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Lower Plasma Vitamin E Levels Are Associated With the Frailty Syndrome: The InCHIANTI Study

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Background. The primary biologic mechanism that causes frailty in older persons has never been adequately explained. According to recent views, oxidative stress may be the driving force of this condition. We tested the hypothesis that, independent of confounders, low plasma levels of vitamin E (α-tocopherol), the main fat-soluble human antioxidant, are associated with the frailty syndrome in older persons free from dementia and disability.

Methods. The study sample included 827 older (≥65 years) persons (women, 54%) who participated in a population-based epidemiological study. Frail participants were identified based on the presence of at least three of five of the following features: self-reported weight loss, low energy, slow gait speed, low grip strength, and low physical activity. Participants with none of these features were considered nonfrail, while participants with one or two were considered intermediate frail. Plasma vitamin E levels were determined using reverse-phase high-performance liquid chromatography. Measured confounders included lower extremity muscle strength, cognitive function, diseases, and factors related to vitamin E metabolism.

Results. Age- and gender-adjusted levels of vitamin E decreased gradually from the nonfrail to the frail group (p for trend = .015). In the logistic model adjusted for multiple potential confounders, participants in the highest vitamin E tertile were less likely to be frail than were participants in the lowest vitamin E tertile (odds ratio, 0.30; 95% confidence interval, 0.10–0.91).

Conclusions. Our findings show an association between low circulating levels of one of the most important components of the human antioxidant system and the presence of frailty.

FRAILTY is a condition typically encountered in older persons and characterized by increased vulnerability to stressors and high risk of adverse outcomes including disability and death (1). Approximately 7% of the older population (≥65 years) is frail (2), and the prevalence of frailty increases up to 40% in persons aged 80 years and older (3). Due to the dramatic increment of the oldest-old population (≥80 years) (4), frailty is becoming increasingly common. Understanding the biologic mechanisms underlying frailty is a critical step toward the development of effective treatment that can prevent or reverse frailty and its consequences (5).

According to recent views, frailty is hypothesized to result from the dysregulation of the physiologic mechanisms involved in the maintenance of homeostasis (6). These mechanisms are aimed at optimizing the production, distribution, and utilization of energy in different physiologic and pathophysiologic conditions and restoring the status quo after a destabilizing environmental stress.

As frailty has been proposed as an accelerated form of aging (6), a process strongly associated with increased oxidative stress (OS) (7), it has been argued that OS might be the driving force behind the dysfunction of the homeostatic mechanisms that underlie frailty (6). However, so far this theory has been supported by scant empirical data.

Vitamin E (α-tocopherol, VE), the major lipophilic antioxidant in humans, is a physiologic scavenger of reactive oxygen species produced during lipid peroxidation (8). Low levels of VE have been associated with cognitive impairment (9,10) and poor lower extremity muscle strength (11,12), two of the major characteristics of the frailty syndrome (13,14). At least theoretically, after controlling for intake, recycling, and excretion of VE, the lower the circulating levels of VE, the higher the conversion of VE into α-tocopheroxyl radical, the post oxidation VE metabolite, which is not detected by current VE assay. Consistently, the higher the conversion, the greater the OS. In this context, lower levels of VE may be considered an indirect marker of high OS burden.

In a large sample of community-dwelling older persons, we tested the hypothesis that, independent of potential

confounders (including factors related to VE metabolism), low VE levels are associated with increased risk of frailty and that such an association is not mediated by the effect of VE on cognitive and muscular function. This hypothesis is consistent with a direct and diffuse involvement of OS in the genesis of frailty.

Methods

Study Population

Data were from InCHIANTI, a large epidemiological study conducted in Italy. The design of the study, approved by the Italian National Institute of Research and Care on Aging review board, has been reported elsewhere (15). Briefly, from the population of two small towns (Greve in Chianti and Bagno a Ripoli), 1260 older persons (≥65 years) were randomly selected. Of these, 1154 (89%) agreed to participate. Participants responded to a structured home interview, underwent full medical and functional examinations, and donated a blood sample. In the present analysis, individuals affected by cancer ($n = 66$) or dementia ($n = 77$) (*Diagnostic and Statistical Manual of Mental Disorders*, Third Edition [DSM III-R] criteria) were excluded. Participants with Mini-Mental State Examination (16) score <18 ($n = 33$), those who reported disabilities in basic activities of daily living (eating, bathing, dressing, transferring from bed to chair, using the toilet, and walking across a small room) ($n = 55$), and those taking VE supplementation ($n = 7$) were also excluded. Disabled individuals were excluded because we wanted to focus on ''at risk'' individuals and because disability strongly affects both the quality and quantity of dietary intake. Participants with severe cognitive impairment were excluded because of the difficulty to accurately assess the condition of frailty and to maintain consistency with the definition of frailty proposed by Fried and coworkers (2). The 827 participants with complete data on VE and frailty status were included in the present analysis.

Frailty Syndrome

The condition of frailty was operationally defined according to Fried and colleagues (2). Their definition included domains of ''shrinking,'' ''weakness,'' ''lack of energy,'' ''slowness,'' and ''sedentariness.'' Using data available in the InCHIANTI study, diagnostic criteria for frailty were operationalized as follows: (a) ''Shrinking'': self-reported unintentional weight loss greater than 4.5 kilograms in the previous year; (b) ''Lack of energy'': self-reported feeling of exhaustion based on two questions from the Italian version of the Center for Epidemiological Studies–Depression scale (CES–D) (17): ''I felt that everything I did was an effort'' and ''I could not get going''; (c) ''Slowness'': usual pace walking speed in the lowest gender- and height-specific quintile. To measure usual walking speed, photocells connected with a recording chronometer were placed at the beginning and at the end of a 4-m course. The time between the activation of the first and the second photocells was recorded, and the average speed of two walks was used in the analysis; (d) ''Sedentariness'': based on self-reported physical activity. Physical activity was assessed during the home interview. According to the level of leisure physical activity performed daily during the last year before the interview, the participant was assigned to one of the following categories: 1) completely inactive or performing light-intensity physical activity (i.e., walking, light housework) less than 1 hour per week; 2) light physical activity: light-intensity physical activity 2–4 hours per week; 3) moderate–high physical activity: light physical activity at least 5 hours per week or moderate physical activity (i.e., gymnastics, playing soccer, gardening) at least 1–2 hours per week (participants in category 1 were considered ''sedentary''); (e) ''Weakness'': grip strength in the lowest sex-specific quintile. Grip strength was measured with a handheld dynamometer (Nicholas Muscle Tester; Sammon Preston, Inc., Chicago, IL) using a standard method. Participants with a combination of at least three of five of these signs and symptoms were classified as being affected by the frailty syndrome. Those with none of these features were considered nonfrail, and those with one or two features were considered ''intermediate frail.'' In our sample, the domain of ''weakness'' measured by hand-grip strength is highly correlated with our measure of lower extremity muscle strength, the knee extension strength. Therefore, because our aim was to verify whether VE status was associated with frailty independent of the effect of VE on muscle strength, in a secondary analysis the domain ''weakness'' was not used as a part of the frailty definition, and lower extremity muscle strength was added in the final model as a confounder. In this sensitivity analysis, we defined frail participants having at least two of the four remaining criteria and ''intermediate frail'' participants with one criterion.

VE and Other Laboratory Measurements

Blood samples were obtained after overnight fasting and centrifuged at 4°C to separate plasma. Plasma aliquots were protected from light with aluminum foil, stored at –80°C, and never thawed until analyzed. Plasma VE concentrations (α-tocopherol) were measured by reversed-phase high-performance liquid chromatography as reported elsewhere (18). Briefly, 100 μl of plasma was mixed with 100 μl of ethanol; after vortexing, tocopherol was extracted into 500 μl of hexane containing 0.002% butylated hydroxyl toluene (BHT; Sigma, St. Louis, MO). Tocol (a gift from Hoffman La Roche, Nutley, NJ) was added to the mixture as an internal standard. Samples were centrifuged at 800 rpm for 5 minutes at 4°C. The supernatant was collected and dried under a stream of nitrogen gas, and reconstituted in 100 μl of methanol. Tocopherols were separated by high-performance liquid chromatography using a 3 μm C18 reverse phase column (Perkin-Elmer, Norwalk, CT). The mobile phase, delivered at a flow rate of 1.0 ml/min, consisted of 1% water in methanol, containing lithium perchlorate at 10 mmol/L. Samples were injected with an autosampler (1100 series; Hewlett-Packard). Eluted peaks were detected at an applied potential of +0.6 V by an LC 4B amperometric electrochemical detector (Bioanalytical Systems, Inc., West Lafayette, IN). Peaks were integrated with ChemStation software (Hewlett-Packard). Tocopherol (α-tocopherol) concentration was expressed in μmol/L. Reproducibility

and accuracy of the procedure used was tested by analyzing representative samples in triplicate from a sample provided by the American Association for Laboratory Accreditation (Washington, DC) containing known concentration of α-tocopherol. Intra- and inter-batch coefficients of variation (CV) were 3% and 4.2%, respectively. For the statistical analysis, VE levels were divided into tertiles (lowest: ≤26.4, middle: 26.4–33.0; highest: ≥33.1 μmol/L). Commercial enzymatic tests were used to determine total cholesterol, triglycerides, serum, and urinary creatinine levels (Roche Diagnostics, GmbH, Mannheim, Germany and Modular P800 Hitachi Autoanalyzer, Hitachi, Maidenhead, U.K.). For serum creatinine, the analytical sensitivity was 0.1 mg/dL, the intra-assay CV was 0.7%, and the inter-assay CV was 2.3%. For urinary creatinine, the intra-assay CV was 0.27%, and the inter-assay CV was 1.7%. Serum creatinine and urinary creatinine from the 24-hour urine collection were used to calculate creatinine clearance. The total cholesterol lower detection limit was 3.0 mg/dL, the intra-assay CV was 0.8%, and the inter-assay CV was 3.3%. The triglycerides lower detection limit was 4.0 mg/dL; the intra-assay CV was 3.1%, and the inter-assay CV was 1.8%. Serum interleukin-6 (IL-6) was measured in duplicate by enzyme-linked immunosorbent assay (Human Ultrasensitive; BioSource International Inc., Camarillo, CA). The minimum detectable concentration was 0.10 pg/mL, and the inter-assay CV was 4.5%.

Other Covariates

Education was assessed as the maximum achieved educational level. Alcohol consumption, total energy, and vitamins E and C intake were assessed using the EPIC questionnaire, a food-frequency questionnaire validated in the Italian population (19), which provides a detailed assessment of food consumption during the previous year through a large number of structured and precoded questions. Originally, the questionnaire was conceived to be self-administered. However, in a pilot study we realized that, in older persons, this method of administration provides ambiguous results, mainly due to the misunderstanding of questions. Thus, in the InCHIANTI study, the EPIC questionnaire was administered by trained interviewers. The information provided by the questionnaire was transformed into average daily intake of macro- and micronutrients (including VE) by software that uses for reference the food composition for Italian epidemiological studies. Although in the InCHIANTI population VE intake tends to be lower in frail compared to nonfrail participants, VE intake distribution in frailty groups showed a wide overlap (nonfrail group: 2–17.8 mg/day, intermediate: 2.5–12.9 mg/day, frail: 2.6–9.9 mg/day). In the present analysis, VE intake is considered a confounder. In fact, we hypothesized that VE circulating levels would be different in frail and nonfrail individuals, independent of VE intake. A measure of smoking exposure that combines intensity and duration (pack-years) was calculated and used in the analysis. Body mass index (BMI), calculated as weight (in kilograms) divided by the square of height (in meters), was considered a measure of general adiposity. Waist circumference was measured at the abdominal point with the largest circumference (usually one centimeter above the iliac crest) and used as indicator of central adiposity. Cognitive status was assessed using the Mini-Mental State Examination (16). Knee extension strength was measured with a handheld dynamometer (Nicholas Muscle Tester; Sammon Preston, Inc., Chicago, IL) using a standard method that has been previously proven to be reliable on the basis of high test–retest and inter-rater reliability (0.85 and 0.74, respectively) (20).

The presence of specific medical conditions was established using standardized criteria that combined information from self-reported history, medical records, and a clinical medical examination. The following diseases or conditions, which either have been associated with poor VE status and/or frailty in the previous literature or potentially affecting VE metabolism, were included in the analysis: angina, acute myocardial infarction, peripheral arterial disease, stroke and/or transient ischemic attacks, hypertension, diabetes, liver diseases, and gastrointestinal surgery. The number of medications taken in the last 2 weeks by participants at the baseline assessment was used in the study as a possible factor affecting VE absorption.

Statistical Analysis

In the descriptive analysis, data were generally reported as mean ± standard deviation; however, due to the skewed distribution, VE, triglycerides, and IL-6 concentrations were reported as median and interquartile range. Differences between groups were tested in age- and gender-adjusted analysis of covariance and tested for linear trend. To approximate a normal distribution, log-transformed data on VE [log (VE)] were used in this analysis and subsequently back-transformed for data presentation (mean ± standard error). Polychotomous logistic regression models were used to assess the association between tertiles of VE and frailty groups. Odds ratios (ORs) adjusted for confounders were calculated, comparing participants with frailty syndrome and participants with intermediate characteristics to those without frailty. Multiple logistic regression analysis was used to assess the association between each individual component of the frailty syndrome and VE tertiles (with the lowest tertile considered the reference group).

Results

Table 1 shows the principal characteristics of the study participants. Women comprised 54% ($n = 446$) of the sample, and the mean age was 73.6 ± 6.4 years. The prevalence of frailty syndrome and intermediate frailty were, respectively, 6.5% and 37.8%.

Figure 1 shows age- and sex-adjusted levels of VE according to frailty status. VE concentrations decreased gradually from the nonfrail to the frail group (p for trend = .015). Nonfrail persons had higher age- and gender-adjusted levels of VE compared with frail ($p = .015$) and intermediate frail persons ($p = .086$).

After adjusting for demographics (Table 2, Model 1), persons in the middle and highest VE tertile were 40% ($p = .156$) and 59% ($p = .015$) less likely to be frail than were those in the lowest tertile. After further adjustment for a number of potential confounders including VE intake, factors affecting absorption, bioavailability and elimination

Table 1. Main Characteristics of the Participants in the InCHIANTI Study Who Were Included in the Present Analysis

Characteristic	Value	
No. of participants	827	
Age [y, mean (standard deviation), range]	73.6 (6.4)	65–92
Gender, No. of women (%)	446 (54.0)	—
Site, No. in Greve in Chianti (%)	389 (47.0)	—
Educational level [y, mean (standard deviation), range]	5.6 (3.2)	0–22
Mini-Mental State Examination score [mean (standard deviation), range]	25.6 (2.7)	18–30
Smoking (pack-years) [mean (standard deviation), range]	12.4 (20.2)	0–120
Alcohol consumption [drinks/wk, mean (standard deviation), range]	107.5 (142.6)	0–1125.0
No. of medications [mean (standard deviation), range]	2.1 (1.9)	0–10
Body mass index [kg/m^2, mean (standard deviation), range]	27.4 (4.0)	18.0–46.6
Waist circumference [cm, mean (standard deviation), range]	93.0 (10.2)	61–124
Leg-extension strength [Kg, mean (standard deviation), range]	16.4 (5.9)	3.5–39.8
Creatinine clearance [mL/min, mean (standard deviation), range]	78.6 (25.2)	8.6–166.3
Frailty status, No. (%)		
Intermediate frail	313 (37.8)	—
Frail	54 (6.5)	—
Frailty features, No. (%)		
Shrinking	40 (4.8)	—
Lack of energy	138 (16.6)	—
Slowness	149 (18.0)	—
Sedentariness	6 (14.0)	—
Weakness	145 (17.5)	—
Myocardial infarction, No. (%)	43 (5.2)	—
Angina, No. (%)	80 (7.3)	—
Stroke or transient ischemic attack, No. (%)	47 (5.7)	—
Peripheral artery disease, No. (%)	116 (14.1)	—
Hypertension, No. (%)	537 (65.0)	—
Diabetes, No. (%)	100 (12.1)	—
Liver diseases, No. (%)	6 (0.7)	—
Gastrointestinal surgery, No. (%)	37 (4.5)	—
Energy intake [Kcal/day, mean (standard deviation), range]	1955.7 (567.5)	650.6–4608.3
Vitamin E intake [mg/day, mean (standard deviation), range]	6.3 (1.9)	1.9–17.8
Vitamin C intake [mg/day, mean (standard deviation), range]	113.5 (48.0)	19.2–406.7
Plasma total cholesterol [mg/dL, mean (standard deviation), range]	219.6 (38.8)	102.0–387.1
Plasma triglycerides [mg/dL, median (interquartile range), range]	109.1 (61.0)	30.9–750.1
Plasma α-tocopherol [μmol/L, median (interquartile range), range]	29.4 (10.8)	7.6–84.5
Interleukin-6 [pg/mL, median (interquartile range), range]	1.36 (1.22)	0.1–19.5

of VE, smoking and alcohol consumption, diseases and inflammation, the association between VE status and frailty became even stronger (Table 2, Model 2). Further adjustment for cognitive status (Table 2, Model 3) did not substantially change the results: participants in the middle and highest VE tertiles were 29% ($p = .454$) and 70% ($p = .035$) less likely to be frail than were participants in the lowest VE tertile.

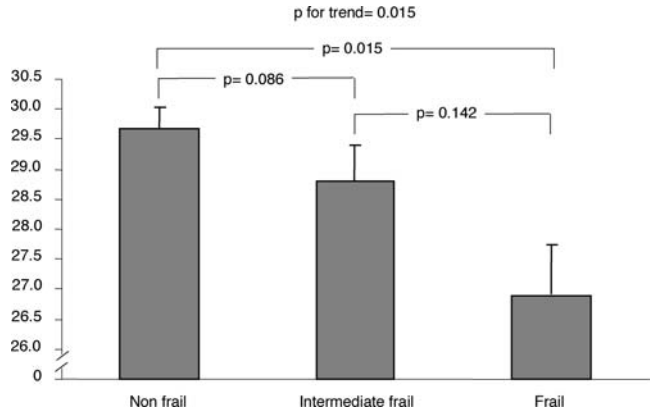

Figure 1. Age- and sex-adjusted levels of vitamin E according to frailty status. The unit of measure for vitamin E (y axis) is μmol/L.

Further analyses were aimed at understanding whether the association between VE status and frailty was mainly due to an association of VE with one or more specific frailty domains. After adjusting for multiple confounders, participants in the highest VE tertile tended to be less likely to be ''sedentary'' (OR: 0.50, 95% confidence interval [CI]: 0.22–1.12), ''slow'' (OR: 0.48, CI: 0.23–1.02), ''exhausted'' (OR: 0.70, CI: 0.34–1.40), or ''weak'' (OR: 0.91, CI: 0.50–1.71) compared with those in the lowest tertile. However, none of these associations was statistically significant. No noteworthy association was found between the domain of ''shrinking'' and VE (OR: 1.19, CI: 0.27–2.37).

Because in our sample log (VE) is significantly related with lower extremity muscle strength ($r = 0.09$, $p = .006$), we tested the association between VE status and frailty independent of the effect of VE on lower extremity muscle strength. This analysis was performed eliminating the domain of ''weakness'' from the operative definition of frailty and adjusting for lower extremity muscle strength. Results were virtually unchanged (Table 3).

Discussion

In a sample of nondisabled, nondemented older persons, we found a significant association between circulating levels of VE and frailty, independent of important confounders including dietary intake, inflammation, and body composition as well as functional parameters that are frequently impaired in frail older persons such as cognitive function and muscle strength (13,14). The association of VE with frailty as a syndrome was not attributable to the effect of VE status on specific features of the frailty syndrome considered singularly. To our knowledge, this is the first study showing a relationship between frailty and a component of the antioxidant system.

Recent theoretical views suggest that OS might be involved in the genesis of frailty (6). Frail older persons show a reduced ability to cope with a challenging environment. This phenomenon has been attributed to the dysregulation of the mechanisms maintaining the homeostatic equilibrium including the sympathetic–parasympathetic balance, the inflammatory response, and the hormonal network

Table 2. Polychotomous Logistic Regression Analysis Testing the Association Between Vitamin E (VE) Status and Frailty

	Nonfrail vs Frail (3–5 Characteristics)			Nonfrail vs Intermediate (1–2 Characteristics)		
VE Tertiles (μmol/L)	OR	95% CI	*p*	OR	95% CI	*p*
Model 1						
Lowest (<26.4)	1			1		
Middle (26.4–33.0)	0.60	0.29–01.21	.156	0.86	0.59–1.26	.461
Highest (>33.0)	0.41	0.20–0.84	.015	0.58	0.40–0.84	.004
Model 2						
Lowest (<26.4)	1			1		
Middle (26.4–33.0)	0.76	0.32–1.80	.544	0.96	0.63–1.47	.868
Highest (≥33.0)	0.32	0.11–0.97	.044	0.61	0.37–1.01	.055
Model 3						
Lowest (<26.4)	1			1		
Middle (26.4–33.0)	0.71	0.30–1.71	.454	0.95	0.62–1.46	.846
Highest (>33.0)	0.30	0.10–0.91	.035	0.60	0.36–1.00	.051

Notes: Model 1 was adjusted for demographics (age, gender, and site). Model 2 factors included those in Model 1 + dietary intake (vitamin E, vitamin C, and caloric intake), factors affecting absorption (number of drugs, gastrointestinal surgery), factors affecting bioavailability (plasma lipids, body mass index, waist circumference), factors affecting elimination (creatinine clearance, liver diseases), smoking and alcohol consumption, diseases and inflammation (angina, acute myocardial infarction, peripheral arterial disease, stroke/transient ischemic attacks, hypertension, diabetes, interleukin-6). Model 3 factors included those in model 2 + Mini-Mental State Examination score.

OR = odds ratio; CI = confidence interval.

Table 3. Polychotomous Logistic Regression Analysis Testing the Association Between Vitamin E (VE) Status and Frailty

	Nonfrail vs Frail (2–4 Characteristics)			Nonfrail vs Intermediate (1 Characteristic)		
VE Tertiles (μmol/L)	OR	95% CI	*p*	OR	95% CI	*p*
Model 1						
Lowest (<26.4)	1			1		
Middle (26.4–33.0)	0.54	0.31–0.93	.028	0.79	0.52–1.20	.285
Highest (>33.0)	0.45	0.26–0.78	.005	0.56	0.41–0.97	.039
Model 2						
Lowest (<26.4)	1			1		
Middle (26.4–33.0)	0.53	0.27–1.04	.067	0.89	0.55–1.44	.647
Highest (>33.0)	0.35	0.15–0.80	.013	0.61	0.34–1.08	.091
Model 3						
Lowest (<26.4)	1			1		
Middle (26.4–33.0)	0.53	0.26–1.08	.084	0.77	0.47–1.27	.319
Highest (>33.0)	0.38	0.15–0.91	.032	0.56	0.31–1.02	.061

Notes: The analysis was performed excluding the feature ''weakness'' from the definition of frailty. Model 1 was adjusted for demographics (age, gender, and site). Model 2 factors included those in model 1 + dietary intake (vitamin E, vitamin C, and caloric intake), factors affecting absorption (number of drugs, gastrointestinal surgery), factors affecting bioavailability (plasma lipids, body mass index, waist circumference), factors affecting elimination (creatinine clearance, liver diseases), smoking and alcohol consumption, diseases and inflammation (angina, acute myocardial infarction, peripheral arterial disease, stroke/transient ischemic attacks, hypertension, diabetes, interleukin-6). Model 3 factors included those in model 2 + Mini-Mental State Examination score and lower extremity muscle strength.

OR = odds ratio; CI = confidence interval.

(6). This dysregulation has been attributed to the excessive production of free radicals that are inadequately opposed by the antioxidant protective system (6). It is interesting that high VE plasma levels have been associated with efficient inflammatory, hormonal, and autonomic response capacity. In particular, VE supplementation partially restores the physiologic cardiac sympathetic–parasympathetic equilibrium that is unbalanced in older people affected by diabetes,

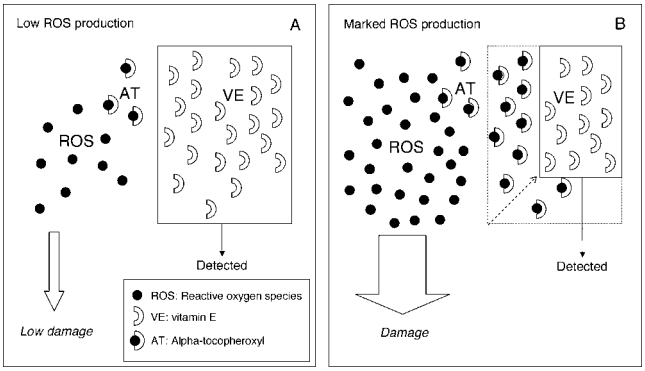

Figure 2. Hypothetical mechanism leading to low detectable levels of vitamin E (VE) in presence of marked level of reactive oxygen species (ROS) production. When the production of ROS is low (**A**), only a small part of the available α-tocopherol (VE) reacts with ROS and is transformed into α-tocopheroxyl radical, the postoxidation VE metabolite, which is not detected by current VE assay. When ROS production increases (**B**), a higher VE quantity is transformed into alpha-tocopheroxyl radical and only a small amount of VE is detected. Thus, under the assumption of constant intake, absorption, bioavailability, recycling, and elimination, levels of VE can be considered an indirect inverse marker of oxidative stress.

a condition characterized by increased OS (21); VE administration reduces serum C-reactive protein and IL-6 (22), two markers of inflammation that are often abnormally high in frail persons (23,24). Moreover, in animal models, VE administration protects against OS-induced damage of testicular function including testosterone production (25), and low VE levels are associated with higher cortisol levels during stress conditions (26). Both reduced testosterone levels (27) and cortisol (28) dysregulation have been conceptually associated with the frailty syndrome.

VE absorption and metabolism are complex and not completely understood processes. We hypothesized that, after accounting for factors affecting VE intake (vitamin E and total caloric intake), absorption (gastrointestinal surgery, number of medications), bioavailability (plasma lipids and body composition such as BMI and muscle strength), recycling and elimination (vitamin C intake, creatinine clearance, and liver diseases), VE circulating levels are inversely related to their utilization (consumption during oxidative processes). In this context, frail persons with low VE circulating levels might have a higher burden of OS (Figure 2). Our finding of an association between VE and frailty suggests that the accumulation of OS damage that naturally occurs with aging on macromolecules such as proteins, lipids, and mitochondrial DNA (7,29) might be accelerated in frail individuals and contribute to reduced efficiency of the cellular processes. However, this is only a speculation, and experimental studies specifically aimed at clarifying this issue are needed.

This study has important limitations. The first and main

limitation is related to the measurement of OS. Direct measures of reactive oxygen species or OS damage were not available in the InCHIANTI study; therefore, we evaluated OS indirectly using adjusted concentrations of the main component of the lipophilic antioxidant barriers. We cannot exclude, however, that VE may be associated with frailty through a still unknown mechanism that is different and independent from its antioxidant property. Second, we accounted for a number of important confounders, but we cannot exclude that residual confounding was still present either because some important factors were not considered or because the measures of confounders lacked sufficient precision. Third, we adjusted for vitamin C intake, but we had no information on current vitamin C plasma levels. Analogously, other important antioxidant pathways, such as the glutathione and ubiquinol pathways were not considered. Finally, we interpreted our results as suggesting that OS induces the onset of frailty; however, due to the cross-sectional nature of the study, a reverse causality mechanism cannot be excluded.

Our findings suggest a possible involvement of OS in the genesis of frailty. Future studies aimed at confirming this hypothesis require a longitudinal design and more global considerations of oxidation by-products and antioxidant systems.

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