Relationship Between Serum Alpha-Tocopherol and Overall and Cause-Specific Mortality

A 30-Year Prospective Cohort Analysis

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Rationale: **Although there has been a long-standing interest in the human health effects of vitamin E, a comprehensive analysis of the association between circulating vitamin E and long-term mortality has not been conducted.**

Objective: **Determine whether serum α-tocopherol (the predominant form of vitamin E) is related to long-term overall and cause-specific mortality and elucidate the dose-response relationships with better quantification of the associations.**

Methods and Results: **We conducted a biochemical analysis of 29 092 participants in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention) that originally tested vitamin E and β-carotene supplementation. Serum α-tocopherol was measured at baseline using high-performance liquid chromatography, and during a 30-year follow-up we identified 23 787 deaths, including deaths from cardiovascular disease (9867), cancer (7687), respiratory disease (2161), diabetes mellitus (119), injuries and accidents (1255), and other causes (2698). After adjusting for major risk factors, we found that men with higher serum α-tocopherol had significantly lower all-cause mortality (hazard ratios=0.83, 0.79, 0.75, and 0.78 for quintile 2 (Q2)–Q5 versus Q1, respectively; P_{trend}<0.0001), and significantly decreased mortality from cardiovascular disease, heart disease, stroke, cancer, respiratory disease, and other causes, with risk reductions from 17% to 47% for the highest versus lowest quintile. The α-tocopherol association with overall mortality was similar across subgroups of smoking intensity, years of smoking, alcohol consumption, trial supplementation, and duration of follow-up. The association was, however, significantly modified by baseline age and body mass index, with stronger inverse associations for younger men and men with a lower body mass index ($P_{interaction}$≤0.006).**

Conclusions: **In this long-term prospective cohort study, higher baseline serum α-tocopherol biochemical status was associated with lower risk of overall mortality and mortality from all major causes. Our data support the long-term health benefits of higher serum α-tocopherol for overall and chronic disease mortality and should be replicated in other more diverse populations. (*Circ Res*. 2019;125:29-40. DOI: 10.1161/CIRCRESAHA.119.314944.)**

Key Words: epidemiology ■ mortality ■ multivariate analysis ■ risk factors ■ vitamin E

Although there has been long-standing interest in the human health effects of vitamin E, a comprehensive analysis of the long-term association between vitamin E status and overall mortality has not been conducted, particularly with respect to cause-specific mortality and characterizations of the dose-response relationship. As an essential fat-soluble vitamin, vitamin E encompasses 8 structurally similar compounds, of which α-tocopherol is the predominant form in humans that is obtained from vegetable oils, some vegetables and fruits, whole grains, nuts (eg, almonds), and seeds (eg, sunflower) as well as supplements.[1–3] As a potent chain-breaking antioxidant, vitamin E can act as a peroxyl radical scavenger that prevents LDL (low-density lipoprotein) cholesterol and lipid peroxidation, which has been implicated in chronic disease risk, including cardiovascular disease (CVD) and cancer. In addition to its antioxidant properties, vitamin E has also demonstrated other important functions including enhancement of anti-inflammation activities, regulation of gene expression, improvement of immune response, inhibition of cell proliferation, and suppression of tumor angiogenesis.[4–9]

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Population-based studies have shown inconsistent associations between circulating α-tocopherol and risk of overall,[10–16] cancer,[10,12,13,16–18] and CVD mortality.[11–13,16–19] A recent meta-analysis of 6 studies suggested that higher α-tocopherol concentrations were related to lower total mortality (for participants in the highest versus lowest vitamin E category, relative risk=0.84, 95% CI, 0.77–0.91).[20] Another recent meta-analysis showed a small inverse association between α-tocopherol

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Novelty and Significance

What Is Known?

- Vitamin E is an essential fat-soluble vitamin encompassing 8 structurally similar compounds, of which α-tocopherol is the predominant form in humans.
- As a potent chain-breaking antioxidant, vitamin E can act as a peroxyl radical scavenger that prevents LDL (low-density lipoprotein) cholesterol and lipid peroxidation. It has anti-inflammatory activities and can regulate gene expression, improve immune response, inhibit cell proliferation, and suppress tumor angiogenesis.
- While some clinical trials report that higher serum α-tocopherol (vitamin E) and greater consumption of vitamin E-rich foods are associated with lower overall mortality and chronic disease risk, others report no beneficial effects, and possible adverse effects.

What New Information Does This Article Contribute?

- During 30 years of cohort follow-up, men in higher quintiles of baseline serum α-tocopherol had decreased all-cause and cardiovascular disease mortality, with risk reductions of 17% to 47% as compared with men in the lowest quintile.
- The associations between serum vitamin E and mortality appeared to be dose-dependent and were not materially attenuated after adjustment for other important risk factors or for consumption of vitamin E-rich foods. The associations were stronger among younger and leaner men, as well as those with no history of cardiovascular disease.

The biochemical status of vitamin E has been inversely associated with overall mortality in some studies, but the precise estimates of the magnitude of the relationship in cause-specific mortality are lacking. We found significant inverse, dose-dependent associations between baseline serum vitamin E (α-tocopherol) concentrations and overall, cardiovascular disease, heart disease, stroke, cancer, and respiratory disease mortality in a large, prospective cohort of over 29 000 men during a 30-year period. The findings were not confounded by other risk factors, including diet. The associations were stable throughout follow-up and were stronger among younger and leaner men, as well as those with no history of cardiovascular disease. These findings support a role for greater dietary vitamin E intake in promoting longevity, but should be reexamined in studies that include women, nonsmokers, and more diverse racial-ethnic groups.

Nonstandard Abbreviations and Acronyms

ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
BMI	body mass index
CVD	cardiovascular disease
HDL	high-density lipoprotein
HR	hazard ratio
ICD	*International Classification of Diseases*
LDL	low-density lipoprotein
VLDL	very-low-density lipoprotein

status and overall mortality, with a 6% risk reduction for each 5 mg/L increase in α-tocopherol concentration.[21] Significant between-study heterogeneity was noted for the risk estimates in these meta-analyses, however.[20,21] Moreover, data remain relatively sparse for elucidating the association between vitamin E status and cause-specific mortality risk, for testing a dose-response association, and for examining consistency of the association across population subgroups.

In the present investigation, we examine whether vitamin E biochemical status is prospectively associated with overall and cause-specific mortality. Based on vitamin E's antioxidant and other beneficial biological properties, we hypothesize that men with higher serum α-tocopherol concentrations would experience lower long-term mortality. The analysis is nested within the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention), a controlled trial that tested vitamin E and β-carotene supplementation for 5 to 8 years. We previously evaluated the same cohort based on 13 380 deaths during 19 years of follow-up and found evidence for an inverse serum α-tocopherol mortality association.[13] Here, we reexamine the vitamin E associations with overall and cause-specific mortality, including deaths from CVD, heart disease, stroke, cancer, respiratory disease, diabetes mellitus, injuries/accidents, and other causes based on nearly 24 000 deaths and over 30 years of cohort observation. Elucidation of the dose-response relationships and hypothesis-generating analyses of key subgroups (eg, based on age and body mass index [BMI]) were of particular interest.

Methods

The data and study materials that support the findings will not be available to other researchers for the purposes of reproducing the results because of the cohort data use agreement between the United States National Cancer Institute and Finland. However, the analytical methods are available from the corresponding author upon appropriate request.

Study Participants

Details of the ATBC Study have been published previously.[22] In brief, 29 133 male smokers of 5 or more cigarettes daily, aged 50 to 69 years, were recruited between 1985 and 1988 from 14 study centers in southwestern Finland. At the first of 2 baseline visits, self-administered questionnaires were used to collect information on demographic, behavioral, and lifestyle characteristics such as age, smoking habits, education, physical activity, medical history (including CVD and diabetes mellitus), and vitamin supplement use. Height, weight, and blood pressure were measured by trained study nurses, and an overnight fasting blood sample was obtained and stored at −70°C until assay. Participants were asked to complete a dietary history questionnaire at home, which collected information on portion size and frequency of consumption of 203 food items and 73 mixed dishes. At the second baseline visit, participants returned the dietary questionnaire (which was checked by the study nurses) and were then randomly assigned to one of the 4 trial supplementation groups: vitamin E (as *dl*-alpha-tocopheryl acetate, 50 mg/day), β-carotene (20 mg/day), both agents, or placebo. Intervention continued for 5 to 8 years (median of 6 years) through the end of the trial (April 30, 1993). Written informed consent was received from all participants, and the study has been approved by the Institutional Review Boards both at the US National Cancer Institute and the Finnish National Public Health Institute.

Laboratory Assays

Serum concentrations of α-tocopherol, retinol, and β-carotene were measured by high-performance liquid chromatography, and the

coefficient of variation of serum α-tocopherol was 2.2%.[22,23] Serum concentrations of total and HDL (high-density lipoprotein) cholesterol were assayed enzymatically (Boehringer Mannheim, Mannheim, Germany). In this study, 29 092 participants were included in the final analysis after excluding subjects with missing data for the serum α-tocopherol measurement (n=41).

Cohort Follow-Up and Outcome Ascertainment

Participants were followed from their randomization date at baseline until date of death or the end of follow-up (December 31, 2015), whichever occurred first. Mortality in the cohort was determined via linkage to the Causes of Death Registry, Statistics Finland (Online Figure I). The underlying causes of death were based on the following eighth, ninth, and tenth revisions of *International Classification of Diseases* (*ICD*-8, *ICD*-9, and *ICD*-10, respectively) codes: CVD (ICD-8, 390–458; ICD-9, 390–459; and ICD-10, I00-I99), heart disease (ICD-8 and ICD-9, 390–398, 401–404, 410–429, and 440–448; ICD-10, I00-I13, I20-I51, and I70-I78), stroke (ICD-8 and ICD-9, 430–438; ICD-10, I60-I69), cancer (ICD-8 and ICD-9, 140–239; ICD-10, C00-D48), respiratory disease (pneumonia, influenza, chronic obstructive pulmonary disease, and other related conditions; ICD-8, 470–474, 480–486, 490–493, and 518; ICD-9, 480–487 and 490–496; ICD-10, J10-J18 and J40-J47), diabetes mellitus (ICD-8 and ICD-9, 250; ICD-10, E10-E14), injuries and accidents (ICD-8, 800–978; ICD-9, E800-E978; ICD-10, U01-U02, U03, V01-X59, X60-X84, X85-Y09, Y35, Y85-Y86, Y87.0, Y87.1, and Y89.0), and other causes (cause of death was missing for 47 subjects, and they were included in the other causes category).

Statistical Analysis

The association between serum α-tocopherol (quintiles) and mortality risk, including cause-specific mortality, was analyzed using Cox proportional hazard regression models, with attained age as the time metric, to estimate hazard ratios (HRs) and 95% CIs. The proportional hazards assumption was not violated, tested by modeling the interaction of follow-up time with serum α-tocopherol. The test for linear trend was examined by entering the median concentration of α-tocopherol of each quintile, as a continuous variable, in a separate Cox proportional hazard regression model. The age-adjusted model adjusted for age at baseline (continuous) and serum total cholesterol (continuous). Multivariate models were additionally adjusted for cigarettes smoked daily (continuous), years of smoking (continuous), intervention assignment (α-tocopherol or no α-tocopherol, β-carotene or no β-carotene), systolic and diastolic blood pressure (continuous), and HDL cholesterol (continuous). History of CVD was further adjusted in the cause-specific mortality models of CVD, heart disease, and stroke end points. We also assessed the following covariates, which were not considered further as they did not materially alter the association between serum α-tocopherol and overall and cause-specific mortality: physical activity, BMI (kg/m^2), educational status, baseline vitamin E supplement use, history of diabetes mellitus, and energy, fat, red meat, and alcohol intake. To address the possibility that an inverse association with mortality may be explained by coexisting bioactive nutrients from a vitamin E-rich diet, we further adjusted for consumption of foods having high vitamin E contents, including vegetable oils, eggs, fish, rye, wheat, total fruit, and total vegetables (as continuous variables).

We generated Kaplan-Meier survival curves and used the log-rank test to evaluate survival differences for end points of interest across quintiles of serum α-tocopherol, in all the subjects and subjects who did not have a history of CVD or diabetes mellitus at baseline. Using cubic-restricted splines, possible nonlinear associations between serum α-tocopherol concentration (as a continuous variable) and overall and cause-specific mortality were assessed, and 4 knots were selected at the 5th, 25th, 75th, and 95th percentiles of the serum α-tocopherol concentration.

Stratified analyses were conducted based on age at baseline (<54, 54–<59, or ≥59 years), daily cigarettes (<16, 16–<20, or ≥20 cigarettes per day), years of smoking (<33, 33–<40, or ≥40 years), alcohol consumption (<5.3, 5.3–<20.4, or ≥20.4 g per day), BMI (<25, 25–<28, or ≥28 kg/m^2), intervention group (α-tocopherol or no α-tocopherol, β-carotene or no β-carotene), and years of follow-up (<13, 13–<23, or ≥23 years). The test for interaction was assessed through likelihood ratio tests entering the cross-product term for each stratified factor and quintiles of serum α-tocopherol. A P value of interaction <0.05 could be due to chance findings as we generated 8 interaction tests for each end point of interest. To minimize the influence of possible bias from reverse causation, we excluded the first 5 years of follow-up in a sensitivity lag analysis (excluded subjects n=2727). We also separately restricted analyses to subjects who did not have a history of CVD or diabetes mellitus at baseline (excluded subjects n=12 482).

Because vitamin E is transported in lipoproteins (primarily, though not exclusively, in the LDL fraction), we conducted a sensitivity analysis that used a non-HDL cholesterol variable (total minus HDL cholesterol), which essentially reflected LDL (and to a lesser degree, VLDLs [very-low-density lipoproteins]). Serum α-tocopherol concentrations were normalized for this serum LDL-proxy concentration, as well as for serum total cholesterol concentration separately, using the residual method,[24] categorized into quintiles, and analyzed through Cox proportional hazards regression models to estimate the HRs for overall and cause-specific mortality. In separate models, we adjusted for the serum LDL-proxy (ie, instead of using total and HDL cholesterol) in the main analytical model.

In addition to the analyses of serum α-tocopherol, we reexamined the ATBC trial α-tocopherol supplementation effect on mortality during this 30-year follow-up, overall and stratified by baseline serum α-tocopherol quintiles and by less than/greater than the median. Crude and multivariate-adjusted HRs and their 95% CIs were estimated from Cox proportional hazards regression models according to the 2×2 factorial design (ie, subjects who received α-tocopherol versus those who did not).

SAS software (version 9.4; SAS Institute Inc, Cary, NC) and the R statistical language version 3.2.3 (Vienna, Austria) were used for all the analyses.

Results

Mean serum concentration of α-tocopherol in the ATBC cohort at baseline was 11.9 mg/L, and the mean value of α-tocopherol in the fifth quintile was double that in the first quintile (Table 1). Compared with men in the lowest quintile, men in higher quintiles of serum α-tocopherol were slightly younger, were more physically active, had higher BMI, and were more likely to use vitamin supplements and have a history of CVD or diabetes mellitus. Serum α-tocopherol was inversely associated with serum HDL cholesterol and alcohol consumption, and positively associated with fruit and vegetable consumption, serum β-carotene (possibly reflecting fruit and vegetable consumption), and total cholesterol (possibly related to α-tocopherol transport in lipoproteins; Table 1).

In the cohort with 514 087 person-years of follow-up, 23 787 men had died (81.8%), of which 9867 deaths were from CVD (8063 heart disease deaths and 1763 stroke deaths), and 7687 deaths were due to cancer. In addition, there were 2161 deaths from respiratory disease, 119 deaths from diabetes mellitus, 1255 deaths from injuries/accidents, and 2698 deaths from other causes combined. The analysis using age-adjusted models showed that compared with those in the lowest quintile of serum α-tocopherol, men with higher quintiles had decreased mortality overall and from CVD, stroke, cancer, respiratory disease, injuries/accidents, and other causes, with 10% to 60% risk reductions (all P_{trend}≤0.04; Table 2). After multivariable adjustment for potential confounding factors, the inverse associations of serum α-tocopherol were observed for mortality from CVD, heart disease, stroke, cancer, respiratory disease, and other

Table 1. Baseline Characteristics by Quintile of Serum α-Tocopherol*

	Quintile of Serum α-Tocopherol				
	Quintile 1 (n=5825)	Quintile 2 (n=5860)	Quintile 3 (n=5794)	Quintile 4 (n=5791)	Quintile 5 (n=5822)
Serum α-tocopherol, mg/L	8.0 (1.2)	10.1 (0.4)	11.5 (0.4)	13.1 (0.6)	16.9 (4.0)
Age, y	57.8 (5.2)	57.3 (5.1)	57.0 (5.0)	57.1 (5.0)	56.9 (4.9)
Cigarettes/d	21 (9)	21 (9)	20 (9)	20 (9)	20 (9)
Years smoked, y	36.8 (8.5)	36.3 (8.3)	35.5 (8.5)	35.5 (8.6)	35.6 (8.3)
Systolic blood pressure, mm Hg	142 (20)	141 (19)	142 (19)	142 (19)	143 (20)
Diastolic blood pressure, mm Hg	87 (11)	87 (11)	88 (11)	88 (11)	89 (11)
Serum total cholesterol, mmol/L	5.2 (0.86)	5.8 (0.81)	6.2 (0.85)	6.6 (0.93)	7.3 (1.17)
Serum HDL cholesterol, mmol/L	1.28 (0.35)	1.23 (0.31)	1.21 (0.31)	1.18 (0.30)	1.09 (0.29)
Serum β-carotene, μg/L	150 (117)	196 (141)	220 (162)	241 (202)	253 (249)
BMI, kg/m^2	25.6 (4.0)	26.0 (3.8)	26.2 (3.7)	26.5 (3.7)	27.1 (3.6)
Education (%, >elementary school)	21.0	21.3	19.5	20.2	23.1
Physically active, %	16.5	19.6	22.6	23.5	21.8
History of CVD, %†	39.4	38.0	38.6	42.1	49.2
History of diabetes mellitus, %	4.2	3.4	3.6	3.7	6.4
Vitamin A supplement use, %	5.5	8.1	10.2	11.9	16.1
Vitamin E supplement use, %	5.0	7.4	9.8	11.7	16.9
Daily dietary intake					
Energy, kcal	2708 (779)	2716 (774)	2710 (745)	2674 (733)	2638 (734)
Fat (triacylglycerol), g	106 (38)	107 (37)	107 (36)	105 (35)	103 (35)
Alcohol (ethanol), g	21.3 (25.2)	18.1 (21.4)	17.1 (20.0)	16.4 (20.1)	17.1 (20.5)
Vitamin E, mg	10.2 (4.7)	11.3 (5.2)	12.1 (5.5)	12.8 (5.9)	13.8 (6.4)
Fruit, g	115 (96)	123 (100)	131 (99)	136 (102)	140 (110)
Vegetables, g	98 (63)	107 (68)	115 (70)	120 (73)	127 (74)
Red meat, g	69 (34)	71 (34)	72 (34)	72 (35)	72 (34)

BMI indicates body mass index; CVD, cardiovascular disease; and HDL, high-density lipoprotein.

*Values are means (SD) unless otherwise indicated.

†CVD includes a history of deep vein thrombosis, superficial venous thrombosis, lung infarction or embolus, hypertension, arterial obstruction, stroke, heart arrhythmia, enlarged heart, valvular heart disease, myocardial infarction, coronary heart disease, and heart failure.

causes combined, with risk reductions from 17% to 47% in the highest quintile as compared with the lowest quintile (all P_{trend}<0.0001; Table 2). Additional adjustment for BMI, alcohol consumption, and educational status did not materially alter the association estimates (Online Table I). The inverse serum α-tocopherol associations with mortality were only slightly attenuated by adjustment for vegetable oils, total fruit, total vegetables, eggs, fish, rye, and wheat in the multivariate models (Online Table II; Q2–5: 0.85, 0.81, 0.78, 0.82; *P* trend <0.0001) compared with the original data in Table 2 (Q2–5: 0.83, 0.79, 0.75, 0.78; *P* trend <0.0001). By contrast, we found that serum α-tocopherol was not related to risk of mortality from diabetes mellitus or injuries and accidents (multivariable model: P_{trend}=0.13 and 0.20, respectively).

Kaplan-Meier survival plots revealed that men in the lowest quintile of serum α-tocopherol experienced a significant excess in overall mortality than those in the higher quintiles (log-rank *P* value <0.0001; Figure 1A). Similar risk patterns were obtained for cause-specific mortality from CVD, heart disease, stroke, cancer, respiratory disease, and other causes combined (all log-rank *P* value <0.0001; Figure 1B through 1D; Online Figure II). In analyses restricted to subjects who did not have a history of CVD or diabetes mellitus, the Kaplan-Meier survival curves showed stronger reductions in mortality for men in the highest quintile (Figure 2; Online Figure III).

We used a restricted cubic spline regression to determine the dose-response association between serum α-tocopherol and mortality, analyzing serum α-tocopherol as a continuous variable. Overall and cause-specific mortality increased when serum α-tocopherol concentrations decreased below 9.3 mg/L, the reference value corresponding to the first quintile cut point (Figure 3). As the serum α-tocopherol concentrations increased, overall and cause-specific mortality was lower and relatively stable for serum α-tocopherol concentrations above 13 mg/L, with the possible exception of deaths from stroke which declined further (Figure 3; Online Figure IV).

Table 2. HRs and 95% CI for All-Cause and Cause-Specific Mortality by Quintile of Serum α-Tocopherol*

Causes of Mortality	Quintile of Serum α-Tocopherol					*P* Value†
	Quintile 1 <9.3 mg/L	Quintile 2 9.3- <10.8 mg/L	Quintile 3 10.8- <12.2 mg/L	Quintile 4 12.2- <14.2 mg/L	Quintile 5 ≥14.2 mg/L	
All-cause						
Deaths, n	5066	4824	4661	4582	4654	
Death rate‡	54.75	46.68	44.07	42.81	44.15	
Age-adjusted HR (95% CI)§	1.0	0.82 (0.79–0.85)	0.77 (0.74–0.80)	0.72 (0.69–0.75)	0.75 (0.71–0.78)	<0.0001
Multivariate HR (95% CI)‖	1.0	0.83 (0.80–0.87)	0.79 (0.76–0.83)	0.75 (0.72–0.78)	0.78 (0.74–0.82)	<0.0001
CVD						
Deaths, n	1845	1881	1950	1948	2243	
Death rate‡	19.94	18.20	18.44	18.20	21.28	
Age-adjusted HR (95% CI)§	1.0	0.86 (0.80–0.91)	0.85 (0.79–0.91)	0.79 (0.73–0.84)	0.90 (0.83–0.97)	0.038
Multivariate HR (95% CI)‖¶	1.0	0.86 (0.80–0.91)	0.83 (0.77–0.88)	0.74 (0.69–0.80)	0.78 (0.72–0.85)	<0.0001
Heart disease						
Deaths, n	1431	1509	1569	1617	1937	
Death rate‡	15.47	14.60	14.83	15.11	18.37	
Age-adjusted HR (95% CI)§	1.0	0.88 (0.82–0.95)	0.87 (0.81–0.94)	0.83 (0.77–0.90)	0.98 (0.90–1.06)	0.82
Multivariate HR (95% CI)‖¶	1.0	0.88 (0.81–0.94)	0.84 (0.78–0.91)	0.77 (0.72–0.84)	0.83 (0.76–0.90)	<0.0001
Stroke						
Deaths, n	407	367	369	319	301	
Death rate‡	4.40	3.55	3.49	2.98	2.86	
Age-adjusted HR (95% CI)§	1.0	0.78 (0.67–0.90)	0.76 (0.66–0.89)	0.63 (0.53–0.74)	0.62 (0.51–0.74)	<0.0001
Multivariate HR (95% CI)‖¶	1.0	0.81 (0.70–0.93)	0.77 (0.66–0.90)	0.64 (0.54–0.75)	0.60 (0.50–0.73)	<0.0001
Cancer						
Deaths, n	1620	1624	1499	1529	1415	
Death rate‡	17.51	15.72	14.17	14.29	13.42	
Age-adjusted HR (95% CI)§	1.0	0.89 (0.83–0.95)	0.80 (0.74–0.86)	0.79 (0.73–0.86)	0.76 (0.70–0.83)	<0.0001
Multivariate HR (95% CI)‖	1.0	0.90 (0.84–0.97)	0.83 (0.77–0.90)	0.84 (0.77–0.91)	0.81 (0.74–0.89)	<0.0001
Respiratory disease						
Deaths, n	621	501	415	349	275	
Death rate‡	6.71	4.85	3.92	3.26	2.61	
Age-adjusted HR (95% CI)§	1.0	0.70 (0.62–0.79)	0.58 (0.51–0.66)	0.47 (0.40–0.54)	0.40 (0.33–0.47)	<0.0001
Multivariate HR (95% CI)‖	1.0	0.76 (0.67–0.86)	0.66 (0.58–0.76)	0.57 (0.49–0.67)	0.53 (0.45–0.64)	<0.0001
Diabetes mellitus						
Deaths, n	24	19	15	24	37	
Death rate‡	0.26	0.18	0.14	0.22	0.35	
Age-adjusted HR (95% CI)§	1.0	0.75 (0.40–1.38)	0.61 (0.31–1.19)	1.00 (0.53–1.88)	1.74 (0.91–3.31)	0.016
Multivariate HR (95% CI)‖	1.0	0.72 (0.39–1.34)	0.56 (0.29–1.10)	0.89 (0.47–1.68)	1.33 (0.69–2.57)	0.13
Injuries and accidents						
Deaths, n	294	275	255	217	214	
Death rate‡	3.18	2.66	2.41	2.03	2.03	
Age-adjusted HR (95% CI)§	1.0	0.85 (0.72–1.01)	0.79 (0.66–0.95)	0.67 (0.55–0.82)	0.69 (0.55–0.85)	0.0002
Multivariate HR (95% CI)‖	1.0	0.92 (0.78–1.10)	0.89 (0.74–1.07)	0.79 (0.65–0.97)	0.89 (0.71–1.12)	0.20

(*Continued*)

Table 2. Continued

Causes of Mortality	Quintile of Serum α-Tocopherol					*P* Value†
	Quintile 1 <9.3 mg/L	Quintile 2 9.3- <10.8 mg/L	Quintile 3 10.8- <12.2 mg/L	Quintile 4 12.2- <14.2 mg/L	Quintile 5 ≥14.2 mg/L	
Other causes						
Deaths, n	662	524	527	515	470	
Death rate‡	7.15	5.07	4.98	4.81	4.46	
Age-adjusted HR (95% CI)§	1.0	0.66 (0.59–0.74)	0.66 (0.58–0.74)	0.62 (0.54–0.70)	0.59 (0.51–0.68)	<0.0001
Multivariate HR (95% CI)‖	1.0	0.68 (0.61–0.77)	0.69 (0.61–0.77)	0.65 (0.57–0.74)	0.64 (0.55–0.74)	<0.0001

CVD indicates cardiovascular disease; HDL, high-density lipoprotein; and HR, hazard ratio.
*Cause of death was missing for 47 subjects.
†*P* value for trend: based on statistical significance of the coefficient of the quintile variable (median value within each quintile).
‡Crude death rate per 1000 person-years.
§Adjusted for age and serum total cholesterol.
‖Adjusted for age, serum total and serum HDL cholesterol, cigarettes smoked per day, years of smoking, intervention assignment, and systolic and diastolic blood pressure.
¶Further adjusted for history of CVD.

Stratified analyses showed similar serum α-tocopherol mortality associations across subgroups of smoking intensity, years of smoking, alcohol consumption, trial intervention, and duration of follow-up (Table 3). The association was significantly modified by age and BMI ($P_{interaction}$=0.0059 and <0.0001, respectively; Table 3), however, with a stronger inverse association among younger (<59 years) and leaner men (BMI <25 kg/m^2). Analyses of cause-specific mortality

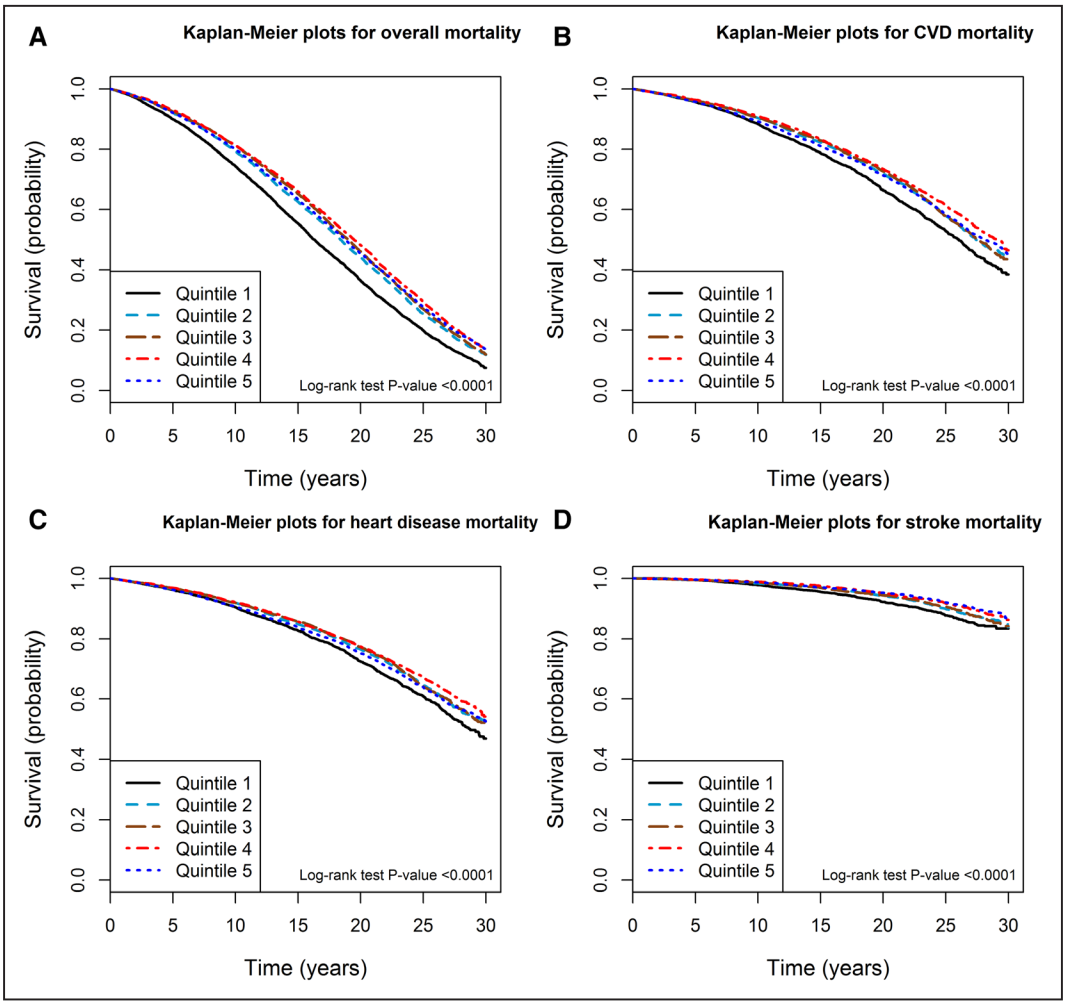

Figure 1. Kaplan-Meier curves of overall and cause-specific mortality according to quintiles of serum α-tocopherol in 29 092 subjects in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention). A, Overall mortality. **B**, Cardiovascular disease (CVD) mortality. **C**, Heart disease mortality. **D**, Stroke mortality.

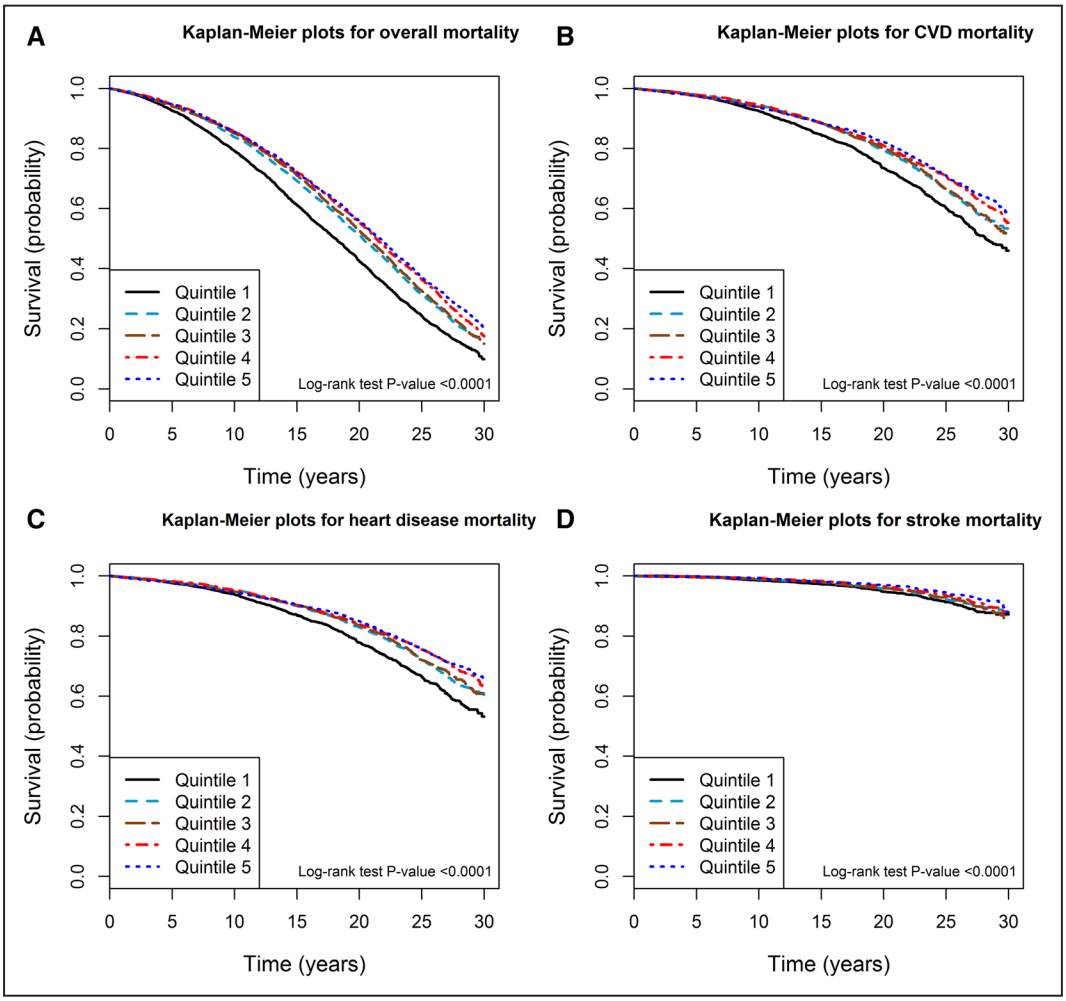

Figure 2. Kaplan-Meier curves of overall and cause-specific mortality according to quintiles of serum α-tocopherol in 16610 subjects who do not have a history of cardiovascular disease (CVD) or diabetes mellitus in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention). A, Overall mortality. **B**, CVD mortality. **C**, Heart disease mortality. **D**, Stroke mortality.

yielded similar patterns for the subgroups (Online Tables III through VIII) with the exception of a stronger inverse heart disease mortality association in younger men ($P_{interaction}$=0.0034; Online Table IV), and more prominent inverse associations for cancer, respiratory disease, and other causes of mortality during the first 13 years of follow-up ($P_{interaction}$=0.005, 0.0021, and <0.0001, respectively; Online Tables VI through VIII). Also, the inverse association with respiratory disease mortality appeared stronger for older and leaner men ($P_{interaction}$=0.0061 and 0.0038, respectively; Online Table VII).

Our findings were not materially altered by excluding the first 5 years of follow-up (overall mortality in the multivariate model, fifth versus first quintile: HR=0.77; 95% CI, 0.73–0.81; Online Table IX). The serum α-tocopherol associations with all-cause, CVD, and heart disease mortality appeared strengthened after excluding the 12482 subjects with a positive history of CVD or diabetes mellitus (mortality from overall, CVD, and heart disease in the multivariate model, fifth versus first quintile: HR=0.69, 0.67, and 0.67, respectively; P_{trend}<0.0001), with the results for other causes remaining unchanged (Online Table X).

Concentrations of serum α-tocopherol and LDL proxy-adjusted serum α-tocopherol were highly correlated (Pearson correlation coefficient=0.77), as were serum α-tocopherol and total cholesterol-adjusted serum α-tocopherol (Pearson correlation coefficient=0.79). The mortality associations did not materially change when we used LDL-proxy–adjusted serum α-tocopherol or total cholesterol-adjusted serum α-tocopherol in the analysis (LDL-proxy–adjusted serum α-tocopherol: overall mortality in the multivariate model, fifth versus first quintile, HR=0.83; 95% CI, 0.79–0.86; Online Table XI; total cholesterol-adjusted serum α-tocopherol: overall mortality in the multivariate model, fifth versus first quintile, HR=0.82; 95% CI, 0.78–0.85; Online Table XII). Our results were also not materially altered when we adjusted for the serum LDL-proxy in the main model (overall mortality in the multivariate model, fifth versus first quintile: HR=0.77; 95% CI, 0.74–0.81; Online Table XIII).

During the 30 years of observation, there were 11951 and 11836 participant deaths in the α-tocopherol and no α-tocopherol trial supplementation arms, respectively. The HR for overall mortality was 1.009 and 1.008 for the α-tocopherol vs no α-tocopherol trial intervention arm in crude and multivariate-adjusted models, respectively (Online Table XIV).

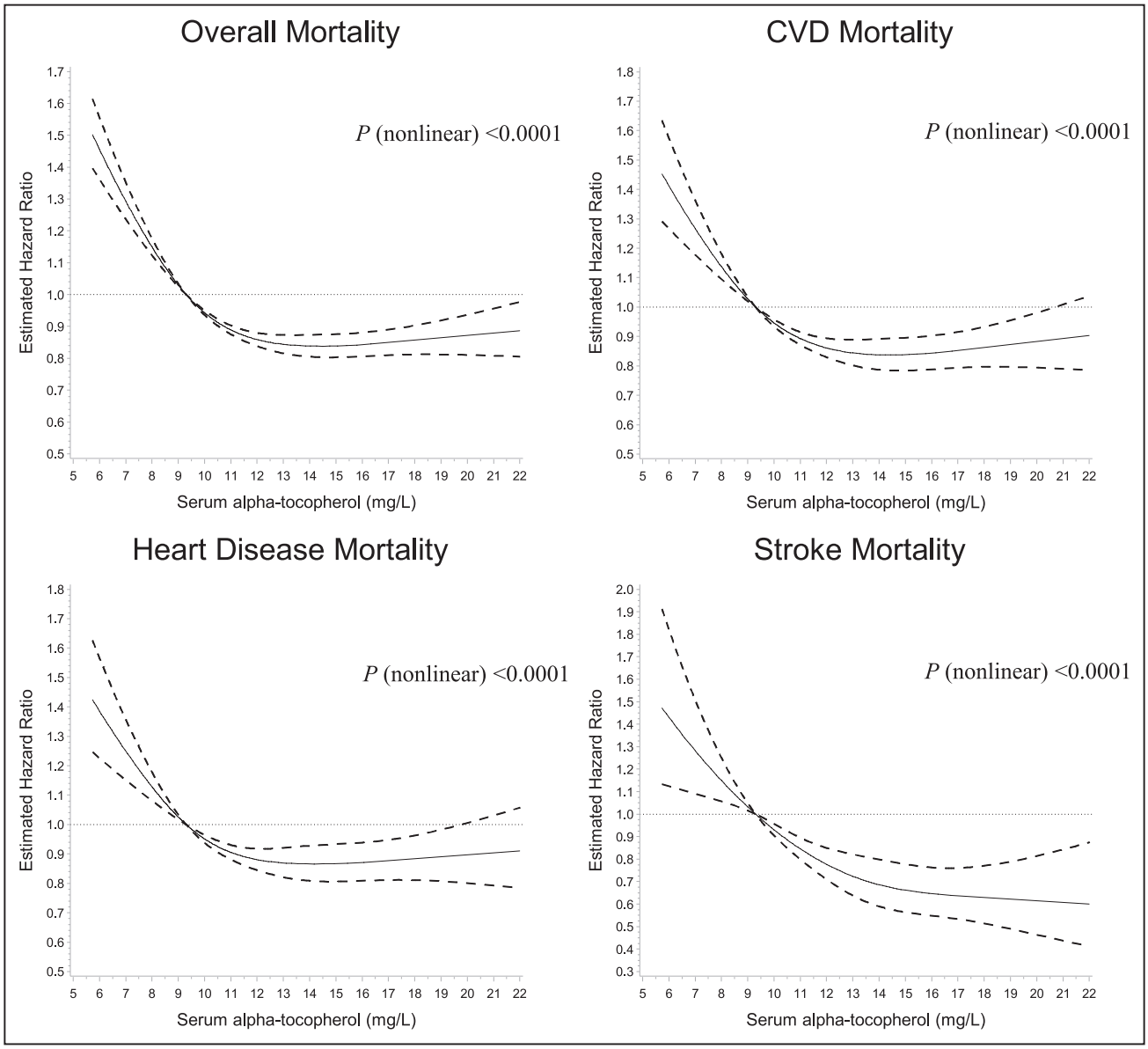

Figure 3. Cubic spline regression for estimated hazard ratios (HRs) of overall and cause-specific mortality according to serum α-tocopherol concentrations in the ATBC Study (Alpha-Tocopherol, Beta-Carotene Cancer Prevention). The reference value (9.3 mg/L; HR=1) corresponds to the cutoff value of the first quintile of serum α-tocopherol concentration. **A**, Overall mortality. **B**, Cardiovascular disease (CVD) mortality. **C**, Heart disease mortality. **D**, Stroke mortality. The solid line suggests the HR for mortality and serum α-tocopherol with a 4-knot spline (knots were selected at the 5th, 25th, 75th, and 95th percentiles of the serum α-tocopherol); dashed lines denoted the 95% CIs. Total number of participants: 29 092. Event number of overall-, CVD-, heart disease-, and stroke death is 23 787, 9867, 8063, and 1763, respectively.

Stratified analyses by baseline serum α-tocopherol quintiles and by median split showed a consistent lack of an α-tocopherol supplementation effect on overall mortality across the serum categories (Online Table XIV).

Discussion

In this large prospective cohort analysis with 30-year follow-up, we observed significant inverse associations between serum α-tocopherol and overall and cause-specific mortality, with a 22% reduction in total mortality for men in the highest versus lowest quintile of serum α-tocopherol. The associations were stable throughout the observation period and were stronger among younger and leaner men, as well as those with no history of CVD.

To our knowledge, this is the largest study to examine α-tocopherol biochemical status in relation to overall and cause-specific mortality. Consistent with a previous study,[25] our findings from the restricted cubic spline analysis support decreasing overall mortality with increasing α-tocopherol concentrations, with a mortality nadir for serum values of 13 to 14 mg/L, and a striking mortality excess for men with concentrations below 10 mg/L. The suggested beneficial role for vitamin E in CVD, heart disease, stroke, cancer, and respiratory disease mortality are supported by recent meta-analyses,[20,21] albeit, the latter data were based on relatively few deaths, insufficient statistical power across studies, population heterogeneity, and inadequate control for important confounders such as serum total cholesterol.[21] Vitamin E status has only

been sporadically investigated in relation to mortality from stroke, respiratory disease,[17] diabetes mellitus, injuries/accidents, or other causes. The present analysis revealed stronger inverse associations in men under the age of 60, those with a lower BMI and those without a history of CVD or diabetes mellitus. The attenuated beneficial association in older men might be explained by age-associated changes in lipoprotein metabolism that may influence the absorption, transport, and metabolism of α-tocopherol; for example, decreased lipoprotein lipase activity could result in greater nonchylomicron lipoprotein vitamin E transport in the older men.[26–28] The stronger inverse vitamin E-mortality association in leaner men and those without a history of CVD or diabetes mellitus could indicate effectiveness of higher α-tocopherol status in the setting of reduced metabolic syndrome-related oxidative stress and inflammation. Also, α-tocopherol bioavailability may be decreased in adults with metabolic syndrome, possibly due to reduced intestinal absorption and impaired hepatic vitamin E transport.[29]

The present findings of a strong inverse serum vitamin E-mortality association stand in stark contrast to the results of controlled trials showing a lack of beneficial effects, and possible adverse effects, for vitamin E supplementation, particularly for the highest supplementation dosages (ie, ≥400 IU/day).[25,30–33] One meta-analysis of antioxidant supplementation trials, for example, showed that vitamin E used alone did not significantly impact overall mortality (relative risk=1.02; 95% CI, 0.98–1.05).[30] However, half of the trials in the analysis had very short follow-up periods (ie, <2 years) that did not permit evaluation of long-term mortality related to vitamin E supplementation, and the majority tested vitamin combinations and administered vitamin E dosages substantially above the recommended daily allowances (15 mg/day for adults), with only 3 of 46 trials testing low-dose supplementation within the recommended daily allowances.[30] Similarly, and most directly relevant to the present vitamin E biochemical analysis showing a mortality benefit, is that the parent ATBC trial which administered a relatively low vitamin E supplement dose of 50 IU/day did not impact overall mortality during or after the intervention.[32,33] In addition, the inverse serum α-tocopherol mortality association in the present analysis did not differ by trial supplementation group (*P* for interaction=0.41). Taken together, the totality of evidence supports a beneficial role in long-term health outcomes for higher diet-based vitamin E status within the physiological range, and particularly, the avoidance of very low serum α-tocopherol concentrations, rather than the improvement of vitamin E status through supplementation. The apparent difference in health effects of higher biochemical status (benefit) versus vitamin E supplementation (no benefit or possible harm) could be due to more than one factor. Vitamin E-rich foods contain a wide range of bioactive substances whose combined long-term effects on multiple metabolic pathways may be required to impact mortality. Supplementation trials have generally tested high-dose α-tocopherol singly or with some other specific vitamins for relatively short periods, which replicates neither the complex dietary vitamin E intake exposure nor its life-long chronicity; it also introduces potential pharmacological disruption of normal homeostatic metabolism.

Vegetable oils, whole grains, nuts, seeds, and some vegetables and fruits are the richest dietary sources of α-tocopherol, and studies demonstrating associations between greater consumption of these foods and lower mortality are consistent with the present cohort biochemical findings.[34–39] Direct biological actions of α-tocopherol and other vitamin E compounds that could impact mortality outcomes are well known and include antioxidant functions related to suppression of free radicals, lipid peroxidation, and oxidative damage to cell membranes, organelles, and DNA that have been related to CVD and carcinogenesis,[40,41] inhibition of protein kinase C in vascular smooth muscle cells and immune/inflammatory responses,[5,40,42] and reduction of the endothelial platelet aggregation and monocyte adhesion that contribute to atherosclerosis.[43–46] Less well known is the dose-dependent nature of these mechanisms in vivo. Beyond such direct effects of vitamin E, serum α-tocopherol is also likely a physiological biomarker of a vitamin E-rich diet that reflects greater intake of fiber, minerals, unsaturated fatty acids, other vitamins, phytosterols, and other antioxidants.[47] These additional bioactive agents have multiple biologic functions including effects on oxidative stress, inflammation, and endothelial function.[48–51] Of note, the inverse association for serum α-tocopherol persisted when we adjusted for intake of vitamin E-rich, multinutrient foods, suggesting that those bioactive substances do not explain the serum α-tocopherol mortality association. We cannot, however, exclude the possibility of residual confounding (eg, due to dietary measurement error).

Major strengths of this investigation include its prospective design, large sample size, and complete follow-up for cause-specific mortality through linkage with national registries for 3 decades. The large sample size enabled examination with substantial statistical power of the vitamin E association both for the less prevalent causes of mortality and across a range of population subgroups. Serum α-tocopherol biochemical concentrations were measured in one institute laboratory through high-quality isocratic high-performance liquid chromatography for the >29 000 men, affording a more accurate and objective assessment of vitamin E status as compared to data from a self-administrated dietary questionnaire. Several limitations of the study also deserve mention. First, because a single assay of serum α-tocopherol concentration at baseline was used, vitamin E biochemical status (and the underlying dietary pattern) could have changed over time. Therefore, bias due to nondifferential misclassification may have led to underestimated associations with mortality. We did, however, observe that serum α-tocopherol concentrations at baseline and 3 years after randomization were highly correlated in participants who were not supplemented with vitamin E (Spearman correlation coefficient=0.72; *P*<0.0001), which suggested its relative stability over time. Second, the homogenous population of Finnish male smokers may limit generalizability of our findings to other populations including women. Third, although we have controlled for several potential confounders, including serum total, HDL, and an LDL cholesterol proxy, we cannot rule out the possibility of residual confounding, such as from unmeasured factors.

In summary, this large prospective cohort analysis with over 30 years of observation provides strong evidence that

Table 3. HRs and 95% CI for All-Cause Mortality by Quintile of Serum α-Tocopherol, Stratified by Selected Factors

	Quintile of Serum α-Tocopherol											
	Quintile 1 <9.3 mg/L		Quintile 2 9.3- <10.8 mg/L		Quintile 3 10.8- <12.2 mg/L		Quintile 4 12.2- <14.2 mg/L		Quintile 5 ≥14.2 mg/L		*P* Value†	*P* for Interaction‡
	Events	HR	Events	HR (95% CI)*	Events	HR (95% CI)*	Events	HR (95% CI)*	Events	HR (95% CI)*		
Age (y)												
<54	1082	1.00	1076	0.81 (0.74–0.88)	1130	0.77 (0.71–0.84)	1028	0.72 (0.65–0.79)	1121	0.72 (0.65–0.81)	<0.0001	0.0059
54–<59	1586	1.00	1559	0.83 (0.77–0.89)	1515	0.74 (0.69–0.80)	1531	0.69 (0.64–0.75)	1597	0.73 (0.66–0.79)	<0.0001	
≥59	2398	1.00	2189	0.85 (0.80–0.90)	2016	0.83 (0.78–0.88)	2023	0.81 (0.75–0.86)	1936	0.84 (0.78–0.90)	<0.0001	
Cigarettes smoked/d												
<16	1459	1.00	1492	0.83 (0.77–0.89)	1516	0.79 (0.73–0.85)	1554	0.72 (0.67–0.78)	1596	0.74 (0.68–0.81)	<0.0001	0.77
16–<20	1692	1.00	1670	0.86 (0.80–0.92)	1602	0.81 (0.76–0.88)	1568	0.81 (0.75–0.88)	1522	0.82 (0.75–0.90)	0.0001	
≥20	1915	1.00	1662	0.82 (0.77–0.88)	1543	0.78 (0.73–0.84)	1460	0.72 (0.67–0.78)	1536	0.78 (0.71–0.84)	<0.0001	
Years of smoking												
<33	1147	1.00	1149	0.82 (0.76–0.90)	1257	0.80 (0.73–0.87)	1177	0.69 (0.63–0.76)	1207	0.74 (0.67–0.82)	<0.0001	0.017
33–<40	1403	1.00	1353	0.81 (0.75–0.88)	1362	0.73 (0.67–0.79)	1320	0.72 (0.66–0.78)	1434	0.72 (0.66–0.79)	<0.0001	
≥40	2516	1.00	2322	0.85 (0.81–0.91)	2042	0.83 (0.78–0.88)	2085	0.80 (0.75–0.86)	2013	0.84 (0.78–0.90)	<0.0001	
Daily alcohol consumption (g)												
<5.3	1332	1.00	1487	0.88 (0.81–0.94)	1425	0.83 (0.77–0.90)	1506	0.82 (0.75–0.89)	1471	0.85 (0.77–0.93)	0.003	0.06
5.3–<20.4	1456	1.00	1389	0.81 (0.75–0.88)	1488	0.79 (0.73–0.86)	1453	0.73 (0.67–0.79)	1503	0.75 (0.68–0.82)	<0.0001	
≥20.4	1829	1.00	1542	0.82 (0.77–0.88)	1431	0.75 (0.70–0.81)	1323	0.70 (0.65–0.76)	1396	0.73 (0.67–0.79)	<0.0001	
BMI, kg/m^2												
<25	2478	1.00	2069	0.79 (0.74–0.84)	1813	0.72 (0.68–0.77)	1655	0.68 (0.63–0.73)	1301	0.68 (0.63–0.74)	<0.0001	<0.0001
25–<28	1352	1.00	1452	0.84 (0.78–0.90)	1512	0.81 (0.75–0.87)	1507	0.75 (0.69–0.81)	1589	0.74 (0.68–0.81)	<0.0001	
≥28	1229	1.00	1301	0.94 (0.87–1.02)	1330	0.91 (0.84–0.98)	1420	0.89 (0.82–0.97)	1761	0.94 (0.86–1.03)	0.25	
Trial intervention arm												
α-tocopherol	2525	1.00	2418	0.87 (0.82–0.92)	2320	0.81 (0.76–0.86)	2327	0.76 (0.71–0.81)	2361	0.80 (0.74–0.86)	<0.0001	0.41
No α-tocopherol	2541	1.00	2406	0.80 (0.76–0.85)	2341	0.77 (0.73–0.82)	2255	0.74 (0.69–0.79)	2293	0.75 (0.70–0.81)	<0.0001	
β-carotene	2520	1.00	2376	0.81 (0.76–0.86)	2285	0.76 (0.72–0.81)	2327	0.73 (0.69–0.78)	2353	0.74 (0.69–0.79)	<0.0001	0.18
No β-carotene	2546	1.00	2448	0.86 (0.82–0.91)	2376	0.82 (0.77–0.87)	2255	0.76 (0.72–0.82)	2301	0.81 (0.76–0.87)	<0.0001	

(*Continued*)

Table 3. Continued

	Quintile of Serum α-Tocopherol											
	Quintile 1 <9.3 mg/L		Quintile 2 9.3- <10.8 mg/L		Quintile 3 10.8- <12.2 mg/L		Quintile 4 12.2- <14.2 mg/L		Quintile 5 ≥14.2 mg/L		*P* Value†	*P* for Interaction‡
	Events	HR	Events	HR (95% CI)*	Events	HR (95% CI)*	Events	HR (95% CI)*	Events	HR (95% CI)*		
Years of follow-up												
0–13	2389	1.00	1945	0.80 (0.75–0.85)	1739	0.72 (0.68–0.77)	1700	0.70 (0.65–0.75)	1849	0.75 (0.70–0.81)	<0.0001	0.42
13–<23	1913	1.00	1949	0.84 (0.79–0.90)	1953	0.82 (0.76–0.87)	1910	0.77 (0.72–0.83)	1866	0.80 (0.74–0.87)	<0.0001	
≥23	764	1.00	930	0.88 (0.80–0.97)	969	0.87 (0.79–0.96)	972	0.80 (0.72–0.89)	939	0.76 (0.68–0.86)	<0.0001	

BMI indicates body mass index; HDL, high-density lipoprotein; and HR, hazard ratio.

*Adjusted for age, serum total and serum HDL cholesterol, cigarettes smoked per day, years of smoking, intervention assignment, and systolic and diastolic blood pressure.

†*P* value for trend: based on statistical significance of the coefficient of the quintile variable (median value within each quintile).

‡*P* value for interaction: according to the likelihood test to assess the statistical significance of the cross-product term entered to the Cox proportional hazard regression model.

men with higher vitamin E status (ie, serum α-tocopherol) experience significantly lower overall and cause-specific mortality for CVD, heart disease, stroke, cancer, and respiratory disease. The associations observed during this long-term follow-up were independent of several other important mortality risk factors. Our findings should be reexamined in other populations that include women, nonsmokers, and other races/ethnicities.

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Disclosures

None.

References

1. Traber MG. Vitamin E regulatory mechanisms. *Annu Rev Nutr.* 2007;27:347–362. doi: 10.1146/annurev.nutr.27.061406.093819
2. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med.* 2014;72:76–90. doi: 10.1016/j.freeradbiomed.2014.03.035
3. Waniek S, di Giuseppe R, Esatbeyoglu T, Plachta-Danielzik S, Ratjen I, Jacobs G, Nothlings U, Koch M, Schlesinger S, Rimbach G, Lieb W. Vitamin e (α- and γ-tocopherol) levels in the community: distribution, clinical and biochemical correlates, and association with dietary patterns. *Nutrients*. 2017;10:E3. doi: 10.3390/nu10010003
4. Azzi A, Stocker A. Vitamin E: non-antioxidant roles. *Prog Lipid Res.* 2000;39:231–255.
5. Pfluger P, Kluth D, Landes N, Bumke-Vogt C, Brigelius-Flohé R. Vitamin E: underestimated as an antioxidant. *Redox Rep.* 2004;9:249–254. doi: 10.1179/135100004225006740
6. Azzi A, Breyer I, Feher M, Pastori M, Ricciarelli R, Spycher S, Staffieri M, Stocker A, Zimmer S, Zingg JM. Specific cellular responses to alpha-tocopherol. *J Nutr.* 2000;130:1649–1652. doi: 10.1093/jn/130.7.1649
7. Shklar G, Schwartz JL. Vitamin E inhibits experimental carcinogenesis and tumour angiogenesis. *Eur J Cancer B Oral Oncol.* 1996;32B:114–119.
8. Sigounas G, Anagnostou A, Steiner M. dl-alpha-tocopherol induces apoptosis in erythroleukemia, prostate, and breast cancer cells. *Nutr Cancer.* 1997;28:30–35. doi: 10.1080/01635589709514549
9. Meydani SN, Beharka AA. Recent developments in vitamin E and immune response. *Nutr Rev.* 1998;56:S49–S58. doi: 10.1111/j.1753-4887.1998.tb01644.x
10. Sahyoun NR, Jacques PF, Russell RM. Carotenoids, vitamins C and E, and mortality in an elderly population. *Am J Epidemiol.* 1996;144:501–511.
11. Fletcher AE, Breeze E, Shetty PS. Antioxidant vitamins and mortality in older persons: findings from the nutrition add-on study to the Medical Research Council Trial of Assessment and Management of Older People in the Community. *Am J Clin Nutr.* 2003;78:999–1010. doi: 10.1093/ajcn/78.5.999
12. Goyal A, Terry MB, Siegel AB. Serum antioxidant nutrients, vitamin A, and mortality in U.S. Adults. *Cancer Epidemiol Biomarkers Prev.* 2013;22:2202–2211. doi: 10.1158/1055-9965.EPI-13-0381
13. Wright ME, Lawson KA, Weinstein SJ, Pietinen P, Taylor PR, Virtamo J, Albanes D. Higher baseline serum concentrations of vitamin E are associated with lower total and cause-specific mortality in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Clin Nutr.* 2006;84:1200–1207. doi: 10.1093/ajcn/84.5.1200
14. De Waart FG, Schouten EG, Stalenhoef AF, Kok FJ. Serum carotenoids, alpha-tocopherol and mortality risk in a prospective study among Dutch elderly. *Int J Epidemiol.* 2001;30:136–143.
15. Huerta JM, González S, Fernández S, Patterson AM, Lasheras C. Lipid peroxidation, antioxidant status and survival in institutionalised elderly: a five-year longitudinal study. *Free Radic Res.* 2006;40:571–578. doi: 10.1080/10715760600580470
16. Buijsse B, Feskens EJ, Schlettwein-Gsell D, Ferry M, Kok FJ, Kromhout D, de Groot LC. Plasma carotene and alpha-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA). *Am J Clin Nutr.* 2005;82:879–886. doi: 10.1093/ajcn/82.4.879
17. Bates CJ, Hamer M, Mishra GD. Redox-modulatory vitamins and minerals that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of people aged 65 years and over. *Br J Nutr.* 2011;105:123–132. doi: 10.1017/S0007114510003053
18. Kilander L, Berglund L, Boberg M, Vessby B, Lithell H. Education, lifestyle factors and mortality from cardiovascular disease and cancer. A 25-year follow-up of Swedish 50-year-old men. *Int J Epidemiol.* 2001;30:1119–1126.
19. Marniemi J, Järvisalo J, Toikka T, Räihä I, Ahotupa M, Sourander L. Blood vitamins, mineral elements and inflammation markers as risk factors of vascular and non-vascular disease mortality in an elderly population. *Int J Epidemiol.* 1998;27:799–807.
20. Jayedi A, Rashidy-Pour A, Parohan M, Zargar MS, Shab-Bidar S. Dietary antioxidants, circulating antioxidant concentrations, total antioxidant capacity, and risk of all-cause mortality: a systematic review and dose-response meta-analysis of prospective observational studies. *Adv Nutr.* 2018;9:701–716. doi: 10.1093/advances/nmy040

21. Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, Tonstad S, Vatten LJ, Riboli E, Norat T. Dietary intake and blood concentrations of antioxidants and the risk of cardiovascular disease, total cancer, and all-cause mortality: a systematic review and dose-response meta-analysis of prospective studies. *Am J Clin Nutr.* 2018;108:1069–1091. doi: 10.1093/ajcn/nqy097
22. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. The ATBC Cancer Prevention Study Group. *Ann Epidemiol*. 1994;4:1–10.
23. Milne DB, Botnen J. Retinol, alpha-tocopherol, lycopene, and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. *Clin Chem.* 1986;32:874–876.
24. Willett W. *Nutritional epidemiology*. 2nd ed. New York, NY: Oxford University Press; 1998.
25. Miller ER III, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med.* 2005;142:37–46.
26. Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Postprandial plasma lipoprotein changes in human subjects of different ages. *J Lipid Res.* 1988;29:469–479.
27. Krasinski SD, Cohn JS, Schaefer EJ, Russell RM. Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. Evidence for delayed plasma clearance of intestinal lipoproteins. *J Clin Invest.* 1990;85:883–892. doi: 10.1172/JCI114515
28. Borel P, Mekki N, Boirie Y, Partier A, Grolier P, Alexandre-Gouabau MC, Beaufrere B, Armand M, Lairon D, Azais-Braesco V. Postprandial chylomicron and plasma vitamin E responses in healthy older subjects compared with younger ones. *Eur J Clin Invest.* 1997;27:812–821.
29. Mah E, Sapper TN, Chitchumroonchokchai C, Failla ML, Schill KE, Clinton SK, Bobe G, Traber MG, Bruno RS. α-Tocopherol bioavailability is lower in adults with metabolic syndrome regardless of dairy fat co-ingestion: a randomized, double-blind, crossover trial. *Am J Clin Nutr.* 2015;102:1070–1080. doi: 10.3945/ajcn.115.118570
30. Bjelakovic G, Nikolova D, Gluud C. Meta-regression analyses, meta-analyses, and trial sequential analyses of the effects of supplementation with beta-carotene, vitamin A, and vitamin E singly or in different combinations on all-cause mortality: do we have evidence for lack of harm? *PLoS One.* 2013;8:e74558. doi: 10.1371/journal.pone.0074558
31. Bjelakovic G, Nikolova D, Gluud C. Antioxidant supplements to prevent mortality. *JAMA*. 2013;310:1178–1179. doi: 10.1001/jama.2013.277028
32. Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin e and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med*. 1994;330:1029–1035. doi: 10.1056/NEJM199404143301501
33. Virtamo J, Taylor PR, Kontto J, Männistö S, Utriainen M, Weinstein SJ, Huttunen J, Albanes D. Effects of α-tocopherol and β-carotene supplementation on cancer incidence and mortality: 18-year postintervention follow-up of the Alpha-tocopherol, Beta-carotene Cancer Prevention Study. *Int J Cancer.* 2014;135:178–185. doi: 10.1002/ijc.28641
34. Bao Y, Han J, Hu FB, Giovannucci EL, Stampfer MJ, Willett WC, Fuchs CS. Association of nut consumption with total and cause-specific mortality. *N Engl J Med.* 2013;369:2001–2011. doi: 10.1056/NEJMoa1307352
35. Eslamparast T, Sharafkhah M, Poustchi H, et al. Nut consumption and total and cause-specific mortality: results from the Golestan Cohort Study. *Int J Epidemiol.* 2017;46:75–85. doi: 10.1093/ije/dyv365
36. Luu HN, Blot WJ, Xiang YB, Cai H, Hargreaves MK, Li H, Yang G, Signorello L, Gao YT, Zheng W, Shu XO. Prospective evaluation of the association of nut/peanut consumption with total and cause-specific mortality. *JAMA Intern Med.* 2015;175:755–766. doi: 10.1001/jamainternmed.2014.8347
37. van den Brandt PA, Schouten LJ. Relationship of tree nut, peanut and peanut butter intake with total and cause-specific mortality: a cohort study and meta-analysis. *Int J Epidemiol.* 2015;44:1038–1049. doi: 10.1093/ije/dyv039
38. Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, Tonstad S, Vatten LJ, Riboli E, Norat T. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. *BMJ.* 2016;353:i2716. doi: 10.1136/bmj.i2716
39. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, Greenwood DC, Riboli E, Vatten LJ, Tonstad S. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. *Int J Epidemiol.* 2017;46:1029–1056. doi: 10.1093/ije/dyw319
40. Ricciarelli R, Zingg JM, Azzi A. Vitamin E: protective role of a Janus molecule. *FASEB J.* 2001;15:2314–2325. doi: 10.1096/fj.01-0258rev
41. Cardenas E, Ghosh R. Vitamin E: a dark horse at the crossroad of cancer management. *Biochem Pharmacol.* 2013;86:845–852. doi: 10.1016/j.bcp.2013.07.018
42. Azzi A, Gysin R, Kempná P, Munteanu A, Villacorta L, Visarius T, Zingg JM. Regulation of gene expression by alpha-tocopherol. *Biol Chem.* 2004;385:585–591. doi: 10.1515/BC.2004.072
43. Rashidi B, Hoseini Z, Sahebkar A, Mirzaei H. Anti-atherosclerotic effects of vitamins D and E in suppression of atherogenesis. *J Cell Physiol.* 2017;232:2968–2976. doi: 10.1002/jcp.25738
44. Devaraj S, Hugou I, Jialal I. Alpha-tocopherol decreases CD36 expression in human monocyte-derived macrophages. *J Lipid Res.* 2001;42:521–527.
45. Devaraj S, Jialal I. Alpha-tocopherol decreases interleukin-1 beta release from activated human monocytes by inhibition of 5-lipoxygenase. *Arterioscler Thromb Vasc Biol.* 1999;19:1125–1133.
46. Ricciarelli R, Zingg JM, Azzi A. Vitamin E reduces the uptake of oxidized LDL by inhibiting CD36 scavenger receptor expression in cultured aortic smooth muscle cells. *Circulation.* 2000;102:82–87.
47. Kris-Etherton PM, Hu FB, Ros E, Sabaté J. The role of tree nuts and peanuts in the prevention of coronary heart disease: multiple potential mechanisms. *J Nutr.* 2008;138:1746S–1751S. doi: 10.1093/jn/138.9.1746S
48. Jenkins DJ, Kendall CW, Josse AR, Salvatore S, Brighenti F, Augustin LS, Ellis PR, Vidgen E, Rao AV. Almonds decrease postprandial glycemia, insulinemia, and oxidative damage in healthy individuals. *J Nutr.* 2006;136:2987–2992. doi: 10.1093/jn/136.12.2987
49. Torabian S, Haddad E, Rajaram S, Banta J, Sabaté J. Acute effect of nut consumption on plasma total polyphenols, antioxidant capacity and lipid peroxidation. *J Hum Nutr Diet.* 2009;22:64–71. doi: 10.1111/j.1365-277X.2008.00923.x
50. Jiang R, Jacobs DR Jr, Mayer-Davis E, Szklo M, Herrington D, Jenny NS, Kronmal R, Barr RG. Nut and seed consumption and inflammatory markers in the multi-ethnic study of atherosclerosis. *Am J Epidemiol.* 2006;163:222–231. doi: 10.1093/aje/kwj033
51. Ma Y, Njike VY, Millet J, Dutta S, Doughty K, Treu JA, Katz DL. Effects of walnut consumption on endothelial function in type 2 diabetic subjects: a randomized controlled crossover trial. *Diabetes Care.* 2010;33:227–232. doi: 10.2337/dc09-1156