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Effects of Vitamin E Supplements and Diet on Colonic α - and γ -tocopherol Concentrations In Persons at Increased Colon Cancer Risk

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Abstract

The available evidence indicates that γ -tocopherol has more potential for colon cancer prevention than α -tocopherol, but little is known about the effects of foods and supplements on tocopherol levels in human colon. This study randomized 120 subjects at increased colon cancer risk to either a Mediterranean or a Healthy Eating diet for six months. Supplement use was reported by 39% of the subjects, and vitamin E intake from supplements was 2-fold higher than that from foods. Serum α -tocopherol at baseline was positively predicted by dietary intakes of synthetic vitamin E in foods and supplements but not by natural α -tocopherol from foods. For serum γ -tocopherol, dietary γ -tocopherol was not a predictor, but dietary α -tocopherol was a negative predictor. Unlike with serum, the data supported a role for metabolic factors, and not a direct effect of diet, in governing concentrations of both α - and γ -tocopherol in colon. The Mediterranean intervention increased intakes of natural α -tocopherol, which is high in nuts, and decreased intakes of γ -tocopherol, which is low in olive oil. These dietary changes had no significant effects on colon tocopherols. The impact of diet on colon tocopherols therefore appears to be limited.

Introduction

Micronutrients such as vitamin E appear to have preventive properties for colon cancer (1, 2). Many of these micronutrients are available in the form of dietary supplements, and the intake of these supplements is prevalent in the U.S. According to data from the Centers for

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Disease Control and the National Center for Health Statistics, use of dietary supplements in the U.S. has been increasing with over half of the U.S. population reporting supplement use during 2003-2006 (3). Fortified foods such as cereals and “power” bars are also common, representing another source of added vitamins.

There are nutritional issues related to using supplements versus foods for increasing micronutrient intakes. The micronutrients most widely available in supplements may not be ideal for cancer prevention. In the case of tocopherols, α -tocopherol appears to have relatively lower, if any, potential for cancer prevention than γ -tocopherol (1, 4). Unfortunately, high α -tocopherol intakes from vitamin E supplements can result in decreased blood γ -tocopherol concentrations (5-8). Supplement intakes also present a problem with the interpretation of dietary intervention studies in which changes in diet are being monitored by measurement of micronutrient concentrations in biological samples.

We conducted a dietary intervention study in persons at increased risk of colon cancer, and subjects were randomized to follow either a standard Healthy Eating diet or a Mediterranean diet for six months. This clinical trial, the Healthy Eating Study, afforded the opportunity to evaluate the relative influence of tocopherol intakes from foods and supplements on serum and colon tocopherol concentrations at baseline and after dietary intervention. There is very little data available on concentrations of tocopherols in the human colon. Colon concentrations of α -tocopherol were decreased in a small study by β -carotene supplementation, and α -tocopherol in colon was increased by vitamin E supplementation (9, 10). Like serum, the concentration of α -tocopherol was higher than that of γ -tocopherol in colon tissue obtained at colonoscopy in a study done using tissue obtained after bowel preparation (11).

Tocopherols may be affected more by supplement intakes than other fat-soluble micronutrients, like carotenoids, since α -tocopherol is commonly consumed in fairly high levels in vitamin E supplements and fortified foods. We hypothesized that supplement use would have detrimental effects on γ -tocopherol concentrations in the colon. A Mediterranean diet intervention also could be predicted to decrease γ -tocopherol since olive oil contains only low amounts of γ -tocopherol. The objective of this study was to better define the relationships between colon α - and γ -tocopherol concentrations with dietary intakes, supplement intakes and serum concentrations of tocopherols.

Methods

Subjects

Enrollment for the study took place from July 2007 to November 2010 in Ann Arbor, MI and surrounding areas. Details of recruitment and retention to the study have been published and the primary aim of the trial was to evaluate changes in colon fatty acids and carotenoids (12, 13). A total of 120 study participants who were at increased risk for colon cancer were recruited and signed informed consent to participate. Increased risk was defined as a strong family history of colon cancer or a personal history of adenomas or colon cancer. The study was approved by the University of Michigan Institutional Review Board (HUM00007622) and was registered at the ClinicalTrials.org (NCT00475722). Potential subjects who were

taking supplements above 200% of the dietary reference intakes for vitamins and minerals at baseline were asked to discontinue supplement use 2 weeks prior to starting on study. Supplement use below that amount was allowed. Questionnaires were used to collect demographic data, health information and physical activity. The diagram recommended by the Consolidated Standards of Reporting Trials (CONSORT) statement has been reported previously (13).

Study subjects were randomized to the Mediterranean diet or a Healthy Eating diet that was based on Healthy People 2010 goals and details of intervention methods have been published previously (14). Individualized counseling for meeting study goals was provided in either case. Subjects in the Healthy Eating arm were asked to consume at least 2 servings/day of fruit, 3 servings/day of vegetables with at least one serving being dark green or orange, at least 3 servings/day from whole grains, less than 10% of calories from saturated fat, and less than 30% of calories from total fat. For the Mediterranean Diet, an exchange list approach was used to decrease intakes of n6-PUFA and increase intakes of MUFA, include high omega-3 fatty acid-containing foods twice a week, three or more servings/day of whole grains, and to replace some of their usual carbohydrates with fruits and vegetables. The fruit and vegetable goal ranged 7-9 servings/day in specific categories to increase variety, and the goal depended on usual energy intakes. Servings were defined using FDA serving sizes as described previously (14).

Assessment of Diet and Supplement Use

Diet was assessed using two days of written food records and one un-announced 24-hour recall. An additional 24-hour recall was obtained at the first study visit, and all four days were averaged to obtain an estimate of baseline diet. At six months, two days of written records and two days of recalls were also used. An interim assessment at 3 months consisted of two written records and one un-announced recall. The food records and recalls were all analyzed by the Nutrition Data System for Research software (NDSR) designed by the University of Minnesota, version 2010. Use of dietary supplements was recorded on the food records and recalls using a separate page that asked about the details of all supplements taken including herbals. These were also entered into the NDSR program for analysis of nutrient intakes from supplements.

The NDSR program calculates vitamin intakes in several different forms using formulas within the program. The mg amount of total α -tocopherol, or vitamin E, was mg natural α -tocopherol plus mg synthetic α -tocopherol*0.45. The total vitamin E activity from supplements and fortified foods was given in IU by the NDS-R program since many supplements continue to label vitamin E in IU, with vitamin E IU = mg natural α -tocopherol/0.67 + mg synthetic α -tocopherol/1.0. The variable "natural α -tocopherol" was the form of α -tocopherol that occurs naturally in foods: RRR- α -tocopherol. The variable "synthetic α -tocopherol" represented the eight 2R stereoisomers of all-rac- α -tocopherol used in fortified foods. Vitamin E intake from supplements was a separate variable. Tocotrienol isomers, another source of vitamin E, are not included in these measures within the NDSR program and are typically low in foods consumed commonly in the U.S. For converting mg amounts of tocopherols in foods to IU so that intakes from foods

could be compared with intakes from supplements, the following were used: natural α -tocopherol or R,R,R- α -tocopherol occurring naturally in foods (1 mg natural α -tocopherol/0.67 = IU vitamin E); Synthetic α -tocopherol in foods (1 mg synthetic α -tocopherol = 1 IU vitamin E = 0.45 mg vitamin E).

Sample Analyses

Fasting blood and colon biopsies were obtained at baseline and after 6 months. Colon biopsies were obtained by flexible sigmoidoscopy without prior preparation of the bowels. Biopsies were obtained in the colon, avoiding sites containing feces as previously described (13). The biopsies were about 1 mm X 4 mm in size consisted mainly of the epithelial and stromal layer of the colon with a small amount of muscularis. Biopsies were flash frozen in liquid nitrogen within 3 seconds of removal from the colon and stored at -80°C until analysis (13). Four biopsies were combined to make one homogenate for analysis of multiple endpoints. Protein in the homogenate was assayed using the Bio-Rad Protein Assay Dye Reagent Concentrate (product #500-0006, Life Sciences, Hercules, CA). Both serum and biopsy tocopherols were extracted and analyzed by high pressure liquid chromatography as previously described (12, 15). Briefly, serum or colon homogenates were extracted with hexane using Tocol as the internal standard. A C30 reverse phase column was used to separate tocopherols, and detection was with an electrochemical detector. Measures of cholesterol, HDL, and triglycerides were performed using a Cobas Mira Chemistry analyzer from Roch Diagnostics Corporation (Indianapolis).

Statistical Analysis

Subject characteristics such as gender, age, BMI, marital status, lifestyle, diet arm assignment and study completion rates were compared across the supplement and non-supplement users by means of chi-square or Fisher's exact test (for categorical characteristics) and two-sample un-pooled t-test (for continuous measures). Two-sample t-tests were also carried out with respect to dietary intakes of tocopherols and tocopherol concentrations in tissue at baseline. A series of linear regression models evaluated the relative contribution of demographic factors on tocopherol concentrations in serum and colon. Partial correlations between dietary intakes and tocopherol concentrations in serum and colon were then evaluated controlling for demographic factors. Change over the study period in dietary intakes of tocopherol as well as in serum and colon concentrations of the same were compared across diet arm and supplement use status by means of linear mixed models using study group, supplement use, time and group-by-time second order interaction as the primary covariates. A third order interaction between diet arm, time and supplement use was also explored when investigating changes in serum and colon tocopherol concentrations.

Other predictors such as BMI, alcohol, smoking, and age were also included in the models based on published studies that identified significant determinants of concentrations of fat-soluble micronutrients (16-19). A random subject effect used in the mixed model accounted for the clustering within subjects. Prior to modeling, micronutrient concentrations were log transformed to achieve normality. All statistical analyses were done in IBM SPSS Statistics

version 20.0.0 (IBM Corporation, Armonk, New York). P-values <0.05 were considered significant. Data shown in tables are mean and SE.

Results

Characteristics of Supplement Users and Non-users at Baseline

Table 1 summarizes the demographic characteristics of subjects who did or did not use dietary supplements containing vitamin E while on study. At baseline, 43 subjects reported taking these supplements, and an additional four subjects reported supplement use either at 3 months (n=2) or at 6 months (n=2). Since supplement use is often not consistent on a daily basis, and the study collected 3–4 days of dietary intake data at each time point, subjects who reported intake of supplements at any time point were combined into a group termed “supplement users”. The 47 subjects who reported use of vitamin E tended to be older ($p=0.09$), of lower BMI ($p=0.06$), and more likely to complete 6 months of study ($p=0.06$), but these differences were not statistically significant. The only difference that was significant was prevalence of arthritis, which was significantly higher in the supplement user group (Table 1). It is not clear why this was the case, but there is a report that vitamin E can prevent rheumatoid arthritis, and it may be that persons with arthritis were using vitamin E supplements to treat their symptoms (20).

Dietary intakes of vitamins and nutrients from foods only in supplement users and non-users also did not differ appreciably at baseline (Table 2). Supplement users had lower mean dietary intakes of β -tocopherol from foods ($p=0.04$). In supplement users, the mean intake of vitamin E from supplements was approximately double the vitamin E intake from all foods, 31 vs. 16 IU/day, as shown in Table 2 ($p<0.001$ by the 2-sample t-test).

Concentrations of α -tocopherol in serum at baseline were significantly higher in the 47 supplement users than the 73 non-users (mean 35.1, SD 14.9 versus mean 27.9, SD 8.6, respectively) with $p<0.001$ from two-sample t-tests, two-tailed. Concentrations of α -tocopherol in colon biopsies at baseline also were significantly higher in supplement users than non-users (mean 884, SD 711 versus mean 586, SD 402, respectively, in pmol/mg protein) with $p=0.001$. Baseline concentrations of γ -tocopherol in serum or colon were not affected by vitamin E supplement use, although the difference for γ -tocopherol in colon was almost significant with $p=0.051$ by the two-sample t-test (mean 230, SD 213 in users versus mean 184, SD 147 in non-users, in pmol/mg protein).

Predictors of Tocopherol Concentrations at Baseline

Linear regression was used to identify significant predictors of baseline tocopherol concentrations (Table 3). For serum α -tocopherol, baseline age, gender, smoking status (current, past, never), usual alcohol intake (g/day from questionnaire) and BMI were not significant predictors. After taking into account the first four demographic factors, serum total cholesterol explained a relatively large proportion of the variance in serum α -tocopherol with a positive beta coefficient indicating that higher serum cholesterol was associated with higher serum α -tocopherol (Table 3). This could be due to the fact that serum cholesterol is a major carrier for vitamin E. Total dietary intake of α -tocopherol was

also a significant predictor but the change in r-square was smaller than for serum cholesterol. Interestingly, this appeared to be due mainly to intakes of synthetic α -tocopherol and vitamin E supplements, with no significant effect of natural α -tocopherol. Dietary γ -tocopherol conversely had a significant inverse relationship with serum α -tocopherol, perhaps due to competition for absorption in the small intestine (5-8). The predictors of colon α -tocopherol were different than for serum. Increased colon α -tocopherol concentrations were significantly predicted by increased age and serum α -tocopherol (Table 3).

For serum γ -tocopherol concentrations, both serum cholesterol and BMI were significant positive predictors, and total dietary α -tocopherol was a negative predictor (Table 3). In evaluating the three different sources of dietary α -tocopherol on serum γ -tocopherol, only synthetic α -tocopherol has a statistically significant effect (not shown). Dietary intake of γ -tocopherol and demographic factors other than BMI were not significant predictors of serum γ -tocopherol. For colon γ -tocopherol, demographic factors (age, smoking, alcohol, and gender) had a borderline significant effect with $p = 0.06$, and the coefficient for age was significant. Both BMI and serum γ -tocopherol were significant positive predictors of colon γ -tocopherol concentrations. These factors accounted for a modest portion of the variance in colon γ -tocopherol concentrations as seen from the change in r-square given in Table 3. Dietary intakes of either tocopherol did not predict colon γ -tocopherol concentrations.

Season of blood draw (defined by the availability of local produce in Michigan: June-November vs. December-May) had no appreciable effect nor did the total carotenoid intakes from food in any case (not shown). The analyses were similar when restricted to supplement users only, indicating that the associations with supplement use were not driven by subjects with no intake of vitamin E from supplements (not shown). To confirm the positive relationship between serum and colon tocopherol concentrations, partial correlations were evaluated. After controlling for age, current smoking, alcohol intake, gender and BMI, serum α -tocopherol was positively correlated with colon α -tocopherol ($r = 0.24$, $p < 0.001$), and serum γ -tocopherol was positively correlated with colon γ -tocopherol ($r = 0.33$, $p < 0.001$). The ratio of α -tocopherol to γ -tocopherol was 5.5 (SE 0.6) in colon and 12.1 (SE 1.7) in serum.

Changes in Tocopherols Over Time

Dietary intakes and concentrations of vitamin E were evaluated for change over time in each diet group: Healthy Eating and Mediterranean (Table 4). There was a significant Group*Time interaction for dietary intake of natural α -tocopherol, with a larger increase at 6 months in the Mediterranean versus the Health Eating group. Dietary intake of γ -tocopherol also displayed a significant Group*Time interaction with a significant decrease in the Mediterranean group only. Mean vitamin E from supplements decreased in both groups, and this was only significant when both diet groups were combined ($p = 0.026$ for the fixed effects of time, data not shown).

Changes in serum and colon concentrations of tocopherols over time are shown in Table 5, as are the results from linear regression for fixed effects of time, supplement use, group assignment and their interactions. Supplement users overall had a significantly higher

concentration ($p < 0.001$) of serum α -tocopherol averaged across study group and time. Similar findings were obtained for colon α -tocopherol although the significance was borderline ($p = 0.05$). Serum concentrations of α -tocopherol exhibited a significant three way interaction between group, time and supplement use. Serum α -tocopherol decreased significantly over time in the Mediterranean group and it increased significantly in the Health Eating group but these differences between diet groups were only present in subjects who were vitamin E supplement users. There were no significant group, time and supplement use interactions for serum γ -tocopherol nor for either tocopherol in the colon. There was a borderline significant decrease in serum γ -tocopherol over time when averaged across diet group and supplement use ($p = 0.05$). Incorporating serum HDL and LDL in the models for serum tocopherols did not change the results appreciably. As reported previously, serum lipoproteins did not exhibit significant changes over time in this study (14).

Discussion

One important mechanism by which dietary changes can exert preventive effects on cancer is via modulation of micronutrient concentrations in target tissues. The effects of diet on serum and tissue micronutrient concentrations can, however, be complicated by high levels of supplement intakes. The objective of this study was to better define the relationships of colon tocopherol concentrations with dietary intakes from food, supplement intakes and serum concentrations of tocopherols. There were interesting findings with regard to differences between the factors that affect serum and colon tissue tocopherols.

In the U.S., intakes of γ -tocopherol from foods generally exceed that of α -tocopherol, as was the case in this study (Table 2). The relatively higher concentration α - versus γ -tocopherol in blood has been attributed to the preferential transfer of α -tocopherol via α -tocopherol transfer protein to very low density lipoprotein, resulting in the relatively more rapid hepatic metabolism and excretion of γ -tocopherol (4, 20). Like serum, the concentration of α -tocopherol in colon was higher than that of γ -tocopherol (Table 5). The observed α - to γ -tocopherol ratio of about 5 in colon was similar to the ratios of 2-8 reported for adipose tissue (21-23). In serum the α - to γ -tocopherol ratio was 12 in our study, indicating a possibility of either specific γ -tocopherol uptake and/or accumulation in colon over time to result in a lower α - to γ -tocopherol ratio in colon versus blood.

Serum concentrations of α -tocopherol at baseline were strongly and positively predicted by total dietary α -tocopherol. This appeared to be mainly due to synthetic α -tocopherol in foods and supplements and not to α -tocopherol naturally occurring in foods (Table 3). This effect might be stronger in the general population since vitamin E supplement use in this trial was limited to those containing less than 200% of the RDA for vitamins and minerals. Serum concentrations of γ -tocopherol, however, were not affected in an analogous way by dietary γ -tocopherol. Interestingly, total dietary α -tocopherol negatively predicted on serum γ -tocopherol and dietary γ -tocopherol negatively predicted α -tocopherol.

For both α - and γ -tocopherol, serum concentrations, but not diet, were important determinants of colon concentrations (Table 3). This is in agreement with the rate-limiting

function of the liver in the distribution of tocopherols to blood (24). A previous study found a stronger correlation of adipose tissue tocopherols with plasma tocopherols than with diet, and this agrees with our results in colon tissue (23). In addition, our results agree with previous studies in that obesity was positively associated with serum γ -tocopherol concentrations (25, 26). Since obesity increases colon cancer risk (27), this is a curious finding and points to the possible importance of other, non-tocopherol, related mechanisms in the obesity-cancer link. This finding contrasts with the lack of effect of obesity on serum α -tocopherol concentrations in our study. Literature reports on the associations of obesity with blood α -tocopherol have not been consistent, but serum cholesterol has been positively associated with blood α -tocopherol (16, 18, 25).

Lastly, we evaluated if supplement use affected tocopherol concentrations over time in the two diet groups. There was a trend ($p < 0.1$) for mean serum and colon γ -tocopherol to decrease over time in the Mediterranean diet group (Table 5). Perhaps with a longer intervention, this may become important. The recommended sources of MUFA in the Mediterranean intervention were olive oil, avocados, macadamia nuts and hazelnuts, none of which contain high amounts of γ -tocopherol (14).

For serum α -tocopherol, there was a significant 3-way interaction of Diet Group*Time*Supplement Use (Table 5). Concentrations of serum α -tocopherol changed significantly only in supplement users over time, and the differences between arms might reflect the very low vitamin E supplement intake in the Mediterranean arm at 6 months (Table 3). Unlike serum, there were no significant changes in concentrations of α - and γ -tocopherol over time in the colon tissue. This could reflect more highly regulated uptake into tissues. Alternatively, more time might be required for colon tissue tocopherol to be changed in response to a dietary decrease as was shown to be the case with adipose tissue (22). The lack of dietary intervention effects on colon tocopherols is consistent with the baseline data indicating that dietary tocopherol intakes did not significantly affect colon tocopherol concentrations.

Limitations of the present study include that only one sample was obtained at baseline and 6 months. For serum, multiple samples may be required for more reliable estimates of tocopherol concentrations (2). In addition, only four days of dietary intakes were captured at each time point, and the intervention was conducted over only six months. Other tocopherol isomers and none of the tocopherol metabolites were quantified, nor were sub-fractions of serum. Strengths of the study include the availability of colon mucosal biopsy tissue obtained without prior preparation of the bowels, which would preserve the normal biology of the tissue, the recruitment of an at-risk population, and the randomized design. The results did show that a Mediterranean diet increased natural α -tocopherol and decreased γ -tocopherol dietary intakes. These dietary changes had little influence on α - and γ -tocopherol concentrations in colonic mucosa. Serum tocopherol concentrations, age and BMI were, however, predictors of colon tocopherols indicating the important role of metabolic factors on colon tocopherol concentrations.

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TABLE 1

Demographic characteristics of subjects stratified by usage of dietary supplements containing vitamin E at any time while on study.

Variable	Non-Supplement Users n = 73 Number (%) or mean $\pm$ SE	Supplement Users n = 47 Number (%) or mean $\pm$ SE	P-value ¹
Mediterranean diet ²	39 (53%)	20 (43%)	0.245
Completed Study ²	53 (73%)	41 (87%)	0.058
Percent Goals ²	85 $\pm$ 3	84 $\pm$ 3	0.940
Female	50 (68%)	36 (77%)	0.336
Age, years	51 $\pm$ 1	55 $\pm$ 2	0.089
White	65 (89%)	40 (85%)	0.525
Current Smoker	11 (15%)	4 (9%)	0.289
College Graduate	56 (77%)	36 (77%)	0.988
Work Outside Home	59 (81%)	43 (91%)	0.110
Married/Committed	49 (67%)	30 (64%)	0.710
Body Mass Index, kg/m ²	27.7 $\pm$ 0.5	26.3 $\pm$ 0.5	0.056
Overweight or Obese	53 (73%)	27 (57%)	0.086
Physical activity ³ , MET/d	19.9 $\pm$ 1.8	19.0 $\pm$ 2.1	0.761
Usual alcohol intake ⁴ , g/d	6.4 $\pm$ 0.9	5.3 $\pm$ 1.3	0.472
Have arthritis	6 (8%)	12 (26%)	0.010
Regular use of NSAIDs ⁵	15 (20%)	10 (23%)	0.924

¹ P-values were derived from the independent t-test for continuous variables or the Pearson Chi-square test for categorical variables (2-sided).

² Subjects were randomized to a Mediterranean or a Healthy Eating diet for 6 months. The number of subjects who completed 6 months of study is given as well as the percentage of counseling goals met at 6 months by persons who completed 6 months of study.

³ Physical activity was calculated in metabolic equivalents/day from measured body weight at baseline and from reported walking, mild, moderate and hard activities in the past week from a questionnaire.

⁴ Usual intakes of beer, wine and liquor were asked on a questionnaire using usual servings sizes for each, and intakes were converted to grams of alcohol/day.

⁵ Regular use of non-steroidal anti-inflammatory agents (NSAIDS) was defined as daily or every other day.

TABLE 2

Baseline dietary intakes of tocopherols in subjects stratified by supplement use.

Dietary intake	Non-Supplement Users n = 73 Mean $\pm$ SE	Supplement Users n = 47 Mean $\pm$ SE ¹	P-value ²
Intakes from foods			
Vitamin E ³ , IU/d	14.8 $\pm$ 0.9	15.6 $\pm$ 1.7	0.672
Natural α -tocopherol, mg/d	9.1 $\pm$ 0.6	7.9 $\pm$ 0.4	0.107
Synthetic α -tocopherol, mg/d	1.3 $\pm$ 0.4	3.8 $\pm$ 1.7	0.127
γ -Tocopherol, mg/d	14.8 $\pm$ 0.9	12.8 $\pm$ 1.0	0.136
δ -Tocopherol, mg/d	3.2 $\pm$ 0.2	2.7 $\pm$ 0.2	0.216
β -Tocopherol, mg/d	0.6 $\pm$ 0.1	0.5 $\pm$ 0.0	0.036
Energy, kcal/d	2120 $\pm$ 73	2017 $\pm$ 88	0.375
Fat, % of energy	35.2 $\pm$ 0.8	33.5 $\pm$ 0.8	0.118
Polyunsaturated Fat, g/d	18.3 $\pm$ 1.0	16.5 $\pm$ 1.0	0.202
Fruits, servings/d	1.5 $\pm$ 0.1	1.8 $\pm$ 0.1	0.122
Vegetables, servings/d	2.9 $\pm$ 0.1	2.9 $\pm$ 0.2	0.956
Intake from supplements			
Vitamin E ³ , IU/d	-	31.4 $\pm$ 3.5	-

¹ A total of 43 subjects reported intakes of vitamins E from supplements at baseline and four additional subjects reported intakes at either 3 or 6 months.

² P-values are from two sample, independent t-tests for continuous variables.

³ The NDS-R program calculates vitamin E in supplements as IU only since many manufacturers provide this information only. Total vitamin E from foods is also given in IU for ease of comparison of the two sources of vitamin E.

TABLE 3

Predictors of serum and colon concentrations of tocopherols at baseline in linear regression models (n=120) ¹. The beta coefficient is given in parenthesis for significant predictors.

Tocopherol Concentration	Predictor	Change in R Square	P-value for F change (β)
Serum α -Tocopherol	Demographic (Age, Smoking, Alcohol, Gender)	0.015	0.789
	Demographic + BMI	0.002	0.661
	Demographic + Serum cholesterol	0.259	<0.001 (0.53)
	Demographic + Total dietary α -tocophero ²	0.113	<0.001 (0.34)
	Demographic + Natural α -tocopherol from food ²	0.002	0.627
	Demographic + Synthetic α -tocopherol from food ²	0.070	0.004 (0.27)
	Demographic + α -Tocopherol from supplements ²	0.080	0.002 (0.29)
	Demographic + Dietary γ -tocopherol	0.076	0.003 (-0.29)
Colon α -Tocopherol	Demographic (Age, Smoking, Alcohol, Gender)	0.120	0.005 ³
	Demographic + BMI	0.023	0.080
	Demographic + Serum cholesterol	0.002	0.631
	Demographic + Total dietary α -tocopherol	0.008	0.295
	Demographic + Natural α -tocopherol from food ²	0.009	0.290
	Demographic + Synthetic α -tocopherol from food ²	0.000	0.880
	Demographic + α -Tocopherol from supplements ²	0.015	0.166
	Demographic + Serum α -tocophereol	0.046	0.014 (0.07)
Serum γ -Tocopherol	Demographic (Age, Smoking, Alcohol, Gender)	0.034	0.411
	Demographic + BMI	0.145	<0.001 (0.40)
	Demographic + Serum cholesterol	0.132	<0.001 (0.38)
	Demographic + Dietary γ -tocopherol	0.012	0.241
	Demographic + Total dietary α -tocopherol	0.046	0.020 (-0.22)
Colon γ -Tocopherol	Demographic (Age, Smoking, Alcohol, Gender)	0.076	0.058 ³
	Demographic + BMI	0.040	0.025 (0.21)
	Demographic + Serum cholesterol	0.004	0.480
	Demographic + Dietary γ -tocopherol	0.002	0.631
	Demographic + Serum γ -tocopherol	0.054	0.009 (0.24)
	Demographic + Serum α -tocopherol	0.009	0.375

¹ Log-transformed serum and colon concentrations were used in the models to achieve a normal distribution. The P-values shown are for the change in F in the linear regression models. The demographic predictors were those determined at baseline: age in years, body mass index in kg/m², current smoking (yes/no) and usual alcohol intake in g/day from a questionnaire.

²Total dietary α -tocopherol intake was the sum of natural and synthetic α -tocopherol from foods and α -tocopherol from supplements, all in International Units (IU). At baseline, 40 subjects had any synthetic vitamin E intake and 43 subjects reported intake of vitamin E from dietary supplements.

³Demographic factors that could influence tocopherol concentrations were entered in the models in one step. For colon α -tocopherol, the coefficient for age was significant with $[\beta = 0.34, p < 0.001]$. For colon γ -tocopherol, the combination of demographic factors was of borderline significance with $p = 0.06$, but the coefficient for age was significant with $\beta = 0.26, p = 0.006$.

TABLE 4

Mean dietary intakes of tocopherols in each diet arm at baseline and 6 months for subjects randomized across two diet groups: Healthy Eating and Mediterranean. Data shown is mean + SE ¹.

Dietary Intakes ²	Healthy Eating Group		Mediterranean Group		P-value Fixed Factors	
	Baseline	6 months	Baseline	6 months	Time	Group*Time
	N=61	N=46	N=59	N=47		
Intakes from Foods						
γ-Tocopherol, mg/d	14.9 ± 1.2	12.7 ± 0.9	15.1 ± 1.1	9.7 ± 0.9 ³	<0.001	0.004
Natural α-tocopherol, mg/d	9.1 ± 0.7	9.6 ± 0.7	9.8 ± 0.8	12.9 ± 0.8 ³	<0.001	0.008
Synthetic α-tocopherol, mg/d	0.8 ± 1.3	3.2 ± 1.2	3.1 ± 1.6	3.6 ± 1.5	0.098	0.319
Vitamin E, IU/d	6.8 ± 1.3	9.6 ± 1.6 ³	9.7 ± 1.3	12.4 ± 1.7 ³	<0.001	0.680
Intake from Supplements						
Vitamin E, IU/d	15.3 ± 3.4	9.7 ± 3.4	8.5 ± 3.2	3.1 ± 3.3	0.473	0.603

¹The mixed linear regression models included the following covariates: age in years, current smoking, alcohol intake in grams per day, gender and BMI in kg/m². The models used data after natural log transformation to achieve normality. Untransformed means and SE from the regression models are shown for ease of interpretation.

²The data shown is the mean and SE for all subjects using a value of zero for the 73 subjects with no reported supplement intakes. Vitamin E from supplements is in International Units/day (IU = mg natural α-tocopherol/0.67 + mg synthetic α-tocopherol). Total vitamin E intakes from foods are also shown in IU for ease of comparison with intakes from supplements.

³Significantly different from baseline within that diet arm (p<0.05).

TABLE 5

Serum and colon tocopherol concentrations in each diet arm at baseline and 6 months for 120 subjects randomized across two diet groups: Healthy Eating and Mediterranean. Data shown is mean $\pm$ SE of tocopherols in serum and in colon mucosal biopsies. The p-values for the fixed factors of time, supplement use and diet group assignment are also shown from linear regression models of the data.

Variable	Healthy Eating Group		Mediterranean Group		P-values for Fixed Factors ¹			
	Baseline	6 months	Baseline	6 months	Time	User	Group*Time	Group*Time*User
Serum Concentration, nmol/l								
α -Tocopherol, all subjects	30.0 $\pm$ 2.0	31.3 $\pm$ 1.8	34.6 $\pm$ 1.9	31.1 $\pm$ 1.7	0.247	<0.001	0.079	0.004
Supplement users	31.1 $\pm$ 2.5	34.6 $\pm$ 2.2 ²	41.3 $\pm$ 2.9	35.3 $\pm$ 2.5 ²	-	-	-	-
Non-users	29.0 $\pm$ 2.4	27.9 $\pm$ 2.2	27.6 $\pm$ 2.0	27.2 $\pm$ 1.9	-	-	-	-
γ -Tocopherol, all subjects	4.29 $\pm$ 0.42	4.15 $\pm$ 0.35	4.56 $\pm$ 0.42	3.29 $\pm$ 0.34	0.054	0.724	0.403	0.814
Supplement users	3.83 $\pm$ 0.56	4.13 $\pm$ 0.43	4.82 $\pm$ 0.65	3.38 $\pm$ 0.48	-	-	-	-
Non-users	4.75 $\pm$ 0.53	4.15 $\pm$ 0.43	4.30 $\pm$ 0.46	3.22 $\pm$ 0.38	-	-	-	-
Colon Biopsy Concentration, pmol/mg protein								
α -Tocopherol, all subjects	687 $\pm$ 95	724 $\pm$ 128	775 $\pm$ 95	782 $\pm$ 128	0.999	0.054	0.246	0.732
Supplement users	803 $\pm$ 121	829 $\pm$ 172	943 $\pm$ 142	1061 $\pm$ 193	-	-	-	-
Non-users	571 $\pm$ 114	620 $\pm$ 165	597 $\pm$ 98	506 $\pm$ 149	-	-	-	-
γ -Tocopherol, all subjects	220 $\pm$ 31	224 $\pm$ 37	214 $\pm$ 31	163 $\pm$ 37	0.082	0.203	0.711	0.882
Supplement users	235 $\pm$ 41	271 $\pm$ 48	252 $\pm$ 48	199 $\pm$ 55	-	-	-	-
Non-users	204 $\pm$ 38	178 $\pm$ 48	177 $\pm$ 33	127 $\pm$ 42	-	-	-	-

¹The mixed linear regression models included the following covariates: age in years, current smoking, alcohol intake in grams per day, gender and BMI in kg/m². Transformations required to achieve normality were log for tocopherol measures and natural log for dietary and supplement nutrient intakes. Untransformed means and SE are shown for ease of interpretation. Of the 120 subjects, 61 were in the Healthy group and 59 in the Mediterranean group. At 6 months, there were 46 and 47 subjects in the two groups, respectively. One serum sample at baseline and one biopsy sample at 6 months were not available for analysis of tocopherols.

²Significantly different from baseline within that group from pairwise analysis.