

Determinants of Plasma Vitamin E in Healthy Males¹

R. Sinha,² B. H. Patterson, A. R. Mangels,
O. A. Levander, T. Gibson, P. R. Taylor, and G. Block

Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Maryland 20892 [R. S., B. H. P., P. R. T.]; Human Nutrition Research Center, United States Department of Agriculture, Beltsville, Maryland 20705 [A. R. M., O. A. L.]; Information Management Services, Silver Spring, Maryland 20906 [T. G.]; and Department of Public Health, University of California, Berkeley, California 94720 [G. B.]

Abstract

Vitamin E or tocopherol, a known antioxidant, may play a role in the etiology of chronic diseases such as cancer and heart disease. This study examined both "internal" (lipids, lipoproteins, and apoproteins) and "external" (dietary components, physical activity, and body mass index) factors which may influence plasma α -tocopherol and γ -tocopherol levels. Analyses were done using dietary questionnaires and plasma obtained from 65 nonsmoking male volunteers aged 30-59 years. Forty-six men did not take any supplements while 19 took supplements containing vitamin E. A positive correlation ($r = 0.32$; $P < 0.01$) between vitamin E intake and α -tocopherol status [(ratio of plasma α - or γ -tocopherol)/(total triglycerides + total cholesterol)] and a negative correlation ($r = -0.33$; $P < 0.007$) between intake and γ -tocopherol status were observed. The main internal factors, or determinants, for plasma α -tocopherol for nonsupplement users were plasma triglycerides and apoproteins, apoA1 and apoB, but neither lipids nor apoproteins appeared to affect tocopherol levels in supplement users. External determinants of α -tocopherol status in nonsupplement users were vitamin E intake, total fat intake, and body mass index, while in supplement users only vitamin E intake was important. Both vitamin E intake and alcohol intake appeared to affect plasma γ -tocopherol status in a negative manner.

Introduction

The protective effect of vitamin E in preventing chronic disease has been attributed to its ability to function highly efficiently as a lipid-soluble antioxidant. Vitamin E, or tocopherol, protects polyunsaturated fatty acids in the cell membranes from free-radical damage and thus maintains the integrity of the cell (1-4). Free radicals include strongly reactive oxygen molecules which can be formed endogenously by normal metabolic processes or by interaction with substances in the environment.

The possible role of free radical damage in the etiology of certain types of cancer, heart and blood vessel disease, arthritis, and cataracts may be minimized by adequate intake of vitamin E (1). Furthermore, high levels of free radicals can be generated by strenuous exercise, by cigarette smoke, and by ozone and nitrogen dioxide present in polluted air. These free radicals can be quenched by vitamin E and other antioxidants (1). In addition, vitamin E at high intakes can enhance immune response and inhibit conversion of nitrates to nitrosamines in the stomach (1, 5, 6).

Although many studies support an inverse association between vitamin E intake and cancer incidence (7-9), contradictory findings have been reported (10). The protective effect of vitamin E may be site specific and may also depend on other factors such as vitamin C intake, amount and type of fat in the diet, and possibly selenium status. Because evidence for vitamin E intake and cancer prevention in humans is inconclusive, clinical trials are underway (9-11) to determine whether supplements of this vitamin separately and in conjunction with other micronutrients lower cancer incidence.

The Recommended Dietary Allowances (12) set by the Food and Nutrition Board of the National Research Council are 8 and 10 mg TE³/day for adult females and males, respectively. Multivitamin tablets typically contain 30 international units/tablet (approximately 20 mg TE). Tocopherol utilization may be higher in individuals who smoke, have high levels of physical activity, or have certain dietary patterns such as high levels of dietary fat intake (1). Thus, plasma tocopherols levels are likely reflective of both vitamin E intake and lifestyle.

There are four forms of dietary vitamin E: α -tocopherol; β -tocopherol; γ -tocopherol; and δ -tocopherol. γ -tocopherol is the most abundant form of vitamin E in the diet, but its activity *in vivo* is appreciably lower than that of α -tocopherol (4). Plasma γ -tocopherol concentration is low in comparison to that of α -tocopherol and an inverse relationship exists between levels of these two isomers in plasma: with vitamin E supplementation (mainly in α - form) plasma α -tocopherol increases and γ -tocopherol decreases (13-16).

In addition to vitamin E intake, plasma levels of tocopherols reflect both absorption and transport (17). Absorption of tocopherols can be relatively inefficient, ranging 20-80% (12), and depends on normal bile secretion and pancreatic function. Tocopherols are transported to the cells in both LDL and HDL (16, 18-20) and, as a result, it is recommended that plasma E be adjusted for total plasma lipids when determining vitamin E status (21, 22).

The primary purpose of this study was to examine the determinants, both "internal" (plasma lipids, lipoproteins,

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² To whom requests for reprints should be addressed, at EPN Rm. 443, National Cancer Institute, 9000 Rockville Pike, Bethesda, MD 20892.

³ The abbreviations used are: TE, tocopherol equivalents; LDL, low density lipoproteins; HDL, high density lipoproteins; BMI, body mass index; apo, apoprotein; R^2 , coefficient of determination.

and apoproteins) and "external" (dietary variables, BMI, physical activity), of plasma α -tocopherol and γ -tocopherol in subgroups defined by supplement use. A secondary objective was to examine the relationship between dietary questionnaire estimates of vitamin E intake and plasma tocopherol levels.

Methods

Men aged 30–59 years were recruited from the greater Beltsville, MD, area, through advertisements in newsletters at local places of employment, newspapers, and fliers posted at local establishments. The blood samples described here were collected at baseline (*i.e.*, before dietary intervention) during prestudy evaluation for a vitamin C depletion/repletion study (23, 24). Screening procedures eliminated those who had smoked cigarettes within the past 6 months or who had chronic diseases or dietary restrictions. The eligible subjects were evaluated by a physician and 68 of the 71 selected completed the study. Of the 68 subjects, 3 were dropped from data analysis: 1 because of an abnormally high daily intake of total calories (7508 kcal); another because of an unusually high level of dietary vitamin E (70 mg TE/day); and a third because of abnormally high plasma triglycerides. Approval for this study was obtained from the Human Studies Review Committees of the National Cancer Institute, United States Department of Agriculture, and Georgetown University.

Subjects initially completed an extensive questionnaire, the Health Habits and History Questionnaire (25, 26). Food frequency data from this questionnaire were used to estimate usual dietary intake of energy, macro- and micro-nutrients, alcohol consumption, and frequency and amount of vitamin supplement use in the 12 months preceding the study. The questionnaire also asked about usual physical activity, smoking history, medical status, family history of cancer, occupational exposure to various materials, perceived stress, and social support systems.

A single fasting blood sample was collected at baseline on each subject, using heparin as an anticoagulant. Samples were kept on ice until processing, a maximum of 30 min. Whole blood was centrifuged at $1800 \times g$ at 4°C for 10–15 min and plasma was stored at -70°C until analysis. Plasma α - and γ -tocopherols were measured by high performance liquid chromatography (27). Plasma triglycerides and HDL cholesterol were analyzed enzymatically at the George Washington University Lipid Research Clinic (28, 29). LDL cholesterol was determined as the difference between total cholesterol and HDL cholesterol. ApoA1 and apoB were determined by rate nephelometry (28).

Total vitamin E intake (diet plus supplements) is expressed in mg TE/day. To convert international units from supplements into TEs, the following factor is used (12):

$$\text{TE} = 0.67 \times \text{international units}$$

Vitamin supplements most often contain vitamin E in the form of α -tocopherol but the actual compound (*e.g.*, *dl*- α -tocopheryl acetate) is not always listed on the label. For most of the analysis, subjects were divided into 2 groups based on vitamin E supplement intake: nonsupplement users (subjects taking less than 1 multivitamin every 2 days, less than 10 mg TE/day); and supplement users (subjects consuming over 10 mg TE/day).

The data were analyzed using the SAS statistical software package (30). Differences between group means were

tested using *t* tests. The associations among the plasma tocopherols, triglycerides, apoA1, apoB, HDL cholesterol and LDL cholesterol were quantified using Spearman correlation coefficients, as were relationships between dietary vitamin E estimates and plasma tocopherols. Spearman coefficients rather than Pearson product-moment coefficients were used because the distributions of these variables were skewed toward higher values. The excessive influence of extreme values on product-moment correlations was eliminated by using rank order techniques.

Linear multiple regression models were used to investigate the association between plasma α - and γ -tocopherols and health habits and biochemical factors. The factors examined in the model for the transport of tocopherols, *i.e.*, "internal" factors, were plasma triglycerides, LDL cholesterol and HDL cholesterol, apoA1, and apoB. ApoA1 was used to represent HDL cholesterol and apoB was used to represent LDL cholesterol in the models as both apoproteins were independently measured and are good predictors of HDL and LDL cholesterol (31–33). The final "internal" factors models took the form:

Plasma α -tocopherol level

$$= \mu + \beta_1 \times (\text{total vitamin E intake}) \\ + \beta_2 \times (\text{plasma triglycerides}) \\ + \beta_3 \times (\text{apoA1}) \\ + \beta_4 \times (\text{apoB}) + \epsilon$$

Plasma α -tocopherol level = $\mu + \beta_1 \times (\text{plasma triglycerides})$

$$+ \beta_2 \times (\text{apoA1}) \\ + \beta_3 \times (\text{apoB}) + \epsilon$$

The regression coefficients presented are all adjusted for the other effects in the models (*e.g.*, coefficients correspond to type II sums of squares) (30).

As tocopherols are lipid soluble, lipid content affects plasma tocopherol levels. Therefore, two methods of adjustment for lipids were considered in the regression model for "external" factors influencing tocopherol status: the ratio of plasma tocopherol over plasma lipids as suggested by Horwitt *et al.* (21, 22); and the residual method used by Stryker *et al.* (34). We define α - and γ -tocopherol status as the ratio of plasma α - or γ -tocopherol to total lipids (*i.e.*, total plasma triglycerides + total plasma cholesterol) (14, 15, 21). Detailed analyses are reported for the ratio method only as regression diagnostics suggested instability of estimates obtained using the residual method.

To develop a model for external factors influencing α - or γ -tocopherol status, variables considered were total vitamin E intake, age, calories consumed, physical activity score (ranging from 1 to 23: 1, sedentary; 23, athletic), BMI, alcohol intake (amount of beer, wine, and liquor per day, week, or month), a stress score (ranging from 1 to 5: 1, felt stress every day; 5, never or rarely felt stress), percentage of calories from sweets, percentage of calories from alcohol, grams of oleic and linoleic acid, grams of saturated fat, and grams of dietary fiber. Models were developed for all subjects together ($n = 65$) and by supplement use: vitamin E nonsupplement users ($n = 46$); and supplement users ($n = 19$). Vitamin E supplement users were defined as those taking at least one multiple vitamin tablet every other day or an equivalent amount from vitamin E capsules. The differences between users and nonusers of vitamin E supplements were examined using *t* tests. The final "external" factors models for α - and γ -tocopherol

Table 1 Subject characteristics

Characteristic	Mean (SE)		
	All	Nonsupplement users	Supplement users
<i>n</i>	65	46	19
Age (yr)	40.6 (1.02)	41.4 (1.31)	38.6 (1.42)
Weight (kg)	80.8 (1.33)	81.0 (1.63)	80.3 (2.35)
Height (cm)	177.3 (0.86)	177.6 (1.11)	176.8 (1.23)
Calories (kcal/day)	2245 (79.8)	2199 (99.8)	2357 (127.1)
Fat intake (g/day)	97.5 (4.62)	95.3 (5.64)	102.8 (8.04)
Saturated fat intake (g/day)	34.2 (1.63)	33.2 (1.92)	36.6 (3.09)
Oleic acid intake (g/day)	35.8 (1.71)	35.2 (2.12)	37.2 (2.84)
Linoleic acid intake (g/day)	18.8 (1.24)	18.4 (1.49)	19.8 (2.27)
Total daily vitamin E intake ^a	30.1 (6.91)	11.3 (0.80) ^b	77.0 (20.17) ^b
Dietary intake	10.9 (0.60)	10.5 (0.75)	11.8 (0.97)
Supplement intake	19.6 (6.86)	0.8 (0.31) ^b	65.2 (20.23) ^b
Plasma α -tocopherol (μ g/dl)	1.16 (0.079)	1.02 (0.035) ^c	1.50 (0.246) ^c
Plasma γ -tocopherol (μ g/dl)	0.18 (0.014)	0.21 (0.017) ^c	0.12 (0.019) ^c

^a Vitamin units in mg of TE.

^b Difference between nonsupplement users and supplement users significant at $P < 0.0001$.

^c Difference between nonsupplement users and supplement users significant at $P < 0.005$.

status, respectively, were of the form:

$$\begin{aligned} \alpha\text{-tocopherol status} = & \mu + \beta_1 \times (\text{total vitamin E intake}) \\ & + \beta_2 \times (\text{total fat intake}) \\ & + \beta_3 \times (\text{BMI}) \\ & + \beta_4 \times (\text{activity}) + \epsilon \end{aligned}$$

$$\begin{aligned} \gamma\text{-tocopherol status} = & \mu + \beta_1 \times (\text{total vitamin E intake}) \\ & + \beta_2 \times (\text{total fat intake}) \\ & + \beta_3 \times (\text{BMI}) \\ & + \beta_4 \times (\text{alcohol intake}) + \epsilon \end{aligned}$$

Results

Subject characteristics are shown in Table 1. Nonsupplement and supplement users did not differ significantly in age, weight, calorie consumption, and amount of fat intake (saturated fat, oleic or linoleic acid) or in dietary intake of vitamin E. The significantly higher total intake of vitamin E in the supplement users was a result of their use of supplements. Plasma α -tocopherol levels were significantly higher in the supplement users but γ -tocopherol levels were significantly lower.

The relationship between the vitamin E intake estimated from the diet questionnaire and plasma α - and γ -tocopherol status [tocopherol/(plasma triglycerides + cholesterol)] is shown in Table 2. For all the subjects, total vitamin E intake (dietary vitamin E plus supplemental vitamin E) and α -tocopherol status were moderately correlated ($r = 0.32$; $P < 0.01$), as were E intake and γ -tocopherol status ($r = -0.33$; $P < 0.007$), although here levels were inversely related. For the subgroups, however, no correlations reached statistical significance, probably due to smaller sample size.

Fig. 1, A-C, show the relationship of vitamin E intake and α -tocopherol status for all subjects together and for subjects by vitamin E supplement use subgroups (i.e., supplement users and nonsupplement users). There is a significant correlation between total vitamin E intake and α -tocopherol status for all subjects (Fig. 1A), which is heavily influenced by those subjects with high supplement use (Fig. 1C). Among nonsupplement users the range of intake is much smaller than that seen in supplement users with no definitive trend (Fig. 1B).

Table 2 Spearman correlations between total vitamin E estimates^a and plasma α - and γ -tocopherol status^b

	Correlation	P value
Total vitamin E intake + α -tocopherol status		
All ($n = 65$)	0.32	0.01
Nonsupplement user ($n = 46$)	0.17	0.26
Supplement user ($n = 19$)	0.37	0.11
Total vitamin E intake + γ -tocopherol status		
All ($n = 65$)	-0.33	0.007
Nonsupplement user ($n = 46$)	-0.08	0.57
Supplement user ($n = 19$)	-0.25	0.31

^a Total vitamin E estimate, diet + supplements.

^b Tocopherol status (μ g/mg).

Tocopherol
(Plasma triglycerides + cholesterol)

When subjects were divided into 3 groups based on vitamin E supplement intake (nonsupplement users, low; multivitamin level users, medium; and vitamin E users, high), α -tocopherol status increased with each group (Table 3). For the two subjects consuming the highest levels of vitamin E, the α -tocopherol status was dramatically higher. Dietary vitamin E intake was similar in all three groups.

Table 4 shows correlations between plasma α - and γ -tocopherols, triglycerides, HDL and LDL cholesterol, and apoA1 and apoB. α -tocopherol is positively correlated with total triglycerides ($r = 0.54$), LDL-cholesterol ($r = 0.34$), and apoB ($r = 0.48$). Correlations between γ -tocopherol and these lipids and lipoproteins are all smaller ($r < 0.16$) and do not approach statistical significance. Total triglycerides are correlated positively with LDL cholesterol ($r = 0.44$) and apoB ($r = 0.60$), and negatively with HDL cholesterol ($r = -0.64$) and apoA1 ($r = -0.34$). HDL cholesterol has a high positive correlation with apoA1 ($r = 0.87$) but a negative correlation with LDL cholesterol ($r = -0.38$) and apoB ($r = -0.50$). In contrast, LDL cholesterol is highly correlated with apoB ($r = 0.92$) and negatively associated with apoA1 ($r = -0.24$).

As mentioned above, two regression models were developed to investigate factors which influence plasma α -tocopherol. One model investigated the role of "internal"

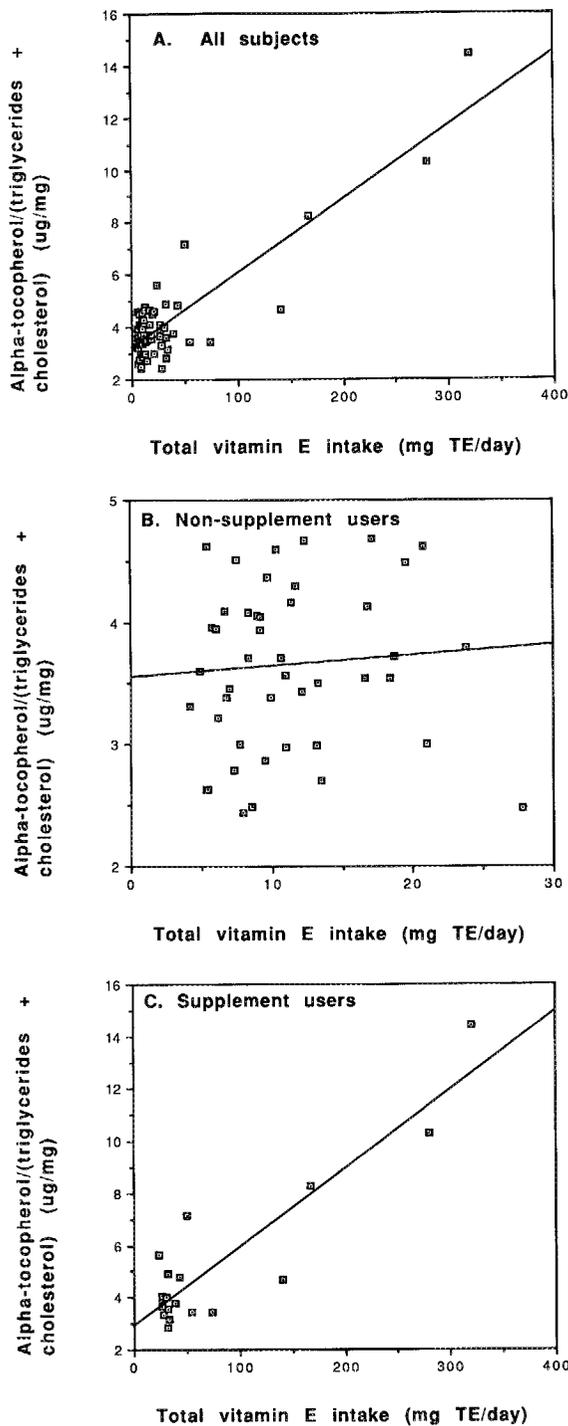


Fig. 1. Daily vitamin E intake and α -tocopherol status in all subjects, non-supplement users, and supplement users.

factors such as plasma lipids, lipoproteins, and apoproteins (with and without total vitamin E intake), while the second model examined the role of "external" factors, e.g., vitamin E intake, fat intake, BMI, and physical activity, on plasma α -tocopherol adjusted for lipids.

The effects of plasma triglycerides, apoproteins, and vitamin E intake on plasma α -tocopherol, as estimated using

Supplement	Daily vitamin E intake ^b		α -Tocopherol status	
	Dict ^c	n	Mean (SE)	Median (Range)
<10	10.5	46	3.66 (0.10)	3.65 (2.44-4.77)
10-42	12.4	14	4.17 (0.31)	3.83 (2.83-7.71)
67+ ^d	10.2	5	8.22 (2.00)	8.25 (3.44-14.43)

^a Tocopherol status ($\mu\text{g}/\text{mg}$):

Tocopherol
(Plasma triglycerides + cholesterol)

^b Vitamin E in mg TE/day.

^c Mean of group (*t* test showed no significant difference).

^d Three subjects in this group were consuming supplements between 67 and 155 mg TE/day with the mean plasma α -tocopherol status of 5.45 (SE, 1.44). The mean plasma α -tocopherol status of the 2 subjects consuming supplement over 155 mg TE/day was 12.37 (SE, 2.06). The dietary vitamin E intake in the two subgroups were 9.2 and 11.8 mg TE/day.

the "internal" factors regression model, are given in Table 5. For all subjects combined, total vitamin E intake, triglycerides, apoA1, and apoB are significant predictors of plasma α -tocopherol and explain 82% of the variance. Among non-supplement users, the effect of vitamin E intake (derived mainly from dietary sources) on plasma α -tocopherol is not significant, but plasma total triglycerides, apoA1, and apoB remain important. By contrast, plasma α -tocopherol in supplement users is strongly associated with vitamin E intake but the plasma triglycerides or apoproteins do not appear to be important. When the "internal" model included only dietary source of vitamin E for all subjects and supplement users the effect on plasma α -tocopherol level was not significant.

Omission of total vitamin E intake from the model decreased the R^2 dramatically for all subjects (from 0.82 to 0.19) and for supplement users (from 0.87 to 0.40). In this model, as in all models for supplement users, the wide range of vitamin E intake produced by use of supplements of varying doses results in the preponderance of the variance being explained by total vitamin E intake. This factor, along with the small number in this group, suggest that model-based results for supplement users be regarded with caution. Similar analyses were performed for γ -tocopherol but no significant relationships were observed (data not shown).

The association between α -tocopherol status and vitamin E intake, fat intake, BMI, and physical activity was examined in the "external" factors regression model (Table 6). The influence of vitamin E intake was significant in both nonsupplement and supplement users, and level of significance was particularly strikingly in supplement users. Fat intake was negatively associated with α -tocopherol status for all subjects and for nonsupplement users, but not for supplement users. BMI was negatively associated with α -tocopherol status in the nonsupplement users, while activity did not appear to be important for any group.

Analyses using the method described by Stryker *et al.* (34) using plasma tocopherol residuals adjusted for lipids (residuals obtained by regressing plasma tocopherol on the sum of total triglycerides and cholesterol) and vitamin E intake, similarly adjusted for calories, showed vitamin E intake alone to be a determinant of plasma α -tocopherol in all subjects and in supplement users. However, regression diagnostics indicated that some of the regression coefficients were not well estimated, and the results are not shown.

Table 4 Spearman correlations^a between plasma tocopherols, lipids, lipoproteins, and apoproteins

	α -tocopherol	γ -tocopherol	total triglycerides	HDL cholesterol	LDL cholesterol	apoA1	apoB
α -tocopherol	1.000						
γ -tocopherol	-0.02 (0.86) ^b	1.000					
Triglycerides	0.54 (0.0001)	0.08 (0.53)	1.000				
HDL-cholesterol	-0.16 (0.21)	0.05 (0.66)	-0.64 (0.0001)	1.000			
LDL cholesterol	0.34 (0.005)	0.16 (0.22)	0.44 (0.0002)	-0.38 (0.002)	1.000		
apoA1	0.06 (0.62)	0.12 (0.32)	-0.34 (0.006)	0.87 (0.0001)	-0.24 (0.05)	1.000	
apoB	0.48 (0.0001)	0.10 (0.42)	0.60 (0.0001)	-0.50 (0.0001)	0.92 (0.0001)	-0.30 (0.01)	1.000

^a $n = 65$.^b Numbers in parentheses, P values.Table 5 Effect of vitamin E intake, plasma lipids, and transport proteins ("internal" factors) on plasma α -tocopherol levels

	Vitamin E intake		Minus vitamin E intake	
	β coefficient (SE)	P value	β coefficient (SE)	P value
All subjects ($n = 65$)	$R^2 = 0.82$		$R^2 = 0.19$	
Total vitamin E intake ^a	9.36 (0.65)	0.0001		
Plasma triglycerides	3.00 (0.90)	0.002	3.11 (1.88)	0.10
apoA1	3.67 (1.70)	0.03	0.10 (3.52)	0.98
apoB	6.13 (2.40)	0.01	10.13 (5.00)	0.05
Nonsupplement users ^b ($n = 46$)	$R^2 = 0.48$		$R^2 = 0.48$	
Total vitamin E intake ^c	-0.19 (5.11)	0.97		
Plasma triglycerides	2.37 (0.65)	0.0008	2.37 (0.64)	0.0007
apoA1	3.17 (1.14)	0.008	3.18 (1.11)	0.007
apoB	3.92 (1.83)	0.04	3.93 (1.76)	0.03
Supplement users ($n = 19$)	$R^2 = 0.87$		$R^2 = 0.40$	
Total vitamin E intake ^a	9.89 (1.38)	0.0001		
Plasma triglycerides	5.36 (3.05)	0.10	5.62 (6.35)	0.39
apoA1	8.77 (10.44)	0.42	-11.76 (20.91)	0.58
apoB	9.65 (8.24)	0.26	20.74 (16.85)	0.24

^a When total vitamin E intake was divided into dietary and supplemental sources, there were no significant effect of dietary vitamin E on the plasma α -tocopherol level in any group (all subjects, nonsupplement, or supplement users).^b Refers to vitamin E supplement use (see text for details).^c Only dietary source of vitamin E.

External factors which affect γ -tocopherol status [γ -tocopherol/(plasma triglycerides and cholesterol)] were different from those for α -tocopherol (Table 7). Total vitamin E intake and alcohol intake were significant for γ -tocopherol status in all subjects, but the R^2 for the model was only 0.14. For vitamin E supplement users, alcohol intake played an important role in determining γ -tocopherol status. The R^2 for the regression model for nonsupplement users, 0.50, was much higher than that for the supplement users.

Discussion

Total vitamin E intake, especially from supplemental sources, plays an important role in determining the plasma tocopherol levels in these subjects. This population can be viewed as two subpopulations, which we have defined as nonsupplement users and supplement users of vitamin E. In this study, 29%, or 19 subjects, used supplements containing

vitamin E, which is similar to proportions seen in the general population (35, 36). Of these, 5 subjects had vitamin E intakes far greater than those achieved by taking multivitamins. These few subjects numbers heavily influence the correlations and the models. However, they provide information on plasma response to high levels of intake that could have an important impact on public health issues such as heart disease (37, 38) and possibly on cancer prevention by its antioxidant properties.

The overall interrelationships observed in this study between the plasma tocopherols, lipids, lipoproteins, and apoproteins into nonsupplement users and supplement users provides useful information about the relative importance of lipid levels in these two subgroups. For subjects who did not take vitamin E supplements, the plasma triglycerides, apoA1, and apoB were strongly associated

Table 6 Influence of "external" factors on plasma α -tocopherol status^a

Model	β coefficient (SE)	P value
$R^2 = 0.78$		
All subjects (n = 65)		
Total vitamin E intake	0.03 (0.002)	0.0001
Total fat intake	-0.01 (0.003)	0.007
BMI	-0.02 (0.04)	0.56
Activity	0.02 (0.02)	0.30
$R^2 = 0.30$		
Nonsupplement users (n = 46)		
Total vitamin E intake	0.07 (0.03)	0.01
Total fat intake	-0.01 (0.004)	0.001
BMI	-0.06 (0.03)	0.04
Activity	-0.02 (0.02)	0.33
$R^2 = 0.87$		
Supplement user (n = 19)		
Total vitamin E intake	0.029 (0.003)	0.0001
Total fat intake	-0.013 (0.009)	0.17
BMI	0.165 (0.11)	0.16
Activity	0.101 (0.06)	0.10

^a Tocopherol status ($\mu\text{g}/\text{mg}$):

$$\frac{\text{Tocopherol}}{\text{(Plasma triglycerides + cholesterol)}}$$

Table 7 Influence of "external" factors on plasma γ -tocopherol status^a

Model	β coefficient (SE)	P value
$R^2 = 0.14$		
All subjects (n = 65)		
Total vitamin E intake	-0.002 (0.001)	0.02
Total fat intake	0.0002 (0.001)	0.89
BMI	-0.019 (0.02)	0.30
Alcohol intake	-0.0007 (0.0003)	0.04
$R^2 = 0.10$		
Nonsupplement users (n = 46)		
Total vitamin E intake	-0.026 (0.02)	0.20
Total fat intake	0.002 (0.003)	0.36
BMI	-0.022 (0.02)	0.34
Alcohol intake	-0.0006 (0.0004)	0.08
$R^2 = 0.50$		
Supplement users (n = 19)		
Total vitamin E intake	-0.001 (0.0008)	0.09
Total fat intake	0.001 (0.002)	0.54
BMI	-0.052 (0.03)	0.07
Alcohol intake	-0.002 (0.001)	0.007

^a Tocopherol status ($\mu\text{g}/\text{mg}$):

$$\frac{\text{Tocopherol}}{\text{(Plasma triglycerides + cholesterol)}}$$

with plasma α -tocopherol levels. However, among subjects who took vitamin E supplements, these factors were not significantly associated with plasma α -tocopherol levels. This suggests that plasma lipids, lipoproteins, and apoproteins may play an important role at lower levels of vitamin E intake, but at high levels, such as seen with supplement users, these factors play a less important role. Saturation of plasma lipids and apoproteins (surrogates for lipoproteins) may be occurring at high levels of vitamin E intake. Alternatively, the amount of variation in the plasma data from this group was so large, and the influence of supplements so great, that it is possible that we could not detect effects of these factors, especially in this small sample size.

Even though vitamin E intake in these analyses is categorized as an "external" factor, its inclusion in the "internal" factors model provides interesting insight into the relative importance of plasma lipids and amount of vitamin E

intake. A strong significant association between estimated total vitamin E intake and plasma α -tocopherol was observed in all subjects and among vitamin E supplement users. Among nonusers of vitamin E supplements, however, vitamin E intake was not associated with plasma α -tocopherol in this study. This is not surprising given the limited range of dietary vitamin E intake of the men in this study. The mean intake of dietary vitamin E, 10.9 mg TE, is similar to the 7.1 mg TE seen in 35–49-year-old men in the 1987 National Health Interview Survey (42). In the "internal" model the effect of dietary vitamin E was insignificant for both supplement users and nonusers; supplemental vitamin E intake appears to be the major determinant of plasma α -tocopherol levels. Other studies (43, 44) also report similar findings.

When we used a lipid adjusted measure of tocopherols (45) to investigate the role of "external" determinants (vitamin E intake, fat intake, BMI, and activity), we found total vitamin E intake again to be important in determining α -tocopherol status, especially for supplement users. The low R^2 for the nonsupplement users may reflect either a limitation of the assessment method or the narrow range of dietary intake of vitamin E. Daily vitamin E intake in nonsupplement users ranged only from 4.2 to 27.8 mg TE, while total intake among supplement users ranged from 23.2 to 320.2 mg TE/day.

Most supplement users obtained their supplemental vitamin E from multivitamins. The five subjects who used vitamin E capsules in our study had higher α -tocopherol status. It appears that dietary vitamin E, at levels typical in American diets, cannot raise plasma α -tocopherol status to the levels seen among supplement users or even as high as among those taking the relatively low levels found in multivitamins. This may have public health implications as recent reports suggest that consumption of vitamin E supplements may be an important factor in reduction of coronary heart disease in both men and women (37, 38).

Other factors found to influence α -tocopherol status were total fat intake and BMI. Total fat intake was inversely associated with α -tocopherol status in all subjects as well as nonsupplement users. With increased intake of polyunsaturated fat, higher levels of lipid peroxidation could result in a "stress" situation, causing loss of tocopherols. The negative relationship between BMI and α -tocopherol status in nonsupplement users may be similar to that reported for vitamin C (46); due to larger body mass there may be a dilution effect in the group not consuming large amounts of vitamin E. Ascherio *et al.* (43) reported a significant negative association between BMI and plasma α -tocopherol in women, while in men, a parallel but nonsignificant trend was observed.

The factors affecting γ -tocopherol status are not clear. As in other reports, a negative relationship between vitamin E intake and γ -tocopherol status is seen (13–16). In people consuming supplements (mainly α -tocopherol), there may be a selective binding and transport of α -tocopherol while γ -tocopherol may be excreted into the bile (17, 47). The negative effect of alcohol consumption on γ -tocopherol status is interesting and needs further exploration.

In conclusion, the main internal factors which influence plasma α -tocopherol in nonsupplement users are plasma triglycerides, apoA1, and apoB but in supplement users vitamin E intake alone was predictive of plasma α -tocopherol. External factors influencing α -tocopherol status in nonsupplement users were vitamin E intake, fat intake, and BMI, while vitamin E intake was the major determinant in supplement users. There was a negative association between

γ -tocopherol status and vitamin E intake as well as alcohol intake, for all subjects.

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