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Association between the plasma proteome and plasma α-tocopherol concentrations in humans☆

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Abstract

Vitamin E is a lipophilic antioxidant that has been inversely associated with certain chronic diseases; however, the biological processes regulated by this vitamin have not been fully elucidated. The objective of the present study was to examine the association between the most biologically active and abundant form of vitamin E in the circulation, α-tocopherol, and the plasma proteome. Subjects were from the Toronto Nutrigenomics and Health Study and included men and women (n=1,022) who completed a general health and lifestyle questionnaire and 196-item food frequency questionnaire, and provided a fasting blood sample. Plasma α-tocopherol concentrations were measured by high-performance liquid chromatography and 54 plasma proteins were assayed by a mass spectrometry-based multiple reaction monitoring method. Analysis of covariance was used to compare mean concentrations of plasma proteins across tertiles of α-tocopherol. Plasma concentrations of apolipoprotein C-III, fibrinogen alpha, beta, and gamma chains, fibronectin and fibrinopeptide A were significantly and positively associated with plasma α-tocopherol, while intermediate levels of α-tocopherol were significantly associated with higher levels of alpha-1B-glycoprotein (all P<.0009). These findings show that circulating levels of α-tocopherol are significantly associated with specific plasma proteins and suggest novel physiological effects of vitamin E.

Keywords: Plasma proteins; Alpha-tocopherol; Vitamin E; Biomarkers; Proteomics

1. Introduction

Recent developments in plasma proteomics has opened its use to absolute measurements of concentrations of numerous proteins in large samples, which may allow for identification of novel biomarkers, mechanisms of pathogenesis and targets for disease intervention [1–3]. Oxidative stress and inflammation have been implicated in a number of chronic diseases and dietary antioxidants may play an important role in reducing disease risk or progression in the general population [4].

Alpha-tocopherol is a dietary antioxidant and one of the eight compounds of the vitamin E family. While γ-tocopherol is the most abundant form of vitamin E in the diet, α-tocopherol is found in the highest concentration in the circulation due to selective incorporation into very low density lipoproteins (VLDL) in the liver by the α-tocopherol transfer protein [5]. Vitamin E is a chain-breaking antioxidant important in the protection of lipid membranes and plasma lipoproteins [6]. Anti-inflammatory properties of vitamin E have been attributed to its ability to inhibit 5-lipoxygenase and subsequent synthesis of inflammatory prostaglandins [7], and reduce the production of inflammatory cytokines [8]. Observational studies have shown that circulating levels of α-tocopherol are inversely associated with chronic diseases such as cardiovascular disease (CVD) [9] and diabetes [10]. However, many vitamin E supplementation trials have shown no benefit, with some meta-analyses reporting potential adverse effects of vitamin E supplementation [11]. Several limitations of previous studies have been proposed to explain these discrepancies between observational and clinical studies. Improved understanding of the role of vitamin E in physiological processes may help clarify the relationship between vitamin E and chronic disease. The objective of the present study was to assess the relationship between plasma α-tocopherol and a proteomic panel of plasma proteins in an ethnically diverse population of young adults. Investigation of plasma proteins that become dysregulated in disease states could potentially help identify the role of vitamin E in disease development and progression and help explain the contradictory findings in the studies noted above.

2. Methods and materials

2.1. Study design

The Toronto Nutrigenomics and Health Study is an ethnoculturally diverse population of men (n=520) and women (n=1,117) ages 20–29 years, recruited

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from the University of Toronto campus. All subjects completed a general health and lifestyle questionnaire, which collected information on subject characteristics including age, sex, medical history, smoking status and ethnocultural group. Subjects were classified into four ethnocultural groups based on their responses: Caucasian, East Asian, South Asian, and Other (all other ethnocultural groups and those reporting more than one ethnocultural groups). Information on physical activity was collected by questionnaire and included the total hours spent in light, moderate, and vigorous physical activity on a typical weekday and weekend day over the past month. Metabolic equivalent task hours per week were then calculated to express modifiable activity. One metabolic equivalent task (MET) hour is equal to 1 kcal expended per kg body weight per hour sitting at rest [12]. Information on habitual dietary intake over the past month was collected using a semi-quantitative food frequency questionnaire that was modified from the Willett questionnaire and included questions on supplement use [13]. Heart rate, systolic and diastolic blood pressure were measured at rest, twice one minute apart, using the OMRON IntelliSense Blood Pressure Monitor (Model HEM-907XL, OMRON Healthcare, Vernon Hills, IL, USA). The means of all measurements taken in duplicate or triplicate were recorded. The study was approved by the Research Ethics Board at the University of Toronto. All subjects provided written, informed consent.

2.2. Biochemical measurements

Subjects provided a blood sample after an overnight fast. Blood samples were collected and analyzed for biomarkers of cardiometabolic disease at LifeLabs Laboratories (Toronto, ON, Canada). Serum ascorbic acid was measured using high-performance liquid chromatography (HPLC) as described in detail elsewhere [13]. The homeostasis model of insulin resistance (HOMA-IR) and beta-cell function (HOMA-β) was calculated from measures of insulin (μU/mL) and glucose (mmol/L). HOMA-IR was calculated using the formula insulin × glucose/22.5 and HOMA-β was calculated using the formula 20×insulin/(glucose−3.5).

Aliquots of plasma from sodium heparin- and EDTA-treated blood samples were shipped from LifeLabs to the University of Toronto (Toronto, ON, Canada) and stored at −80°C. A reversed-phase isocratic HPLC method with fluorescence detection was used to measure plasma concentrations of α-tocopherol and is described in detail elsewhere [14]. Proteomic analysis was conducted at the Genome British Columbia Proteomics Centre at the University of Victoria (Victoria, BC, Canada). Frozen plasma samples were shipped for mass spectrometry-based multiple reaction monitoring (MRM) proteomics analysis to measure 57 common plasma proteins linked to chronic disease. Details of this method are described in detail elsewhere [1]. Of the 57 proteins measured, 3 were excluded from subsequent analyses because they had a coefficient of variability greater than 20% as previously described [15].

2.3. Statistical Analyses

Statistical analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina). A total of 1637 subjects completed the study, with 1126 samples being available for proteomic analysis at the time it was conducted. Subjects were excluded if they reported being a current smoker (n=2) due to the potentially confounding effects

Table 1
Subject characteristics by tertiles of plasma α-tocopherol

	Total population	Plasma α-tocopherol			P value *
		Tertile 1	Tertile 2	Tertile 3	
	n=1,022	n=340	n=341	n=341	
Age, years	22.7±2.5	22.4±2.4[a]	22.7±2.3[ab]	22.9±2.6[b]	.04
Sex					
Male	302 (30)	104 (31)	98 (29)	100 (29)	.9
Female	720 (70)	236 (69)	243 (71)	241 (71)	
Ethnocultural group					
Caucasian	483 (47)	141 (41)	170 (50)	172 (50)	.1
East Asian	359 (35)	136 (40)	106 (31)	117 (34)	
South Asian	105 (10)	37 (11)	35 (10)	33 (10)	
Other	75 (7)	26 (8)	30 (9)	19 (6)	
Physical activity, MET-hours/week	7.7±3.1	7.3±3.0	7.8±3.1	7.9±3.2	.06
Body mass index, kg/m^2	22.7±3.5	22.5±3.4	23.1±3.8	22.7±3.3	.1
Waist circumference, cm	73.8±8.9	73.1±9.0	74.3±8.8	74.0±9.0	.2
Systolic blood pressure, mm Hg	113.7±11.3	113.4±11.0	113.7±11.0	114.0±11.9	.8
Diastolic blood pressure, mm Hg	69.0±8.0	69.0±7.8	68.3±7.7	69.5±8.5	.2
Oral contraceptive use (among women)					
No	497 (69)	178 (75)	172 (71)	147 (61)	.002
Yes	223 (31)	58 (25)	71 (29)	94 (39)	
Season of blood draw					
Spring	277 (27)	115 (34)	89 (26)	73 (21)	<.0001
Summer	280 (27)	71 (21)	85 (25)	124 (36)	
Autumn	273 (27)	104 (31)	95 (28)	74 (22)	
Winter	192 (19)	50 (15)	72 (21)	70 (21)	
HOMA-IR	1.4±0.9	1.4±1.0	1.5±0.9	1.4±0.8	.07
HOMA-β	109.7±72.2	106.7±73.4	114.2±75.1	108.3±68.0	.2
Total serum cholesterol, mmol/L	4.2±0.8	4.0±0.7[a]	4.2±0.6[b]	4.6±0.8[c]	<.0001
Serum HDL cholesterol, mmol/L	1.6±0.4	1.5±0.4[a]	1.6±0.4[ab]	1.6±0.4[b]	.0001
Serum LDL cholesterol, mmol/L	2.2±0.6	2.1±0.6[a]	2.2±0.6[a]	2.4±0.7[b]	<.0001
Triglycerides, mmol/L	1.0±0.5	0.8±0.3[a]	0.9±0.4[b]	1.1±0.7[c]	<.0001
Serum ascorbic acid, μmol/L	30.4±17.2	29.5±18.0[a]	28.3±16.3[a]	33.4±17.0[b]	<.0001
Plasma α-tocopherol, μmol/L	30.0±11.8	18.7±4.3[a]	28.3±2.4[b]	42.8±9.8[c]	<.0001
Energy, calories/day	1952±631	1946±609	1923±638	1987±646	.4
Alcohol, g/day	5.0±8.4	4.2±6.5	5.3±8.6	5.6±9.8	.9
Fibre intake, g/day	23.5±11.8	22.8±11.0	23.2±11.7	24.4±12.5	.3
Dietary α-tocopherol, mg/day					
Including supplements	12.2±11.5	11.0±11.2[a]	11.3±8.1[a]	14.3±14.1[b]	<.0001
Excluding supplements	8.4±4.4	8.1±4.0[a]	8.2±4.3[a]	9.0±4.8[b]	.01
Dietary vitamin E adequacy §					
Less than RDA	169 (17)	276 (81)	262 (77)	227 (67)	<.0001
Meets RDA	853 (83)	64 (19)	79 (23)	114 (33)	
Multivitamin or vitamin E supplement use					
No	648 (63)	265 (78)	234 (69)	208 (61)	<.0001
Yes	374 (37)	75 (22)	107 (31)	133 (39)	

All values are mean±standard deviation or n (%).
Means with different superscript letters are significantly different from one another (P<.05) after Tukey-Kramer adjustment.
* P value calculated from χ^2 analysis for categorical variables and ANOVA for normalized continuous variables.
§ The current RDA for vitamin E is 15 mg/day α-tocopherol for males and females ≥14 years of age.

Table 2
Mean plasma protein concentration by tertiles of plasma α-tocopherol

Plasma proteins, μmol/L	Plasma α-tocopherol			*P* value
	Tertile 1	Tertile 2	Tertile 3	
Apolipoprotein C-III	2.12±0.04[a]	2.37±0.04[b]	2.69±0.05[b]	**<.0001**
Fibrinogen alpha chain	11.05±0.32[a]	12.47±0.33[b]	12.79±0.36[b]	**<.0001**
Fibrinogen beta chain	8.78±0.21[a]	9.92±0.22[b]	10.14±0.24[b]	**<.0001**
Fibrinogen gamma chain	8.75±0.23[a]	9.93±0.24[b]	10.04±0.26[b]	**<.0001**
Fibronectin	0.51±0.06[a]	0.64±0.05[b]	0.73±0.07[c]	**<.0001**
Fibrinopeptide A	6.58±0.16[a]	7.33±0.17[b]	7.42±0.16[b]	**<.0001**
Alpha-1B-glycoprotein	1.57±0.03[a]	1.75±0.03[b]	1.66±0.03[a]	**.0002**
Complement C3	18.88±0.23	20.46±0.29	19.62±0.26	.001
Alpha-1-anti-trypsin	10.58±0.14	11.47±0.18	11.22±0.16	.002
Alpha-2-HS-glycoprotein	8.67±0.11	9.07±0.13	8.65±0.10	.002
Gelsolin, isoform 1	1.19±0.02	1.26±0.02	1.18±0.02	.002
Transferrin	12.07±0.15	12.96±0.20	12.53±0.15	.004
Complement C9	2.63±0.05	2.84±0.05	2.63±0.04	.005
Plasminogen	1.18±0.01	1.26±0.02	1.26±0.02	.01
Alpha-2-antiplasmin	1.87±0.02	1.97±0.03	1.90±0.02	.02
Inter-alpha-trypsin inhibitor HC	0.62±0.01	0.63±0.01	0.61±0.01	.02
Retinol-binding protein	0.86±0.01	0.93±0.02	0.98±0.02	.02
Complement factor H	0.57±0.01	0.62±0.01	0.61±0.01	.03
Clusterin	1.47±0.02	1.55±0.02	1.54±0.02	.03
Hemopexin	9.90±0.11	10.38±0.13	10.07±0.12	.03
Zinc-alpha-2-glycoprotein	0.99±0.02	1.07±0.02	1.07±0.02	.04
Complement factor B	1.41±0.02	1.50±0.02	1.44±0.02	.04
Heparin cofactor II	0.68±0.01	0.71±0.01	0.70±0.01	.04
Alpha-2-macroglobulin	5.83±0.09	6.01±0.10	5.75±0.09	.07
Albumin	947.92±7.91	970.46±9.03	950.05±7.89	.07
Antithrombin-III	3.53±0.03	3.60±0.04	3.53±0.03	.07
Complement C4 gamma chain	1.58±0.03	1.61±0.03	1.55±0.03	.08
Alpha-1-antichymotrypsin	3.28±0.04	3.46±0.05	3.36±0.04	.09
Apolipoprotein A-I	41.92±0.48	44.17±0.60	44.95±0.56	.1
Afamin	0.25±0.003	0.26±0.004	0.26±0.004	.2
Alpha-1-acid glycoprotein 1	1.68±0.03	1.82±0.04	1.77±0.04	.2
Apolipoprotein A-II precursor	24.02±0.28	25.42±0.34	26.04±0.32	.2
Apolipoprotein A-IV	1.39±0.02	1.46±0.02	1.42±0.03	.2
Apolipoprotein B-100	0.73±0.01	0.80±0.01	0.87±0.01	.2
Apolipoprotein C-I	3.02±0.04	3.24±0.05	3.39±0.05	.2
Complement C1 inactivator	4.62±0.06	4.73±0.07	4.67±0.07	.2
Ceruloplasmin	2.21±0.05	2.41±0.06	2.38±0.06	.2
Vitamin D binding protein	2.73±0.04	2.89±0.04	2.90±0.04	.2
Complement C4 beta chain	1.41±0.03	1.48±0.03	1.44±0.03	.3
Haptoglobin beta chain	10.40±0.29	11.14±0.32	10.38±0.30	.3
Transthyretin	5.58±0.07	5.77±0.07	5.79±0.07	.3
Vitronectin	3.59±0.04	3.78±0.06	3.81±0.05	.3
Apolipoprotein D	0.34±0.005	0.35±0.005	0.34±0.005	.4
L-Selectin	0.07±0.001	0.07±0.001	0.07±0.001	.4
Prothrombin	0.56±0.01	0.58±0.01	0.59±0.01	.4
Apolipoprotein L1	0.40±0.01	0.43±0.01	0.42±0.01	.5
Beta-2-glycoprotein I	2.72±0.03	2.81±0.04	2.80±0.04	.5
Serum amyloid P-component	0.43±0.01	0.45±0.01	0.46±0.01	.5
Apolipoprotein E	0.49±0.01	0.49±0.01	0.52±0.01	.6
Kininogen-1	2.07±0.03	2.18±0.03	2.24±0.03	.6
Coagulation factor XIIa HC	0.25±0.01	0.27±0.01	0.28±0.01	.7
Histidine-rich glycoprotein	1.33±0.02	1.32±0.02	1.28±0.02	.8
Adiponectin	0.06±0.001	0.07±0.002	0.07±0.002	.9
Angiotensinogen	0.90±0.04	0.98±0.04	1.05±0.04	.9

Data presented are crude plasma protein means±standard errors.
HC, heavy chain; HS, Heremans–Schmid.
†*P*-value from ANCOVA with log or square-root transformed plasma protein concentrations where necessary, adjusted for sex, ethnocultural group, season of blood draw, oral contraceptive use among women, waist circumference, total serum cholesterol and serum ascorbic acid. *P* values that are bolded and italicized meet the Bonferroni significance level of .0009 for 54 comparisons.
Means with different superscript letters are significantly different from one another (*P*<.05) after Tukey-Kramer adjustment.

on circulating α-tocopherol concentrations [16]. Subjects who may have over- (>3,500 kcal/day for women, >4,000 kcal/day for men) or under-reported (<800 kcal/day) their energy intake were excluded from the analysis (n=7). Subjects with incomplete data for plasma α-tocopherol (n=37), serum ascorbic acid (n=50), or any protein from the proteomics panel (n=8) were also excluded from the analysis. A total of 1022 subjects were available for analysis after the above exclusions.

Continuous variables were log or square-root transformed to improve normality where necessary. Plasma α-tocopherol was categorized into tertiles. chi-Square analysis and analysis of variance (ANOVA) was used to examine subject characteristics by tertiles of plasma α-tocopherol for categorical and continuous variables, respectively. All *P* values less than .05 were considered significant. Means compared between tertiles were adjusted for multiple comparisons using the Tukey–Kramer procedure.

Analysis of covariance (ANCOVA) was used to compare mean plasma protein concentrations by tertiles of plasma α-tocopherol. Several covariates were considered; however, only those that were significant in most models or materially altered the results were retained. The final model included adjustments for sex, ethnocultural group, season of blood draw, oral contraceptive use among women, waist circumference, serum ascorbic acid, and total cholesterol. Significance was set at .0009, applying a Bonferroni adjustment to take into account the 54 tests performed. Subsequent comparisons of means between tertiles were adjusted for multiple comparisons using the Tukey-Kramer procedure.

3. Results

Plasma α-tocopherol values were categorized into tertiles with cut points of <24.234 μmol/L for tertile 1, 24.234–32.250 μmol/L for tertile 2, and >32.250 μmol/L for tertile 3. Mean α-tocopherol concentrations were 18.7, 28.3 and 42.8 μmol/L for tertiles 1, 2 and 3, respectively (*P*<.0001, Table 1). Season of blood draw, dietary vitamin E adequacy [meeting recommended dietary allowance (RDA)], multivitamin and vitamin E supplement use, and oral contraceptive use among women were significantly associated with plasma α-tocopherol. Mean age and serum HDL cholesterol were significantly higher in tertile 3 compared to tertile 1, while mean serum LDL cholesterol, and serum ascorbic acid were significantly higher in tertile 3 compared to tertile 1 and 2. Total serum cholesterol and triglycerides were significantly different among all tertiles of α-tocopherol, with the highest concentrations in tertile 3 and the lowest in tertile 1. Mean dietary vitamin E intakes (with and without supplements) were significantly higher in tertile 3 compared to tertiles 1 and 2.

Significant differences (*P*<.0009) in mean protein concentrations among tertiles of α-tocopherol were identified for 7 of the 54 proteins examined in models adjusted for sex, ethnocultural group, season of blood draw, oral contraceptive use among women, waist circumference, serum ascorbic acid, and total cholesterol (Table 2). These findings remained statistically significant (*P*<.0009) in models additionally adjusted for dietary vitamin E adequacy, vitamin E intake (with and without supplements), supplement use (multivitamin and vitamin E), and age (data not shown). Mean protein concentrations in the lowest tertile of α-tocopherol were significantly lower than tertiles 2 and 3 for apolipoprotein C-III, fibrinogen alpha, beta, and gamma chains, and fibrinopeptide A after Tukey-Kramer adjustment for multiple comparisons. Mean plasma concentration of fibronectin was significantly different among all the tertiles of α-tocopherol and was highest in tertile 3 and lowest in tertile 1. Mean alpha-1B-glycoprotein concentration for α-tocopherol tertile 2 was significantly higher than tertile 1 and tertile 3.

4. Discussion

Vitamin E is an essential nutrient with important antioxidant and anti-inflammatory properties and plays a role in cell signalling and gene expression [8]. This vitamin may have roles in additional biological pathways not yet identified. In the present investigation, we compared circulating levels of α-tocopherol to a number of plasma proteins in order to identify biological pathways that might be regulated by this vitamin. Using a mass-spectrometry based MRM proteomics assay, the concentrations of 54 plasma proteins involved in various physiological processes were measured simultaneously. Plasma levels of α-tocopherol were significantly associated with seven of the plasma proteins, five of which are involved in coagulation

and hemostasis including fibrinogen alpha, beta, and gamma chains, fibronectin and fibrinopeptide A.

Fibrinogen is a positive acute phase protein that plays a critical role in coagulation and hemostasis. The protein is made up of three pairs of polypeptide chains (Aα, Bβ and γ) linked by disulfide bridges [17]. Thrombin cleaves the Aα and Bβ chains producing fibrinopeptide A and B, respectively. The remaining monomer polymerizes to form fibrin, leading to clot formation. During this process, the adhesive glycoprotein fibronectin is covalently bound to fibrin by the action of factor XIIIa [18]. In addition to being a precursor to fibrin, fibrinogen also plays an important role in coagulation by linking activated platelets in the process of platelet aggregation, and it also contributes to blood viscosity [19,20].

A meta-analysis of long-term prospective studies published before 1998 found subjects in the top tertile of fibrinogen levels to be at significantly increased risk of coronary heart disease (CHD) compared to those in the bottom third [21]. A second meta-analysis of prospective, cross-sectional and case–control studies confirmed a higher risk of cardiovascular events for subjects in the highest compared to the lowest tertile of fibrinogen [22]. However, it is not clear from these studies whether fibrinogen, as a critical component of thrombus formation, is contributing to CVD risk, or if, as an acute phase protein, it is reflecting inflammation associated with CVD risk [20,23].

In the present study, mean circulating levels of the fibrinogens were significantly lower in the lowest tertile of α-tocopherol compared to tertiles 2 and 3. This was unexpected because of the inhibitory effect on platelet aggregation, protective effect on CVD, and the anti-inflammatory properties ascribed to α-tocopherol [8,24], and the role of fibrinogen in coagulation and inflammation. In one study, supplementation with increasing doses of α-tocopherol over 9 weeks among 12 subjects with CHD and 12 controls resulted in significantly lower levels of plasma fibrinogen compared to baseline levels at supplementation [25]. However, the reduction in fibrinogens was only noted among patients and not controls [25]. In contrast, no association between dietary vitamin E intake and fibrinogen levels was found in over 5,000 subjects from the Multi-Ethnic Study of Atherosclerosis [26]. In addition to fibrinogen alpha, beta and gamma chains, other markers of the coagulation pathway, including fibronectin and fibrinopeptide A, showed associations in a similar direction with tertiles of α-tocopherol in this study.

Levels of fibronectin were significantly lower in α-tocopherol tertile 1 compared to tertiles 2 and 3, and significantly lower in tertile 2 compared to 3. In addition to coagulation, fibronectin can form a complex with macrophages which leads to increased release of inflammatory cytokines such as interleukin-1 [27]. Although less consistent than the association observed with fibrinogen, some studies also suggest a positive association between fibronectin levels and CVD [28]. Fibronectin also plays an important role in the extracellular matrix. Interestingly, α-tocopherol has been shown to increase expression of connective tissue growth factor in vascular smooth muscle cell lines [29], which may stimulate the production of extracellular matrix components including fibronectin [30]. It remains unknown, however, what impact circulating α-tocopherol has on plasma levels of fibronectin in vivo.

Fibrinopeptide A levels were also significantly lower in the first tertile of α-tocopherol compared to tertiles 2 and 3. Although fibrinopeptide A does not directly participate in clot formation, it is used as a marker of thrombin-mediated conversion of fibrinogen to fibrin. One study reported a positive association between levels of fibrinopeptide A and the carbonyl content of plasma proteins, a marker of protein oxidation [31]. However, subsequent selection of a subset of the study population to receive 600 mg α-tocopherol-acetate daily for 18 days did not alter protein carbonyl content, fibrinopeptide A, or fibrinogens, even though α-tocopherol levels were significantly increased [31]. Alpha-tocopherol has also been shown to attenuate thrombin generation by oxidized LDL [32]; therefore, it is unclear why fibrinopeptide A levels were highest among the higher tertiles of α-tocopherol.

Apolipoprotein C-III (apo C-III) is a marker of triglyceride metabolism found in circulating triglyceride-rich particles such as chylomicrons and VLDL, as well as HDL. Apo C-III inhibits lipoprotein lipase hydrolysis of triglyceride-rich particles [33,34], preventing their uptake and contributing to the development of hypertriglyceridemia [35]. Apo C-III has been linked to the development of atherosclerosis through stimulation of monocyte adhesion to endothelial cells and endothelial dysfunction [36]. Several studies have linked higher levels of apo C-III to CVD risk [37]. In one study, higher apo C-III levels were also associated with an increased propensity for thrombin generation in coronary artery disease patients [38]. In the present study, circulating levels of apo C-III were significantly lower in the lowest tertile of α-tocopherol compared to tertiles 2 and 3. As both apo C-III and α-tocopherol circulate in the plasma associated with lipoproteins, the observed association was not surprising; however, it remained significant after adjustment for total serum cholesterol. In support of our findings, a previous study has identified a single nucleotide polymorphism (SNP) in the *APOC3* gene to be associated with levels of α-tocopherol in a group of Caucasian women [39]. A subsequent genome wide association study and meta-analysis also identified a SNP in a region near *APOC3*, *A1*, *A4*, and *A5* to be associated with circulating levels of α-tocopherol [40]. Adjustments were also made for total cholesterol in each of these studies [39,40].

In the present study, circulating levels of alpha-1B-glycoprotein were significantly higher in tertile 2 compared to tertiles 1 and 3. The function of alpha-1B-glycoprotein is unclear, although the protein is believed to be a member of the immunoglobulin family [41]. Several proteomic analyses have associated this protein with cancer, including pancreatic [42], bladder [43], liver [44], endometrial and cervical cancer [45]. Lower levels of this protein have also been found in the cerebral spinal fluid of patients with Alzheimer's disease [46]. Although the specific role of this protein is unknown, our results suggest α-tocopherol levels are associated with levels of alpha-1B-glycoprotein.

In the present study, a positive association was noted between α-tocopherol and several markers of coagulation as well as apo C-III, most of which have been positively associated with CVD. This is in contrast to the inverse association between circulating levels α-tocopherol and CVD noted in observational studies, although not all studies have found such an association [9]. Furthermore, α-tocopherol has been shown to inhibit platelet aggregation, but this has been attributed to inhibition of protein kinase C [47] with additional anticoagulant effects that may involve interactions with vitamin K [48]. No measures of blood coagulation or platelet aggregation were taken in the present study, and therefore, it is unclear whether the higher levels of coagulation factors in the upper tertiles of vitamin E have any functional consequences. In addition, the cross-sectional nature of this study prevents us from drawing any conclusions of a causal effect of vitamin E on the concentrations of these proteins. We cannot rule out the possibility of residual confounding, despite testing a wide range of potential covariates. Circulating levels of α-tocopherol and plasma proteins may be confounded by other factors not adjusted for here. The population used consists of generally healthy adults between the ages of 20–29 years. The benefit of using such a population is that it reduces the likelihood of confounding by prevalent chronic disease. However, we cannot rule out the possibility of additional confounding by differences in subject characteristics not measured and accounted for here. In addition, these associations may not exist in other age groups and the findings should thus be replicated in additional populations. Furthermore, only single measurements of α-tocopherol and plasma proteins were taken at

one time point and may not be truly reflective of long term status or disease risk. Additional research, including prospective and experimental studies, will be required to validate these findings and identify potential mechanisms.

This is the first study, to our knowledge, to examine the association between plasma α-tocopherol and a large proteomic panel of plasma proteins involved in several physiological pathways. Plasma levels of α-tocopherol were significantly associated with several of these proteins suggesting potential roles for vitamin E in these pathways. These findings contribute to our knowledge of vitamin E in the regulation of physiological processes and generate hypotheses to be tested in future experiments.

References

[1] Kuzyk MA, Smith D, Yang J, et al. Multiple reaction monitoring-based, multiplexed, absolute quantitation of 45 proteins in human plasma. Mol Cell Proteomics 2009;8:1860-77.
[2] Didangelos A, Simper D, Monaco C, Mayr M. Proteomics of acute coronary syndromes. Curr Atheroscler Rep 2009;11:188-95.
[3] Mayr M, Mayr U, Chung YL, Yin X, Griffiths JR, Xu Q. Vascular proteomics: linking proteomic and metabolomic changes. Proteomics 2004;4:3751-61.
[4] García-Bailo B, El-Sohemy A, Haddad P, et al. Vitamins D, C, and E in the prevention of type 2 diabetes mellitus: Modulation of inflammation and oxidative stress. Biologics 2011;5:1–13.
[5] Traber MG, Arai H. Molecular mechanisms of vitamin E transport. Annu Rev Nutr 1999;19:343-55.
[6] Thomas SR, Stocker R. Molecular action of vitamin E in lipoprotein oxidation: Implications for atherosclerosis. Free Radic Biol Med 2000;28:1795-805.
[7] Devaraj S, Jialal I. α-Tocopherol decreases interleukin-1β release from activated human monocytes by inhibition of 5-lipoxygenase. Arterioscler Thromb Vasc Biol 1999;19:1125-33.
[8] Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress, and inflammation. Annu Rev Nutr 2005;25:151-74.
[9] Gaziano JM, Vitamin E. and cardiovascular disease: observational studies. Ann N Y Acad Sci 2004;1031:280-91.
[10] Mayer-Davis EJ, Costacou T, King I, Zaccaro DJ, Bell RA. Plasma and dietary vitamin E in relation to incidence of type 2 diabetes: The Insulin Resistance and Atherosclerosis Study (IRAS). Diabetes Care 2002;25:2172-7.
[11] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: Systematic review and meta-analysis. J Am Med Assoc 2007;297: 842-57.
[12] Ainsworth BE, Haskell WL, Leon AS, et al. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc 1993;25:71-80.
[13] Cahill L, Corey PN, El-Sohemy A. Vitamin C deficiency in a population of young canadian adults. Am J Epidemiol 2009;170:464-71.
[14] Liu Z, Lee H, Garofalo F, Jenkins D, El-Sohemy A. Simultaneous measurement of three tocopherols, all-*trans*-retinol, and eight carotenoids in human plasma by isocratic liquid chromatography. J Chromatog Sci 2011;49:221-7.
[15] García-Bailo B, Brenner D, Nielsen D, et al. Dietary patterns and ethnicity are associated with distinct plasma proteomic groups. Am J Clin Nutr 2012;95: 352-61.
[16] Bruno RS, Ramakrishnan R, Montine TJ, Bray TM, Traber MG. Alpha-tocopherol disappearance is faster in cigarette smokers and is inversely related to their ascorbic acid status. Am J Clin Nutr 2005;81:95–103.
[17] Fuss C, Palmaz JC, Sprague EA. Fibrinogen: structure, function, and surface interactions. J Vasc Interv Radiol 2001;12:677-82.
[18] Corbett SA, Schwarzbauer JE. Fibronectin-fibrin cross-linking: a regulator of cell behavior. Trends Cardiovasc Med 1998;8:357-62.
[19] Lowe GDO, Rumley A, Mackie IJ. Plasma fibrinogen. Ann Clin Biochem 2004;41: 430-40.
[20] Folsom AR. Hemostatic risk factors for atherothrombotic disease: an epidemiologic view. Thromb Haemost 2001;86:366-73.
[21] Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. J Am Med Assoc 1998;279:1477-82.
[22] Maresca G, Di Blasio A, Marchioli R, Di Minno G. Measuring plasma fibrinogen to predict stroke and myocardial infarction: An update. Arterioscler Thromb Vasc Biol 1999;19:1368-77.
[23] Packard CJ, O'Reilly DSJ, Caslake MJ, et al. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. N Eng J Med 2000;343: 1148-55.
[24] Keaney Jr JF, Simon DI, Freedman JE. Vitamin E and vascular homeostasis: Implications for atherosclerosis. FASEB J 1999;13:965-75.
[25] Leichtle A, Teupser D, Thiery J. Alpha-tocopherol distribution in lipoproteins and anti-inflammatory effects differ between CHD-patients and healthy subjects. J Am Coll Nutr 2006;25:420-8.
[26] de Oliveira Otto MCC, Alonso A, Lee DH, et al. Dietary micronutrient intakes are associated with markers of inflammation but not with markers of subclinical atherosclerosis. J Nutr 2011;141:1508-15.
[27] Schmidt DR, Kao WJ. The interrelated role of fibronectin and interleukin-1 in biomaterial-modulated macrophage function. Biomaterials 2007;28:371-82.
[28] Maurer LM, Tomasini-Johansson BR, Mosher DF. Emerging roles of fibronectin in thrombosis. Thromb Res 2010;125:287-91.
[29] Villacorta L, Graça-Souza AV, Ricciarelli R, Zingg JM, Azzi A. α-tocopherol induces expression of connective tissue growth factor and antagonizes tumor necrosis factor-α-mediated downregulation in human smooth muscle cells. Circ Res 2003;92:104-10.
[30] Frazier K, Williams S, Kothapalli D, Klapper H, Grotendorst GR. Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor. J Invest Dermatol 1996;107:404-11.
[31] De Cristofaro R, Rocca B, Marchioli R, Landolfi R. Plasma protein oxidation is associated with an increase of procoagulant markers causing an imbalance between pro- and anticoagulant pathways in healthy subjects. Thromb Haemost 2002;87:58-67.
[32] Rota S, McWilliam NA, Baglin TP, Byrne CD. Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: modulation by oxidation and vitamin E. Blood 1998;91:508-15.
[33] Ginsberg HN, Ngoc-Anh L, Goldberg IJ. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. J Clin Invest 1986;78:1287-95.
[34] Wang CS, McConathy W, Kloer HU, Alaupovic P. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. J Clin Invest 1985;75: 384-90.
[35] Shachter NS. Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. Curr Opin Lipidol 2001;12:297-304.
[36] Kawakami A, Yoshida M. Apolipoprotein CIII links dyslipidemia with atherosclerosis. J Atheroscler Thromb 2009;16:6–11.
[37] Chan DC, Chen MM, Ooi EMM, Watts GF. An ABC of apolipoprotein C-III: a clinically useful new cardiovascular risk factor? Int J Clin Pract 2008;62:799-809.
[38] Olivieri O, Martinelli N, Girelli D, et al. Apolipoprotein C-III predicts cardiovascular mortality in severe coronary artery disease and is associated with an enhanced plasma thrombin generation. J Thromb Haemost 2010;8:463-71.
[39] Borel P, Moussa M, Reboul E, et al. Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, cholesteryl ester transfer protein, hepatic lipase, intestinal fatty acid binding protein and microsomal triacylglycerol transfer protein. Br J Nutr 2009;101: 680-7.
[40] Major JM, Yu K, Wheeler W, et al. Genome-wide association study identifies common variants associated with circulating vitamin E levels. Hum Mol Genet 2011;20:3876-83.
[41] Ishioka N, Takahashi N, Putnam FW. Amino acid sequence of human plasma α1B-glycoprotein: homology to the immunoglobulin supergene family. Proc Natl Acad Sci U S A 1986;83:2363-7.
[42] Tian M, Cui YZ, Song GH, et al. Proteomic analysis identifies MMP-9, DJ-1 and A1BG as overexpressed proteins in pancreatic juice from pancreatic ductal adenocarcinoma patients. BMC Cancer 2008;8:241.
[43] Kreunin P, Zhao J, Rosser C, Urquidi V, Lubman DM, Goodison S. Bladder cancer associated glycoprotein signatures revealed by urinary proteomic profiling. J Proteome Res 2007;6:2631-9.
[44] Yoon SY, Kim JM, Oh JH, et al. Gene expression profiling of human HBV- and/or HCV-associated hepatocellular carcinoma cells using expressed sequence tags. Int J Oncol 2006;29:315-27.
[45] Abdul-Rahman PS, Lim BK, Hashim OH. Expression of high-abundance proteins in sera patients with endometrial and cervical cancers: analysis using 2-DE with silver staining and lectin detection methods. Electrophoresis 2007;28:1989-96.
[46] Puchades M, Hansson SF, Nilsson CL, Andreasen N, Blennow K, Davidsson P. Proteomic studies of potential cerebrospinal fluid protein markers for Alzheimer's disease. Mol Brain Res 2003;118:140-6.
[47] Freedman JE, Farhat JH, Loscalzo J, Keaney Jr JF. α-tocopherol inhibits aggregation of human platelets by a protein kinase C-dependent mechanism. Circulation 1996;94:2434-40.
[48] Traber MG, Frei B, Beckman JS. Vitamin E revisited: do new data validate benefits for chronic disease prevention? Curr Opin Lipidol 2008;19:30-8.