant by adding the scores in the food categories (range: 0–10); a higher score suggested a greater adherence to the traditional Mediterranean dietary habits.

Statistical analysis

Variables are presented as means ± SDs (continuous) or as number and percentage (categorical). Preliminary analyses showed no association between cognitive impairment and the variables of interest considered as absolute value. However, there is previous evidence that blood availability of vitamin E metabolites is dependent on the concurrent concentrations of lipids (34). Therefore, plasma concentrations of tocopherols, αTQ, and 5NGT are reported here only per cholesterol unit (absolute concentration divided by serum total cholesterol). (For full data on the variables of interest considered as absolute value, see Tables S1–S4 under “Supplemental data” in the current online issue.)

Differences among participants stratified by baseline cognitive status and those among cognitively normal subjects stratified into 3 groups according to their MMSE score (ie, scores of 24–25, 26–28, and >28) were evaluated using by analysis of variance (Tukey’s test for pairwise comparisons were performed whenever the main effect was significant) or the chi-square test. Correlations between selected continuous variables were studied by using Pearson correlation coefficient. Logistic regression was used to estimate odds ratios (ORs) (and 95% CIs) for cognitive status at baseline across tertiles of plasma tocopherols and their derivatives. Because the dependent variable had 3 levels (cognitively normal, MCI, and dementia), a multinomial model was used to simultaneously estimate ORs for both MCI and dementia. Cognitively normal subjects in the lowest tertile were used as the reference category. The model was adjusted for age, sex, education, APOE genotype, smoking habit, sedentarness, BMI, Mediterranean diet score, and history of stroke and cardiovascular disease. Cox proportional hazards regression was used to estimate hazard ratios (HRs) (and 95% CIs) for incident dementia across plasma concentrations of tocopherols and their derivatives. The date of the onset of dementia was estimated as the midpoint of the interval from baseline to follow-up or death. Only subjects with a baseline diagnosis of normal cognitive function were included in this analysis. Subjects in the bottom tertile were used as the reference category. The model was adjusted for the same covariates used for logistic regression plus MMSE score.

The P value for linear trend was calculated for both logistic and Cox models to verify whether there was a linear dose-related association between risk of MCI or dementia and tertiles of vitamin E metabolites. The statistical significance of individual ORs and HRs across tertiles was used to detect nonlinear patterns of association.

Because of the small number of cases, separate analyses for the specific dementia types were not performed. Statistical analyses were performed with SYSTAT software (version 10; SPSS Inc, Chicago, IL). All tests were 2-tailed. P < 0.050 was considered significant.

Baseline data on drugs and conditions potentially affecting the endogenous synthesis of cholesterol (ie, liver disease, diabetes, thyroid disease, and the use of statins and steroids) also were available. However, because preliminary analyses showed that they did not affect the final results, they were not included in the present report.

RESULTS

Cross-sectional associations

Laboratory data for the current investigation were available for 761 participants in the baseline examination. The characteristics of the cohort, both as a whole and grouped according to baseline cognitive status, are shown in Table 1. Only 6.3% of the subjects reported use of vitamin supplements in the 6 mo before the study, and only 3.7% were users of supplements at baseline. Only 4% of the cohort had an α-tocopherol deficit according to a commonly used threshold [α-tocopherol÷cholesterol < 2.2 (35)]. Prevalent MCI and dementia cases (24 AD, 17 VD, 2 dementias from other causes) were older and had lower MMSE scores than than cognitively normal subjects. In addition, dementia cases were more likely to be women, nonsmokers, and sedentary and to have a history of stroke and lower values of BMI, serum cholesterol, and Mediterranean diet score. The Mediterranean diet score was not associated with plasma concentration of any measured tocopherol form (P > 0.40 for all). Dementia was associated with significantly higher plasma concentrations per cholesterol unit of α- and δ-tocopherol, αTQ, and 5NGT. No difference in plasma tocopherols and their derivatives was found between subjects with AD and those with VD (data not shown).

Among cognitively normal subjects, plasma concentrations of tocopherols, αTQ, and 5NGT did not differ across MMSE score groups (Table 2). Multivariate-adjusted analyses did not find any significant independent association of the Mediterranean diet score with baseline cognitive status or with MMSE among cognitively normal subjects (data not shown).

Multivariate-adjusted ORs for prevalent MCI and dementia across plasma tertiles of tocopherol forms are shown in Table 3.
With respect to those in the corresponding lowest tertile, dementia was more likely among participants in the highest tertile of δ-tocopherol + cholesterol (OR: 3.87; 95% CI: 1.46, 10.27; \( P = 0.02 \)) and αTQ + cholesterol (4.02; 1.45, 11.14; \( P = 0.007 \)). No other significant association was found across tertiles of the remaining variables.

No significant linear trend was found for risk of prevalent MCI across tertiles of tocopherols and their derivatives. However, analysis of the individual ORs showed a U-shaped association for 5NGT + cholesterol: participants in the middle tertile of 5NGT + cholesterol were less likely to have MCI than were those in the lowest tertile (OR: 0.39; 95% CI: 0.17, 0.91; \( P = 0.029 \)).

**Longitudinal associations**

Information on cognitive outcome at follow-up was collected for 615 of the 666 participants who were cognitively normal at baseline. Subjects lost at follow-up were older than those included in the study, but they did not differ in plasma concentrations of tocopherols and their derivatives (data not shown). The final at-risk cohort included 88 persons who did not undergo follow-up examination but for whom we were able to collect enough reliable information to establish or exclude the occurrence of dementia. These subjects were older than those actually reexamined, but they did not differ in sex, education, or plasma tocopherols (data not shown).

### TABLE 2

<table>
<thead>
<tr>
<th>MMSE score</th>
<th>(&lt;26 (n = 50))</th>
<th>(26–28 (n = 127))</th>
<th>(&gt;28 (n = 489))</th>
<th>(P^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol + cholesterol</td>
<td>6.1 ± 2.1(^2)</td>
<td>6.2 ± 1.7</td>
<td>5.9 ± 1.2</td>
<td>0.354</td>
</tr>
<tr>
<td>β-Tocopherol + cholesterol</td>
<td>0.38 ± 0.14</td>
<td>0.40 ± 0.12</td>
<td>0.38 ± 0.13</td>
<td>0.280</td>
</tr>
<tr>
<td>γ-Tocopherol + cholesterol</td>
<td>0.37 ± 0.13</td>
<td>0.39 ± 0.10</td>
<td>0.36 ± 0.11</td>
<td>0.170</td>
</tr>
<tr>
<td>δ-Tocopherol + cholesterol</td>
<td>0.05 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.294</td>
</tr>
<tr>
<td>α-Tocopherylquinone + cholesterol</td>
<td>9.0 ± 2.2</td>
<td>8.8 ± 2.2</td>
<td>8.5 ± 1.9</td>
<td>0.128</td>
</tr>
<tr>
<td>5-Nitro-γ-tocopherol + cholesterol</td>
<td>3.7 ± 1.1</td>
<td>3.7 ± 0.9</td>
<td>3.5 ± 0.9</td>
<td>0.428</td>
</tr>
</tbody>
</table>

\(^1\) \(P\) values are from a one-factor ANOVA model adjusted for age, sex, education, APOE4 genotype, smoking habit, physical activity, BMI, Mediterranean Diet score, and history of cardiovascular disease and stroke. All tests are 2-tailed.

\(^2\) \(\bar{x} ± SD\) (all such values).
During an average of 3.8 ± 0.9 y, 73 incident dementia cases occurred in the cohort at risk (45 AD, 26 VD, and 2 dementias from other causes). Eight cases were diagnosed among participants without a formal reassessment. With respect to participants who did not develop dementia, incident dementia cases were more likely to be older (77.1 ± 6.1 compared with 72.8 ± 5.8 y, respectively; *P* < 0.001), female (67.1% compared with 50.7%, respectively; *P* = 0.009), less educated (45.2% with education ≤3 y compared with 25.4%, respectively; *P* < 0.001), and sedentary (52.0% compared with 39%, respectively, *P* = 0.033) and to have lower MMSE (26.8 ± 1.7 compared with 28.4 ± 1.3, respectively) and Mediterranean diet (6.8 ± 1.3 compared with 6.2 ± 1.3, respectively; *P* = 0.002) scores. No difference between the groups was found for any other study covariate. The risk of incident dementia across tertiles of the measured tocopherol forms is shown in Table 4. No significant linear association was found, but the analysis of individual HRs showed a U-shaped association for γ-tocopherol + cholesterol, with a lower dementia risk for subjects in the middle tertile than for subjects in the lowest tertile (HR: 0.42; 95% CI: 0.22, 0.84; *P* = 0.014). In all multivariate-adjusted Cox models, Mediterranean diet scores remained consistently associated with incident dementia independent of all the other study variables, with an average 16% reduction in dementia risk for every 1-point increase (*P* < 0.05).

**DISCUSSION**

In this elderly Italian population–based cohort, the prevalence of dementia was highest among participants with the higher plasma concentrations per cholesterol unit of δ-tocopherol and αTQ. Prevalent MCI was less frequent among participants in the middle tertile of plasma 5NGT per cholesterol unit. In longitudinal analyses, a lower 4-y risk of dementia was found for the middle tertile of plasma γ-tocopherol per cholesterol unit with respect to the lowest. The associations were weak but independent of several possible sociodemographic, genetic, nutritional, and medical confounders.

Plasma concentrations of α- and γ-tocopherol in the CSBA are similar to those reported in European and other Italian cohorts of elderly (36). In contrast, the CSBA cohort has higher plasma α-tocopherol and lower plasma γ-tocopherol than does the elderly population of the United States (35). This difference is likely to reflect the high consumption of olive oil that is characteristic of Mediterranean diet habits, rather than the high consumption of soybean and corn oils that is characteristic of the US diet (2). No reference population-based plasma values are available for the other measured tocopherols, but they are usually much lower than those for α-tocopherol (2).

No association between cognitive function and plasma α-tocopherol was found in the CSBA cohort. In previous
case-control studies, plasma \( \alpha \)-tocopherol of patients with AD and 
VD were either decreased or unchanged compared with 
controls (19). In 2 studies of Italian elderly subjects, one of 
ambulatory patients (18) and one involving a population-
based cohort (20), plasma \( \alpha \)-tocopherol was lower in 
MCI and dementia cases than in controls. However, because of 
their cross-sectional design, these investigations could not 
exclude the possibility that the lower plasma \( \alpha \)-tocopherol of 
cognitively impaired participants simply mirrored changes in 
dietary habits or impairment in nutritional status. Indeed, 
prevalent dementia cases in the CSBA had lower serum cholesterol 
concentrations, BMIs, and Mediterranean diet scores than did 
cognitively normal participants.

A lower risk of developing AD was reported among cognitively 
normal subjects with higher \( \alpha \)-tocopherol food intake (7) 
or consuming \( \alpha \)-tocopherol supplements (9), but other dietary 
studies failed to confirm these associations (10–12). A nested 
case-control study within the French PAQUID (Personnes Agées 
QUID) cohort reported an inverse association between blood 
\( \alpha \)-tocopherol and risk of incident dementia (17). However, the 
association was based on a very small number of dementia cases. 
Moreover, plasma \( \alpha \)-tocopherol in the PAQUID study was lower 
than that found in the CSBA, which suggests different dietary 
habits of the subjects. Alternatively, the narrower distribution of 
plasma \( \alpha \)-tocopherol in the CSBA cohort may have hindered the 
detection of associations with cognitive function.

Previous cross-sectional studies of cognitively normal elderly 
persons found an association between low plasma \( \alpha \)-tocopherol 
and poor performance on tests of memory (13, 16) and general 
cognitive function (15). No similar association was found in the 
CSBA by using MMSE scores as a measure of global cognitive 
function. Of course, it cannot be excluded that the MMSE may 
not be sensitive enough to detect the association of interest. 
However, in agreement with our results, a population-based 
study of elderly Swiss subjects found no association of circulating 
\( \alpha \)-tocopherol with cognitive performance at baseline or at a 
22- \( \gamma \) follow-up (14).

There are no studies of plasma \( \alpha \)TQ and \( 5 \text{-NTG} \) in relation to 
cognitive impairment in the elderly. Under physiologic condi-
tions, \( \alpha \)-tocopherol is the main scavenger of both oxygen and 
nitroxide radicals (37). Therefore, the association found in the 
CSBA between dementia and high plasma concentrations of the 
\( \alpha \)-tocopherol oxidation product \( \alpha \)TQ agrees with the hypothesis 
that oxidative stress is a feature of this disease (4, 5). However, 
it is not yet clear whether oxidative stress is actually a cause or 
just an epiphenomenon of brain damage (4), and the lack of 
relation between plasma \( \alpha \)TQ and cognitive status at follow-up 
in the CSBA needs further investigation.

There is no previous report of plasma \( \delta \)-tocopherol in relation to 
cognitive impairment. Only one study investigated dementia 
risk in relation to dietary intake of all tocopherol forms, and no 
effect was found for \( \delta \)-tocopherol (11). In the CSBA, the cross-
sectional association between plasma \( \delta \)-tocopherol and dementia 
was not confirmed in longitudinal analyses, so no causal 
relation can be established. However, different tocopherols mixed 
in vitro in different proportions have different antioxidant effects 
(38). Therefore, an excess of plasma \( \delta \)-tocopherol as a result of 
alterations in dietary habits may damage the brain through low-
ering the global vitamin E antioxidant defenses.

The only investigation including measurement of plasma 
\( \gamma \)-tocopherol had a cross-sectional design and reported no 
association of this tocopherol form with concurrent cognitive 
performance (15). In contrast, the inverse association found in 
the CSBA cohort between risk of incident dementia and plasma 
\( \gamma \)-tocopherol agrees with results from a study suggesting that 
dietary \( \gamma \)-tocopherol may have a protective role against dementia 
(11). Although it is a less powerful chain-breaking antioxidant 
than is \( \alpha \)-tocopherol, \( \gamma \)-tocopherol is superior as a scavenger of

### Table 4

Multivariate-adjusted hazard ratios (HRs) for any dementia by tertile (T) of plasma tocopherols, \( \alpha \)-tocopherylquinone, and 5-nitro-\( \gamma \)-tocopherol per unit of cholesterol

<table>
<thead>
<tr>
<th>Tocopherol + cholesterol</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident dementia cases</td>
<td>25 (12.1)</td>
<td>18 (9.0)</td>
<td>30 (14.3)</td>
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</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>0.65 (0.34, 1.23)</td>
<td>0.81 (0.45, 1.47)</td>
<td>0.526</td>
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<table>
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<tbody>
<tr>
<td>Incident dementia cases</td>
<td>22 (10.9)</td>
<td>28 (12.6)</td>
<td>23 (12.0)</td>
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</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>1.16 (0.65, 2.09)</td>
<td>0.95 (0.50, 1.79)</td>
<td>0.873</td>
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<th>P</th>
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<tbody>
<tr>
<td>Incident dementia cases</td>
<td>26 (14.0)</td>
<td>19 (8.5)</td>
<td>28 (13.6)</td>
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</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>0.42 (0.22, 0.84)</td>
<td>0.79 (0.45, 1.40)</td>
<td>0.547</td>
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<table>
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<th>T3</th>
<th>P</th>
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<tbody>
<tr>
<td>Incident dementia cases</td>
<td>24 (11.8)</td>
<td>26 (11.4)</td>
<td>23 (12.5)</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>0.87 (0.49, 1.56)</td>
<td>0.76 (0.40, 1.47)</td>
<td>0.420</td>
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<th>T2</th>
<th>T3</th>
<th>P</th>
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<tbody>
<tr>
<td>Incident dementia cases</td>
<td>23 (10.9)</td>
<td>26 (12.3)</td>
<td>24 (12.5)</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>0.96 (0.51, 1.78)</td>
<td>1.06 (0.57, 1.96)</td>
<td>0.841</td>
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<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident dementia cases</td>
<td>21 (10.2)</td>
<td>23 (10.6)</td>
<td>29 (15.0)</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.00</td>
<td>1.08 (0.57, 2.04)</td>
<td>1.31 (0.70, 2.43)</td>
<td>0.393</td>
</tr>
</tbody>
</table>

\(^1\) \( n = 615 \) subjects. Incident dementia cases were 73. HRs and 95% CIs are from a Cox model adjusted for age, sex, education, \( APOE4 \) genotype, smoking status, physical activity, BMI, Mediterranean Diet score, history of cardiovascular disease and stroke, and baseline Mini-Mental State Examination score. Subjects in the lowest tertiles were used as the reference group. \( P \) values are for linear trend across tertiles.

\(^2\) \( n \) in brackets (all such).
reactive nitrogen species, which are thought to contribute significantly to neurodegenerative diseases via oxidative damage (39). In addition, γ-tocopherol has antiinflammatory properties that may be important in dementia prevention (3). It is not clear why, in the present study, the beneficial effect of γ-tocopherol was evident only in the middle tertile. A possible explanation is that higher γ-tocopherol status may be associated with unhealthy dietary behaviors, such as higher intakes of saturated and trans unsaturated fats (11).

The lower risk of prevalent MCI for the middle tertile of plasma 5GNT+cholesterol with respect to the corresponding lowest tertile is a somewhat puzzling finding and, perhaps, due only to chance. However, on the basis of our results for dementia risk, the specificity of 5GNT as a γ-tocopherol nitration product, and the latter’s activity as a scavenger of nitrogen species, we hypothesize that subjects with an optimal range of γ-tocopherol may be better able to scavenge nitrogen radicals than may those with a less-than-optimal range, and this effect may already be evident at the preclinical stages of dementia.

The strengths of the present study are the longitudinal design, the population-based setting, the concurrent measurement of all natural tocopherols along with markers of their antioxidant activity, and the large number of confounders taken into account. Identification of baseline MCI cases and their exclusion from analyses for incident dementia represent other important strengths. Of course, it cannot be excluded that the very early stage of subclinical dementia, undetected by the diagnostic work-up for MCI used in the present study, may cause changes in dietary habits. Indeed, among participants with baseline normal cognitive function, incident dementia cases were characterized by a looser adherence to a Mediterranean diet. However, all statistical analyses were performed by taking this variable into account.

The present study has also several limitations. First, vitamin E dietary intake was not measured in the CSBA, and only semiquantitative dietary data were available. Second, the variables of interest were measured only once and were assumed to be stable during the years of follow-up. Third, because of the small number of dementia cases, no specific analyses could be performed for AD and VD. Fourth, antioxidants work as a system and are affected by many other nutrients, so that measurements of a single species may not reflect its role in the antioxidant network. Fifth, our findings may not apply to a population with different dietary habits or sociocultural background. Finally, significant results were found only after adjustment for serum cholesterol concentrations, and, in addition to a lower dietary intake, unmeasured confounders related to a reduced endogenous synthesis may be relevant to explain the lower cholesterol of participants with dementia. In preliminary analyses, we tested for the effect of several drugs and diseases potentially affecting cholesterol metabolism, but APOE4 polymorphism was the only genetic confounder taken into account.

In conclusion, the present study shows that, in the elderly, both prevalent and incident cognitive impairments are associated with variations in blood concentrations of non-α-tocopherol forms of vitamin E. This finding support the hypothesis that dietary interventions aimed at modifying plasma vitamin E status may affect the risk of cognitive impairment in older persons. It also prompts further longitudinal investigations and clinical trials in an area where, up to now, research has been focusing almost solely on α-tocopherol.

The authors’ responsibilities were as follows—all authors: participated in developing the study design and the final version of the manuscript and gave final approval for manuscript submission; GR: contributed to the first draft of the manuscript and acquisition of funds; PF: contributed to statistical data analysis and to the first draft of the manuscript; AL, NP, ER, FMo, FMa, and RC: contributed to data collection; and PM and CP: assisted with data interpretation and contributed to the final version of the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES