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# Plasma α-Tocopherol, γ-Tocopherol and Incident, Sporadic Colorectal Adenoma Risk: A Pooled Case-Control Study

By

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Master of Public Health

Department of Epidemiology

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Bachelor of Administration
Nanjing Medical University
2012

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# Abstract

Plasma α-Tocopherol, γ-Tocopherol and Incident, Sporadic Colorectal Adenoma Risk: A Pooled Case-Control Study

By Liang Tao

**Background:** Since evidence on an association between tocopherols and colorectal cancer is limited, and mechanistic results from cell and animal models have been inconsistent with those from human studies, we investigated associations of plasma α-tocopherol (α-T) and γ-tocopherol (γ-T) concentrations with risk of incident, sporadic colorectal adenomas in a pooled colonoscopy-based case-control study.

**Methods:** We used pooled data collected in two previously conducted case-control studies (n = 184 adenoma cases and 250 controls). Multivariable logistic regression was used to estimate the associations of tocopherols with adenoma risk. Stratified analyses and the likelihood ratio test were used to examine effect modification by various risk factors and adenoma characteristics.

**Results:** No clear patterns of associations between the two tocopherols and incident, sporadic colorectal adenomas were found. Participants in the highest relative to those in the lowest tertile of plasma α-T levels were at an estimated 20% lower risk for adenoma, and those in the highest relative to the lowest tertile of plasma γ-T were at an estimated 24% higher adenoma risk. However, these results were not statistically significant, and there were no dose-response patterns to the associations across the tertiles. In stratified analyses, among those who regularly took NSAIDs, there was a stronger inverse association of α-T with adenomas (OR for those in the highest vs. the lowest tertile = 0.45 (95% CI 0.21, 1.00; $P_{trend}$ 0.03). Associations of plasma α-T and γ-T did not substantially differ by other demographic, lifestyle, dietary risk factors or by adenoma characteristics.

**Conclusion:** The results from this small case-control study suggest that high circulating α-T may be associated with lower, and high circulating γ-T may be associated with higher risk of incident sporadic colorectal adenomas, and support further investigation in a larger, preferably prospective study.

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# Chapter 1. Background

Colorectal cancer, the third most commonly diagnosed cancer in the world, but more common in developed countries, is the second leading cause of cancer deaths in the US. While the molecular basis and physiological mechanisms of colon carcinogenesis is becoming clearer, the prognosis of advanced disease has not been improved over the last 20 years (1). Two primary environmental exposures, diet and physical activity play important roles in the etiology of the disease based on the international ecologic and migration studies.

As reviewed before (2, 3), the strongest risk factors are autosomal dominant familial adenomatosis polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC) genetic syndromes, and extensive ulcerative colitis. Some environmental risk factors associated with colorectal cancer include age, inflammatory diseases, pelvic irradiation, smoking and alcohol intake, obesity, cholecystectomy, hormone replacement therapy (HRT), physical activity, NSAID use, and high red meat, fat, vegetable fruit, fiber, folate, and calcium diet *etc*.(2)

As a multi-step process including step-wise acquisition of molecular and genetic defects by the colon epithelial cells (1), most sporadic colorectal cancer arises from adenomas: approximately 50% of the Western population develops an adenoma by the age of 70 (4). Some mechanisms have been proposed to explain the initiation and progression of colorectal cancer. For example, based on the adenoma/carcinoma sequence described by Hill (5), loss or mutation of the Adenomatous Polyposis Coli (*APC*) gene will induce polyp formation. During this period, β-catenin and T-cell factor will be involved, resulting in failure of proper adhesion and migration of cells as well as the transcription of a proliferative signal that can affect the *c-MYC* gene (6).

Other mechanisms include 1) hypermethylation of multiple genes (e.g., the mismatch repair genes *MLH1* and *MSH2*) resulting in mutations in the *BAX* and *TGFβRII* genes; 2) chronic inflammation, resulting in aneuploidy and *p53* gene mutation; 3) age- and HRT-related hypermethylation of the estrogen receptor (*ER*) gene (7).

Discovered more than 80 years ago, vitamin E (VE) has been extensively studied as an essential component in human health. VE families consist of two categories, tocopherols and tocotrienols. Depending upon the difference in the number and the position of methyl groups substituted on the ring, both tocopherols and tocotrienols can be grouped into four categories: α, β, γ, and δ. Alpha-tocopherol (α-T) is trimethylated at the 5, 7, and 8 positions on the chromanol ring, while gamma-tocopherol (γ-T) is methylated at the 7 and 8 positions.

In nature, the most abundant and richest source of VE is plant seeds and products derived from them, such as soybeans, corn, and sesame, as well as nuts such as walnuts, peanuts, and pecans, *etc*. In the human body, α-T is the primary form absorbed and accumulated. Upon ingestion, tocopherols are indiscriminately transported to the liver through the lymphatic system. But in the liver, the α-T transfer protein preferentially combines with α-T, and then returns it to the plasma, while very few of γ-T and other forms can be transferred by this mechanism. The latter forms in liver are more actively metabolized through side-chain degradation by the ω-oxidation/β-oxidation pathway (8). Metabolites of α-T and γ-T, α-CEHC [2,5,7,8- tetramethyl-2-(2′ -carboxyethyl)-6-hydroxychroman] and γ- CEHC [2,7,8-trimethyl-2- (2′ carboxyethyl)- 6-hydroxychroman], respectively, are excreted and can be found in plasma, urine, and bile.

Past studies found that high intakes of α-T lower blood and tissue γ-T levels, and high intakes of γ-T lower blood and tissue α-T levels. For instance, in a randomized, placebo-

controlled trial in 184 adult nonsmokers, supplementing diets with α-T acetate (400 IU/d) reduced serum γ-T concentrations by a median change of 58% (9). Sundl *et al*. found that a daily supplement of 1,000 IU of α-T for 4 days could decrease γ-T concentrations and the ratio of α-/γ-T in plasma (10). In another trial by Yoshikawa *et al.* with 13 health adult male volunteers, participants who took 2g γ-T/day were found to have decreased plasma α-T levels after 28 days (11). In addition, in the Women's Health Initiative study (12), serum α-T was found to be inversely correlated with γ-T intake from food ($r = - 0.09$, $P< 0.01$) in 1,047 postmenopausal women aged 50-79.

Because VE has antioxidant effects, which is mainly due to its ability to donate phenolic hydrogens (electrons) to lipid radicals and terminate chain reactions of oxidation of polyunsaturated fatty acids as peroxyl radical scavengers (13), and the presence of it in feces is able to block the process of lipid peroxidation and thereby reduce the formation of mutagenic peroxidation products arising from the oxidation (14), it is considered to potentially play an important role in decreasing risk for colorectal cancer. Since α-T is the primary form in the human body and in VE supplements, it is the form most studied in relation to colorectal cancers. However, the results from these studies are inconsistent and some of them even yielded negative results.

Longnecker and colleagues conducted a meta-analysis of five prospective, nested case-controls studies including 289 cases of colorectal cancer and 1,267 matched controls, and found that the odds ratio for those in the highest relative to those in the lowest quintile of serum α-T was 0.6 (95% CI: 0.4, 1.0) for cancers of the colon and rectum (15). However, none of the results of the individual studies was statistically significant. In the Physicians' Health Study II

Randomized Control Trial, supplementation with 400 IU of α-T every other day in physicians for 8 years did not reduce the incidence of prostate cancer or of all other cancers combined (16). In the Women's Health Study with 39,876 healthy U.S. women aged 45 years or older, the administration of 600 IU of α-T on alternate days had no significant effects on the incidence of colon cancer (17). In addition, in the HOPE trial, which included more than 9,000 male and female participants, participants were asked to take α-T (400 IU/day) and were followed for an average of 4.5 years. At the end of the study, 11.5% (n = 552) of the participants given α-T were diagnosed with cancer (including colon cancer) compared to 12.3% (n = 586) of those taking placebo (relative risk [RR]: 0.94, 95% confidence interval [95% CI]: 0.84-1.06) (18). After an additional study, HOPE-TOO, 4% lower risk was found in the α-T intake group compared to the placebo group (RR: 0.96, 95%CI: 0.84-1.09) (18). Therefore, in general, the inconsistent and unconvincing results from the observational and clinical studies may reflect that circulating α-T may not be associated with colorectal cancer risk.

Interestingly, γ-TmT, a γ-tocopherol-rich natural mixture of tocopherols consisting of 568 mg γ-T, 130 mg α-T, 15 mg β-T, and 243 mg δ-T, has shown surprising effects against lung, colon, mammary gland, and prostate cancer in rats and human cell lines (19). Moreover, the results from basic science studies in animal models suggest that γ-T may be more potent in colorectal cancer chemoprevention. For example, Campbell and colleagues found that treatment with γ-T resulted in significant cell death in all colon cancer cell lines tested (SW480, HCT-15, HCT-116 and HT-29), while α-T did not (20). Fei Guan *et al.* also reported that γ-T but not α-T inhibited colon carcinogenesis in aoxymethane-treated F344 rats when given in identical doses (0.2%) in their diets (21). Jiang *et al.* found that γ-T could inhibit protein nitration, pro-inflammatory eicosanoids, attenuate inflammation-induced ascorbate oxidation, suppress pro-

inflammatory cytokines in rats, and inhibit COX activity in human macrophages and epithelial cells and rats (22-24). Gysin *et al.* found that γ-T was more effective in inhibiting cell proliferation, cell cycle progression, and DNA synthesis in CaCo-2 colon cell lines (25).

Based on the results of the above and other experimental and animal model studies, some mechanisms in relation to chemical reactivity, biological activity and metabolism have been proposed to explain the anti-carcinogenic mechanisms of α-T and γ-T. For example, both α-T and γ-T have similar antioxidant properties, but γ-T is more capable of inactivating the genotoxic substances generated in the gastrointestinal tract from dietary constituents, and neutralizing lipid peroxides, bile acids and other auto-oxidation products. Sjohol and colleagues showed that γ-T but not α-T could prevent IL-1β induced RINm5F toxicity (26). Through a chemical reaction, γ-T can more actively terminate the modifications and nitrosation reactions in DNA caused by reactive nitrogen species from fecal bacteria, or by the production of mutagenic aldehydes such as malondialdehyde from lipids containing PUFA. In addition, γ-T is found to possess better antioxidant-independent functions. For instance, γ-T and γ-CEHC, but not α-T, can suppress prostaglandin $E_2$ synthesis in lipopolysaccharide (LPS)-stimulated macrophages and in interleukin-Iβ-activated epithelial cells, as well as directly inhibit COX-2 activity in intact cells without affecting the expression of the COX-2 protein (24). Prostaglandins and COX-2 expression are known as biomarkers related to inflammation that may play a role in reducing carcinogenesis. Therefore, γ-T should be more potent in decreasing inflammation and risk of colorectal cancer and adenomas. Other mechanisms included inducing colon cancer cellular apoptosis (27), down regulating the *Ras* genes (mutations of which is an early event in colorectal carcinogenesis) (28), and inhibiting proliferation of colon cancer cell lines through reducing in the levels of cyclin D1 and cyclin E (29), *etc.*

However, few studies had been conducted to assess the association of γ-T with colorectal cancer. In a case-control study with 332 colorectal adenomas and 363 controls, Ingles *et al.* found that higher plasma α-T and lower plasma γ-T levels were associated with decreased occurrence of large (≥ 1cm) but not of small (< 1 cm) adenomas (30), but the results were not statistically significant.

In conclusion, since few related human studies are available, and mechanistic results from cell and animal models have been inconsistent with those from human studies, further investigation in humans is needed. Herein we report an investigation of associations of circulating γ-T and α-T with risk of incident, sporadic colorectal adenomas in a pooled colonoscopy-based case-control study.

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# Chapter 2: Manuscript

Plasma α-Tocopherol, γ-Tocopherol and Incident, Sporadic Colorectal Adenoma Risk:
A Pooled Case-Control Study

By Liang Tao

## Abstract

**Background:** Since evidence on an association between tocopherols and colorectal cancer is limited, and mechanistic results from cell and animal models have been inconsistent with those from human studies, we investigated associations of plasma α-tocopherol (α-T) and γ-tocopherol (γ-T) concentrations with risk of incident, sporadic colorectal adenomas in a pooled colonoscopy-based case-control study.

**Methods:** We used pooled data collected in two previously conducted case-control studies (n = 184 adenoma cases and 250 controls). Multivariable logistic regression was used to estimate the associations of tocopherols with adenoma risk. Stratified analyses and the likelihood ratio test were used to examine effect modification by various risk factors and adenoma characteristics.

**Results:** No clear patterns of associations between the two tocopherols and incident, sporadic colorectal adenomas were found. Participants in the highest relative to those in the lowest tertile of plasma α-T levels were at an estimated 20% lower risk for adenoma, and those in the highest relative to the lowest tertile of plasma γ-T were at an estimated 24% higher adenoma risk. However, these results were not statistically significant, and there were no dose-response patterns to the associations across the tertiles. In stratified analyses, among those who regularly took NSAIDs, there was a stronger inverse association of α-T with adenomas (OR for those in the highest vs. the lowest tertile = 0.45 (95% CI 0.21, 1.00; $P_{trend}$ 0.03). Associations of plasma α-T and γ-T did not substantially differ by other demographic, lifestyle, dietary risk factors or by adenoma characteristics.

**Conclusion:** The results from this small case-control study suggest that high circulating α-T may be associated with lower, and high circulating γ-T may be associated with higher risk of incident sporadic colorectal adenomas, and support further investigation in a larger, preferably prospective study.

# Introduction

Since being discovered more than 80 years ago, vitamin E (VE) has been extensively studied as an essential component in human health. Depending upon the difference in the number and the position of methyl groups substituted on the ring, tocopherols can be grouped into four categories: α, β, γ, and δ. Alpha-tocopherol (α-T) is trimethylated at the 5, 7, and 8 positions of the chromanol ring, while gamma-tocopherol (γ-T) is methylated at the 7 and 8 positions. In nature, the most abundant and richest sources of VE are plant seeds and products derived from them, such as soybeans, corn, and sesame, as well as nuts such as walnuts, peanuts, and pecans *etc*. Among these vegetables oils, γ-T is the most prevalent form of VE whereas α-T accounts for appreciable amounts only in almonds and sunflower and olive oils (31). For example, soybean oil is relatively high in Americans' diets. This vegetable oil contains about 70 mg γ-T per 100 g, but only 7 mg α-T (32). Traber *et al.* reported that more than 70% of the VE consumed in USA is γ-T (8).

Unlike other fat-soluble vitamins, VE is not accumulated in the human body. Upon ingestion, tocopherols are indiscriminately transported to the liver through the lymphatic system. But in the liver, α-T transfer protein preferentially combines with α-T, and then returns it to the plasma, while very few γ-T and other forms can be transferred by this mechanism. The latter forms in the liver are more actively metabolized through side-chain degradation by the ω-oxidation/β-oxidation pathway (8). Metabolites of α-T and γ-T, α-CEHC [2,5,7,8-tetramethyl-2-(2′ -carboxyethyl)-6-hydroxychroman] and γ-CEHC [2,7,8-trimethyl-2- (2′ carboxyethyl)- 6-hydroxychroman], respectively, are excreted and can be found in plasma, urine, and bile.

Previous studies found α-T and γ-T in the body to be inversely associated with each other after dietary and supplementary consumption. Intake of α-T and γ-T will increase the level of

themselves and their metabolites, α-CEHC and γ-CEHC, respectively, in blood and urine, but intake of a dose of supplementary α-T will decrease the blood level of γ-T while increasing γ-CEHC, and *vice versa* for α-T and α-CEHC when taking γ-T supplements. In a randomized, placebo-controlled trial in 184 adult nonsmokers, supplementing diets with α-T acetate (400 IU/d) reduced serum γ-T concentrations by a median change of 58% (9). Sundl *et al*. also reported that a daily supplement of 1,000 IU of α-T for 4 days could decrease γ-T concentrations and the ratio of α-/γ-T in plasma (10).

Colorectal cancer, the third most commonly diagnosed cancer in the world, but more common in developed countries, mostly develops in pre-cancerous adenomatous polyps (2, 3). Because VE has antioxidant effects, mainly due to its ability to donate phenolic hydrogens (electrons) to lipid radicals and terminate chain reactions of oxidation of polyunsaturated fatty acids as peroxyl radical scavengers (13), and the presence of it in feces is able to block the process of lipid peroxidation and thereby reduce the formation of mutagenic peroxidation products arising from the oxidation (14), it is considered to potentially play an important role in decreasing the risk of colorectal cancer. In *vitro* and *vivo*, VE in some studies reduced the number of chemically-induced colonic adenomas and carcinomas in mice and in human colon cancer cell lines (33). Bostick *et al.* found that high VE intake was associated with lower risk of colorectal cancer in women under the age of 65 years (34). However, other epidemiology studies found no association of VE with colorectal neoplasms (35, 36).

Unlike its distribution in nature, α-T is the most abundant form of VE in the human body. Also, it has been the most commonly used VE supplement intervention studies, the results of which have yielded negative results. Interestingly, γ-TmT, a γ-tocopherol-rich natural mixture of tocopherols consisting of 568 mg γ-T, 130mg α-T, 15mg β-T, and 243 mg δ-T, has shown

surprising effects against lung, colon, mammary gland, and prostate cancer in rats and human cell lines (19). Moreover, the results from basic science studies in animal models suggest that γ-T may be more effective in inhibiting colorectal carcinogenesis. Campbell and colleagues found that treatment with γ-T resulted in significant cell death in all colon cancer cell lines tested (SW480, HCT-15, HCT-116 and HT-29), while α-T did not (20). Fei Guan *et al.* reported that γ-T but not α-T inhibited colon carcinogenesis in aoxymethane-treated F344 rats when given in identical doses (0.2%) in the diet (21). Thus, γ-T may be a potential better candidate and biomarker for preventing colorectal cancer.

In our previous case-control studies we found that plasma γ-T level was higher in adenoma cases. Since few related human studies are available, and the mechanistic results from cell and animal models have been inconsistent with those from human studies, herein we report an investigation of associations of circulating γ-T and α-T concentrations with risk of incident, sporadic colorectal adenomas in a pooled colonoscopy-based case-control study.

## Methods

### Study Population

In this study we used pooled data collected in two previously conducted colonoscopy-based case-control studies of incident, sporadic colorectal adenoma. The two studies were carried out in two different U.S. states by the same principal investigator (RMB). The Markers of Adenomatous Polyps I study (MAP I), was conducted in community gastroenterology practices in Winston-Salem and Charlotte, North Carolina from 1994-1997 to assess the validity of colonic epithelial cell proliferation as a biomarker of the risk for sporadic colorectal adenomas; and the Markers of Adenomatous Polyps II study (MAP II), using the same design of the former was

conducted at Consultants in Gastroenterology, PA, a large and private practice in Columbia, South Carolina in 2002, to investigate associations of the expression patterns of various genes and cell cycle markers in the normal-appearing colorectal mucosa with risk for incident, sporadic adenomas,.

Participants in these two case-control studies were 30-74 years of age, with no prior history of colorectal adenomas, known genetic syndromes associated with colonic neoplasia, or inflammatory bowel diseases. They were all recruited from the patients who were scheduled to undergo outpatient, elective colonoscopy at the study sites. Detailed study protocols and methods for the two studies were previously published. (MAP I: (37, 38), MAP II: (39, 40)).

In the MAP I study, 669 (30% of a total of 2,246) colonoscopy patients identified were eligible to participate, and of these, 617 (92%) were contacted and 417 (68%) agreed to participate. In the MAP II study, 305 (87% of a total of 351) colonoscopy patients identified were eligible to participate, and of these, 232 (76%) were contacted and agreed to participate. Participants who met final eligibility criteria for this analysis (excluding those who reported consuming less than 600 kcal/day or more than 6,000 kcal/day total energy intake) consisted of 181 adenoma cases, and 179 polyp free controls in the MAP I study and 46 adenoma cases and 119 polyp free controls in the MAP II study.

For both of the studies, fasting peripheral venous blood was collected at the clinic visit. Blood samples were drawn into red-coated, pre-chilled vacutainer tubes, and then immediately placed on ice and shielded from light. Blood fractions were aliquotted into amber-colored cryopreservation tubes, the air was displaced with an inert gas (nitrogen in MAP I and argon in MAP II), and then the aliquots were stored in a -70$^{o}$ C freezer until analysis. Since the present

study was conducted after most of the stored plasma samples were exhausted in previous studies, not all samples were available. Therefore, the analyses we report herein only included those with plasmas samples. The final number of participants with plasma samples in MAPI was 144 adenoma cases and 144 controls, and in MAP I it was 40 adenoma cases and 106 controls. Data from the two studies were combined for the following data analysis.

The study protocols for the two studies were approved by the respective Institutional Review Boards of the corresponding institutions. Participants were all able to understand and provide informed consent.

**Data Collection**

Before undergoing colonoscopy, patients in the two studies all completed questionnaires to elicit information on demographics, family history of colon cancer and other diseases, medications such as aspirin and other nonsteroidal anti inflammatory drugs (NSAID), lifestyle, physical activity, body size characteristics such as BMI and WHR, use of alcohol and tobacco, and *etc.* Dietary and nutritional supplement intakes were obtained using a modified 153-item Willett food frequency questionnaire (41).

**Case-control assignment**

The results of complete colonoscopies were recorded on standardized forms and included information on the colon site and size and shape of any polyp. Upon detection and removal, adenomas and hyperplastic polyps were verified by an index study pathologist using diagnostic criteria established for the National Polyp Study (42). Information of adenoma characteristics

was also collected during verification, including histologic type, location of the largest adenoma, multiplicity, size of the largest adenoma, shape, and degree of atypia of the worst adenoma.

According to the results of the colonoscopy and pathology exams, participants without adenomas and hyperplastic polyps were considered controls, and others were assigned into the following case groups: 1) adenoma cases and 2) hyperplastic polyp cases. Since the primary outcome of interest was adenoma, and the sample size or the hyperplastic polyp cases was limited, we included only the adenoma cases in this report.

**Laboratory methods**

Plasma α- and γ-T, along with carotenoids (including α- and β-carotene, β-cryptoxanthin, lutein + zeaxanthin, and lycopene) were measured using high-performance liquid chromatography-based as described in detail previously (43-45). All laboratory assays were performed at the Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota Medical Center, Fariview.

**Statistical Analysis**

Prior to analysis, all continuous variables were log or square root transformed to normalize their skewed distributions. Basic characteristics were compared between cases and controls using the t-test for continuous variables and Fisher's exact test for categorical variables. Since both plasma α- and γ-T levels were statistically different between the two studies, they were compared using general linear models (GLM) adjusted for study, age, and sex.

Study-specific tertiles of plasma α- and γ-T levels were calculated based on their distributions in the controls in their respective study. We used unconditional logistic regression

models to assess the associations between tertiles of plasma α- and γ-T levels and risk of colorectal adenoma, with control for confounding as described below. In addition, we investigated the associations stratified by potential effect-modifying variables such as age, sex, aspirin or NSAID use, family history of colorectal cancer in a first degree relative, current smoking/drinking status, and obesity, which were dichotomized based on the study-specific median distributions in the controls. A corresponding interaction term was included in the model to test the statistical significance of the term using the log-likelihood ratio test. We also examined the associations between plasma α- and γ-T tertiles and adenomas according to adenoma characteristics (location and size of the largest adenoma, multiplicity, and pathologic subtype).

Based on biologic plausibility and the significant difference in plasma α- and γ-T levels between the two studies, we included age, sex, study, circulating cholesterol level, and total energy intake (kcal/day) into the crude model. Other covariates were added if including them in the model changed the estimate for the primary exposure by ≥ 10%. Some of the latter variables were excluded if they were highly correlated with other model variables (Pearson correlation > 0.6). Next, some covariates were deleted from the final model if dropping them did not appreciably affect the estimate for the primary exposure variable. Final covariates included in the multivariate-adjusted model for α-T were age, sex, study, and serum cholesterol level; and for γ-T, they were age, sex, study, and serum cholesterol, α-carotene, and β-carotene levels.

The results were expressed as odds ratios with 95% confidence intervals. Tests for linear trend were performed by creating a continuous variable using the median value within the tertiles of plasma α-T and γ-T, respectively, and using that variable as the predictor of interest in the logistic models. All statistical tests were two-sided and considered statistically significant at P <

0.05. All statistical analyses were conducted with SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina).

## Results

Selected characteristics of the cases and controls are summarized in Table 1. Adenoma cases were more likely than controls to be male and be current smokers, and, on average, have a higher waist-hip ratio and greater intakes of total energy, fiber, cholesterol, dietary and supplementary vitamin E, dietary vitamin D, and all forms of fat. Compared to adenoma cases, controls were more likely to have a family history of colorectal cancer in a first-degree relative, take NSAIDs regularly (more than once a week), and take a multivitamin supplement; less likely to smoke; and, on average, to be younger and have higher intakes of fruits and vegetables. Hyperplastic polyp cases compared to controls were more likely to be male, currently smoke, and, on average, have a higher BMI and waist-hip ratio, and consume more caffeine and soy products (data not shown). In addition, the controls had higher mean plasma β-carotene and β-cryptoxanthin levels than did the adenoma cases, and a higher plasma α-carotene level than did the hyperplastic polyp cases. Overall, the mean plasma γ-T level in the adenoma cases was statistically significantly 10.5% higher than in the controls. There were no substantial differences in the characteristics between participants with and without plasma samples (data not shown).

In the pooled analyses (Table 2), those in the highest relative to the lowest tertile of plasma α-T were at an estimated 20% lower risk for colorectal adenoma; however, the finding was not statistically significant and there was no dose-response pattern to the association across the tertiles. In contrast, those in the highest relative to the lowest tertile of plasma γ-T were at an estimated 24% higher risk for adenoma, but this finding was not statistically significant and there

was no dose-response pattern to the association across the tertiles. After multivariable adjustment, these associations remained similar (Table 2).

We also examined the associations stratified by age, sex, family history of colorectal cancer in a first degree relative, regular NSAID (including aspirin) use, current smoking status, and BMI (Table 3). The sample size for these analyses was limited and there were no clear patterns of differences between the strata and no statistically significant multiplicative interactions. Among those who regularly took an NSAID, there was a stronger inverse association of α-T with adenomas (OR for those in the highest vs. the lowest tertile = 0.45 (95% CI 0.21, 1.00; $P_{trend}$ 0.03); however, this finding was one among the many comparisons made.

Among all cases, 27.8% had their largest adenoma located in the right colon, 40.2% had multiple adenomas, 14.7% had at least one adenoma with a diameter ≥ 1 cm, and 38% had a mild degree of atypia in their worst adenoma. The worst adenoma had a pedunculated shape in 10.9% of cases and was of villous or tubulovillous histology in 28.7% of cases.

Associations of plasma α-T and γ-T levels with adenoma according to adenoma characteristics are shown in Table 4. As for the stratified analyses described above, the sample size was small, especially for some strata, the confidence intervals were extremely wide, and there were no statistically significant multiplicative interactions. In light of the markedly overlapping confidence intervals and multiple comparisons, no clear patterns of differences across strata were noted.

## Discussion

In this pooled study, we observed that cases in the highest relative to the lowest tertile of plasma α-T were at an estimated 20% lower risk, and those in the highest relative to the lowest tertile of plasma γ-T were at an estimated 24% higher risk, respectively, for incident sporadic colorectal adenomas. Neither of the associations was statistically significant and there was no dose-response pattern. The sample size for stratified analyses in our study was limited; only one of the multiple comparisons across the various strata of demographic, lifestyle, and dietary risk factors, or according to adenoma characteristics suggested an association among a particular sub-group of individuals: among those who regularly took NSAIDs, there was a stronger inverse association of α-T with adenomas.

Previous results of experimental and animal model studies suggest that all tocopherols may play a role in preventing cancers, while γ-T may be a more potent form than α-T, and some mechanisms in relation to its chemical reactivity, biological activity and metabolism have been proposed to explain this. For example, both α-T and γ-T have similar antioxidant properties, but γ-T is more capable in inactivating the genotoxic substances generated from dietary constituents in the gastrointestinal tract, and in neutralizing lipid peroxides, bile acids, and other auto-oxidation products. Sjohol *et al*. found that γ-T but not α-T could prevent IL-1β induced RINm5F toxicity (26). Through a chemical reaction, γ-T can more actively terminate the modifications and nitrosation reactions in DNA caused by reactive nitrogen species from fecal bacteria, or by the production of mutagenic aldehydes, such as malondialdehyde, from lipids containing PUFA. In addition, γ-T was found to possess better antioxidant-independent functions. For instance, γ-T and γ-CEHC, but not α-T, can suppress prostaglandin $E_2$ synthesis in lipopolysaccharide (LPS)-stimulated macrophages and in interleukin-Iβ-activated epithelial cells, as well as directly inhibit

COX-2 activity in intact cells without affecting the expression of the COX-2 protein (24). Prostaglandins and COX-2 expression are known as biomarkers related to inflammation that may play a role in reducing carcinogenesis. Therefore, γ-T should be more potent in decreasing inflammation and risk of colorectal cancer and adenomas. Jiang *et al.* found that only γ-T could inhibit the pro-inflammatory eicosanoids and cytokines, and attenuate inflammation-mediated damage in a rat model (23). Other mechanisms included inducing colon cancer cellular apoptosis (27), down-regulating *Ras* genes (mutations of which is an early event in colorectal carcinogenesis (28), and inhibiting proliferation of colon cancer cell lines through reducing levels of cyclin D1 and cyclin E (25), *etc.*

Our results support those from some previous studies that indicated that a high concentration of circulating α-T is associated with lower risk of colorectal cancer. For example, in a meta-analysis of five prospective, nested case-control studies, including 289 cases of colorectal cancer and 1,267 matched controls, the odds ratio for the highest quintile of serum α-T relative to the lowest was 0.6 (95% CI: 0.4, 1.0) for cancers of the colon and rectum (15). Similar to ours, the results of the individual studies in this meta-analysis were also not statistically significant. However, we found no such associations for plasma γ-T. In our study, high plasma γ-T levels were associated with higher adenoma risk, which was inconsistent with the previous experimental and animal results, although our results were not statistically significant.

The associations of intakes of α-/γ-T with their corresponding changes in circulating levels may contribute to our confusing results for high plasma γ-T. As described before, high intakes of α-T would lower blood and tissue γ-T level, and high intakes of γ-T would also lower blood and tissue α-T levels. Since the primary form in supplementary VE is α-T, high-dose VE supplements will result in increasing circulating α-T and decreasing γ-T, which is consistent with

the results in our study in that both high plasma α-T and low γ-T were associated with lower risk of adenoma (Table 2b). Likewise, the positive association of low α-T and high γ-T with higher adenoma risk may be caused by the high intakes of γ-T. Via this mechanism, the protective effects of one may be offset by a lower level of the other, and such a phenomenon has been described before (19). Whereas the reason why high circulating γ-T level would be associated with higher risk of adenoma even with better cancer chemopreventive effects is still unknown. In addition, other risk factors for colorectal cancer may contribute to the results. For example, the difference between the association of α-T with colorectal adenomas among NSAIDs users and non-users suggest that the cancer chemoprevention effects of tocopherols are in relation to inflammation and oxidative stress risk factors. Although most past studies found γ-T to be a strong anti-inflammation agent, Berdnikovs *et al.* (46) recently found that γ-T could elevate inflammation in experimental asthma and even ablate the anti-inflammatory benefit of α-T in an amount as little as 10% of that of α-T during inflammation by regulating leukocyte recruitment in cell lines and mice. Therefore, γ-T at a high circulating level may possess pro-inflammatory effects, which may neutralize the cancer chemopreventive effects of itself and of α-T, or even directly increase adenoma risk by a pro-inflammatory process. To fully understand these complicated associations, future studies should assess the interaction of dietary/circulating α-T and γ-T, while taking other risk factors related to colorectal cancer and tocopherols into consideration.

For the associations of tocopherols with adenoma characteristics, our study indicated a different result from those of previous studies. In the study conducted by Ingles *et al.* including 332 colorectal adenoma cases and 363 controls, higher plasma α-T and lower γ-T levels were associated with a lower occurrence of large (≥ 1 cm) adenomas but not small (< 1 cm) adenomas

(30). In contrast, our study indicated that both higher plasma α-T and lower γ-T levels were associated with having multiple adenomas, while only lower α-T was associated with lower risk (Table 4). However, because of our small sample size for these analyses, our results had limitations, and further studies on these associations are required.

This study has some other limitations. Because most of the participants were white (> 90%), the results from this analysis may not be representative of the general population. Since we had depleted our stored plasma samples in previous studies, data on plasma α-T and γ-T on 19% adenoma cases and 16% of controls were unavailable, but we found no differences in the demographic and dietary characteristics between participants with and without tocopherol measurements (data not shown). In addition, because our study is a case-control study, the temporality of circulating α-T and γ-T levels with respect to adenoma incidence cannot be established. Also, we did not measure α-T and γ-T levels in colon tissue.

Our study was one of the few case-control studies to assess the association of tocopherols with colorectal adenomas in human. We minimized outcome misclassification and reduced recall bias and unmeasured confounding through careful and detailed colonoscopy to verify adenoma status and the polyp-free status of controls, collected detailed information on all of the multiple covariates before case-control status ascertainment.

In conclusion, the results from this small case-control study suggest that high circulating α-T may be associated with lower, and high circulating γ-T may be associated with higher risk of incident sporadic colorectal adenomas, and support further investigation in a larger, preferably prospective study.

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# Tables

**Table 1.** Selected Characteristics of Cases and Controls in the Pooled MAP Studies, United States, 1994-2002.

| Characteristics | Cases (n = 184) | Controls (n = 250) | *P* value[a] |
|---|---|---|---|
| Age, years | 57.8 (8.1) | 55.5 (9.4) | < 0.01 |
| Male (%) | 58[a] | 37 | < 0.01 |
| Caucasian race (%) | 99 | 92 | 0.92 |
| College education (%) | 23 | 28 | 0.17 |
| Family history of colorectal cancer in 1st degree of relative(%) | 20 | 30 | 0.02 |
| Regularly take an NSAID (≥ once a week) (%) | 23 | 36 | < 0.01 |
| Regularly take aspirin (≥ once a week) (%) | 34 | 36 | 0.76 |
| Postmenopausal (women only) (%) | 85 | 78 | 0.29 |
| HRT user (%) (women only) | 73 | 74 | 0.86 |
| Currently smoke (%) | 31 | 16 | < 0.01 |
| Currently drink (%) | 54 | 46 | 0.14 |
| BMI, $kg/m^2$ | 27.9 (6.4) | 27.9 (6.2) | 0.96 |
| Physical activity[b], MET-hours/week | 197 (139) | 190 (123) | 0.58 |
| Multivitamin supplement user (%) | 30 | 41 | 0.03 |
| Total vitamin E, mg/day | 75 (168) | 72 (147) | 0.50 |
| Supplements | 65 (167) | 64 (147) | 0.03 |
| Dietary | 9.8 (6.2) | 8.6 (6.2) | < 0.01 |
| Total energy, kcal/day | 2,016 (769) | 1,840 (775) | < 0.01 |
| Folate, µg/day | 443 (237) | 480 (269) | 0.32 |
| Fiber, gm/day | 21.6 (9.0) | 20.3 (10.6) | 0.02 |
| Total calcium, mg/day | 802 (427) | 871 (457) | 0.16 |
| Total fruits & vegetables, servings/week | 5.8 (3.3) | 5.5 (3.6) | 0.19 |
| Total fat, gm/day | 73.9 (38.8) | 65.3 (33.9) | < 0.01 |
| Lutein/zeaxanthin, µg/day | 3,642 (2915) | 3,282 (3220) | 0.09 |
| Red and processed meat, servings/week | 8.3 (8.3) | 7.0 (5.8) | 0.24 |
| Carotenoids, IU/day | 8,837 (9002) | 9,013 (7913) | 0.69 |
| Vitamin C, mg/day | 276 (353) | 288 (310) | 0.59 |
| Lycopene, µg/day | 4,539 (4,049) | 4,802 (5,146) | 0.46 |
| Plasma concentrations | | | |
| Alpha-carotene, µg/dL | 2.8 (2.8) | 3.7 (4.0) | 0.13 |
| Beta-carotene, µg/dL | 14.8 (19.2) | 16.9 (14.8) | 0.03 |
| β-cryptoxanthin, µg/dL | 6.2 (5.3) | 7.2 (6.0) | < 0.01 |
| Lutein/zeaxanthin, µg/dL | 16.9 (6.9) | 16.5 (8.3) | 0.33 |
| Lycopene, µg/dL | 25.9 (13.4) | 25.7 (12.3) | 0.83 |
| Alpha-tocopherol, mg/dL | 1.138 (0.507) | 1.191 (0.529) | 0.21 |
| Gamma-tocopherol[b], mg/dL | 0.231 (0.105) | 0.209 (0.110) | 0.04 |
| Cholesterol, mg/dL | 205 (36) | 205 (40) | 0.87 |
| Ferritin, mg/dL | 152 (131) | 139 (158) | 0.14 |
| 25(OH)-vitamin $D_3$, ng/mL | 25.5 (11.9) | 26.1 (11.3) | 0.52 |

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; 25-OH-vitamin D3, 25 hydroxylvitamin-D3; BMI, body mass index; MET, metabolic equivalents; MAP, Markers of Adenomatous Polyps.

[a] By Fisher's exact test for categorical variables and t test for continuous variables except for plasma α- and γ-T which were compared using ANOCOVA with GLM method adjusted for study, age, and sex.

[b] Indicates variables were transformed by square root for normality, others transformed by natural logarithm.

**Table 2.** Crude and Multivariable-Adjusted Associations of Plasma α- and γ-Tocopherol With Colorectal Adenoma in the Pooled MAP Studies, United States, 1994-2002.

| Tocopherol Tertiles | Tertile Range | | No. of Cases | No. of Controls | Crude | | Adjusted | |
|---|---|---|---|---|---|---|---|---|
| | MAP1 | MAP2 | | | OR | 95% CI | OR | 95% CI |
| **Alpha Tocopherol, mg/dL** | | | | | | | | |
| T1 | <= 0.918 | <= 0.883 | 62 | 83 | 1.00 | | 1.00 | |
| T2 | 0.918 – 1.213 | 0.883 – 1.422 | 72 | 82 | 1.18 | (0.75, 1.86) | 1.33[a] | (0.80, 2.22) |
| T3 | >= 1.213 | >= 1.422 | 50 | 84 | 0.80 | (0.49, 1.29) | 0.75[a] | (0.42, 1.34) |
| $P_{trend}$ | | | | | *0.37* | | *0.37* | |
| **Gamma Tocopherol, mg/dL** | | | | | | | | |
| T1 | <= 0.172 | <= 0.132 | 63 | 85 | 1.00 | | 1.00 | |
| T2 | 0.172 – 0.262 | 0.132 – 0.201 | 43 | 80 | 0.73 | (0.44, 1.19) | 0.75[b] | (0.44, 1.29) |
| T3 | >= 0.262 | >= 0.201 | 78 | 85 | 1.24 | (0.79, 1.94) | 1.37[b] | (0.81, 2.30) |
| $P_{trend}$ | | | | | *0.32* | | *0.32* | |

Abbreviations: CI, confidence interval; MAP, Markers of Adenomatous Polyps; OR, odds ratio.
[a] Adjusted for age (continuous), sex, plasma cholesterol and study.
[b] Adjusted for age (continuous), sex, plasma cholesterol, alpha-carotene, beta-carotene and study.

**Table 3.** Multivariable-adjusted Associations of Plasma α-Tocopherol and γ-Tocopherol with Colorectal Adenoma by Demographic and Lifestyle Characteristics in the Pooled MAP Studies, United States, 1994-2002.

| Characteristic and Tertiles of Plasma Tocopherols | α-Tocopherol | | | | γ-Tocopherol | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of Cases/Controls | OR[a] | 95% CI | $P_{interaction}$ | No. of Cases/Controls | OR[b] | 95% CI | $P_{interaction}$ |
| Age[c], years | | | | 0.38 | | | | 0.15 |
| < 55 | | | | | | | | |
| 1 | 26/46 | 1.00 | | | 19/40 | 1.00 | | |
| 2 | 24/45 | 1.03 | (0.48, 2.21) | | 10/38 | 0.51 | (0.19, 1.36) | |
| 3 | 13/25 | 0.86 | (0.33, 2.24) | | 34/39 | 1.95 | (0.78, 4.85) | |
| $P_{trend}$ | | *0.83* | | | | *0.06* | | |
| ≥ 55 | | | | | | | | |
| 1 | 36/35 | 1.00 | | | 44/44 | 1.00 | | |
| 2 | 48/37 | 1.61 | (0.80, 3.23) | | 33/42 | 0.82 | (0.42, 1.62) | |
| 3 | 36/59 | 0.73 | (0.35, 1.52) | | 43/45 | 1.02 | (0.53, 1.96) | |
| $P_{trend}$ | | *0.07* | | | | *0.88* | | |
| Sex | | | | 0.19 | | | | 0.83 |
| Male | | | | | | | | |
| 1 | 39/39 | 1.00 | | | 40/32 | 1.00 | | |
| 2 | 38/29 | 1.43 | (0.70, 2.92) | | 26/28 | 0.68 | (0.31, 1.48) | |
| 3 | 29/22 | 0.94 | (0.42, 2.13) | | 40/31 | 1.03 | (0.48, 2.21) | |
| $P_{trend}$ | | *0.41* | | | | *0.93* | | |
| Female | | | | | | | | |
| 1 | 23/42 | 1.00 | | | 23/52 | 1.00 | | |
| 2 | 34/53 | 1.24 | (0.59, 2.61) | | 17/52 | 0.71 | (0.32, 1.58) | |
| 3 | 21/62 | 0.60 | (0.25, 1.41) | | 38/53 | 1.63 | (0.77, 3.43) | |
| $P_{trend}$ | | *0.16* | | | | *0.11* | | |
| Family History of Colorectal Cancer[f] | | | | 0.82 | | | | 0.27 |
| Yes | | | | | | | | |
| 1 | 11/21 | 1.00 | | | 16/21 | 1.00 | | |
| 2 | 17/25 | 1.86 | (0.62, 5.62) | | 5/25 | 0.29 | (0.09, 1.00) | |
| 3 | 7/26 | 0.48 | (0.13, 1.77) | | 14/26 | 1.07 | (0.36, 3.21) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $P_{trend}$ | | *0.25* | | | | *0.47* | | |
| No | | | | | | | | |
| 1 | 49/59 | 1.00 | | | 47/62 | 1.00 | | |
| 2 | 52/55 | 1.24 | (0.68, 2.27) | | 35/53 | 0.87 | (0.46, 1.66) | |
| 3 | 43/56 | 0.92 | (0.47, 1.79) | | 62/56 | 1.49 | (0.81, 2.73) | |
| $P_{trend}$ | | *0.79* | | | | *0.15* | | |
| NSAIDs or aspirin | | | | 0.62 | | | | 0.76 |
| Use[e] | | | | | | | | |
| Yes | | | | | | | | |
| 1 | 35/37 | 1.00 | | | 36/50 | 1.00 | | |
| 2 | 32/50 | 0.69 | (0.34, 1.40) | | 22/51 | 0.71 | (0.34, 1.47) | |
| 3 | 29/61 | 0.45 | (0.21, 0.99) | | 38/47 | 1.35 | (0.67, 2.72) | |
| $P_{trend}$ | | *0.03* | | | | *0.71* | | |
| No | | | | | | | | |
| 1 | 26/46 | 1.00 | | | 27/35 | 1.00 | | |
| 2 | 38/31 | 3.05 | (1.35, 6.90) | | 18/29 | 0.62 | (0.26, 1.47) | |
| 3 | 21/23 | 1.90 | (0.73, 4.98) | | 40/37 | 1.25 | (0.55, 2.86) | |
| $P_{trend}$ | | *0.14* | | | | *0.29* | | |
| Current Smoking | | | | 0.49 | | | | 0.95 |
| Yes | | | | | | | | |
| 1 | 17/19 | 1.00 | | | 20/13 | 1.00 | | |
| 2 | 24/12 | 2.67 | (0.84, 8.52) | | 10/11 | 0.59 | (0.15, 2.29) | |
| 3 | 15/7 | 1.72 | (0.39, 7.59) | | 26/14 | 1.01 | (0.30. 3.43) | |
| $P_{trend}$ | | *0.08* | | | | *0.66* | | |
| No | | | | | | | | |
| 1 | 42/63 | 1.00 | | | 43/70 | 1.00 | | |
| 2 | 45/69 | 1.11 | (0.61, 2.00) | | 30/69 | 0.70 | (0.37, 1.30) | |
| 3 | 35/73 | 0.74 | (0.38, 1.43) | | 49/67 | 1.37 | (0.75, 2.52) | |
| $P_{trend}$ | | *0.25* | | | | *0.50* | | |
| Current Drinking | | | | 0.41 | | | | 0.31 |
| Yes | | | | | | | | |
| 1 | 27/31 | 1.00 | | | 36/41 | 1.00 | | |
| 2 | 40/44 | 1.18 | (0.55, 2.54) | | 18/39 | 0.50 | (0.22, 1.12) | |
| 3 | 29/39 | 0.69 | (0.28, 1.69) | | 42/35 | 1.36 | (0.63, 2.93) | |
| $P_{trend}$ | | *0.65* | | | | *0.33* | | |
| No | | | | | | | | |
| 1 | 33/51 | 1.00 | | | 27/43 | 1.00 | | |
| 2 | 29/38 | 1.32 | (0.63, 2.75) | | 22/41 | 0.93 | (0.42, 2.05) | |
| 3 | 21/44 | 0.74 | (0.33, 1.67) | | 34/49 | 1.28 | (0.60, 2.72) | |
| $P_{trend}$ | | *0.43* | | | | *0.74* | | |
| Obese[d] | | | | 0.11 | | | | 0.80 |
| Yes | | | | | | | | |
| 1 | 23/28 | 1.00 | | | 10/17 | 1.00 | | |
| 2 | 25/21 | 1.84 | (0.76, 4.50) | | 9/20 | 0.66 | (0.20, 2.23) | |
| 3 | 7/22 | 0.44 | (0.13, 1.53) | | 36/34 | 1.80 | (0.66, 4.95) | |
| $P_{trend}$ | | *0.14* | | | | *0.11* | | |
| No | | | | | | | | |
| 1 | 38/55 | 1.00 | | | 53/67 | 1.00 | | |
| 2 | 44/59 | 1.20 | (0.63, 2.29) | | 30/60 | 0.64 | (0.34, 1.21) | |
| 3 | 43/6 | 0.96 | (0.48, 1.94) | | 42/49 | 1.21 | (0.63, 2.32) | |
| $P_{trend}$ | | | | | | *0.88* | | |

Abbreviations: CI, confidence interval; MAP, Markers of Adenomatous Polyps; OR, odds ratio; NSAIDs: Non-steroidal anti-inflammatory drug. Study-specific tertiles of plasma α-Tocopherol (mg/dL): MAP I (T1: ≤ 0.918, T2: 0.918 – 1.213, T3: ≥ 1.213), MAP II (T1: ≤ 0.883, T2: 0.883 – 1.422, T3: ≥ 1.213). Study-specific tertiles of plasma γ-Tocopherol (mg/dL): MAP I (T1: ≤ 0.918, T2: 0.918 – 1.213, T3: ≥ 1.213), MAP II (T1: ≤ 0.883, T2: 0.883 – 1.422, T3: ≥ 1.213).

[a] Odds ratios with 95% confidence intervals were adjusted for age (continuous), sex, plasma cholesterol, and study. The stratification variable was not included in the model.

[b] Odds ratios with 95% confidence intervals were adjusted for age (continuous), sex, plasma cholesterol, α-carotene, and β-carotene level and study.
The stratification variable was not included in the model.

[c] Study-specific cut points were calculated on the basis of the median distribution in controls.

[d] Obesity was defined as a body mass index of ≥ 30 kg/m$^2$.

[e] Regularly intake (≥ once/week).

[f] In 1st degree relative

**Table 4.** Multivariable-adjusted Associations of Plasma α-Tocopherol and γ-Tocopherol With Colorectal Adenoma by Demographic and Lifestyle Characteristics in the Pooled MAP Studies, United States, 1994-2002.

| Adenoma Characteristic and Tertiles of Plasma Tocopherols | α-Tocopherol | | | γ-Tocopherol | | |
|---|---|---|---|---|---|---|
| | No. of Cases/Controls | OR[a] | 95% CI | No. of Cases/Controls | OR[b] | 95% CI |
| Location | | | | | | |
| Right colon[c] | | | | | | |
| 1 | 11/83 | 1.00 | (0.82, 4.48) | 16/85 | 1.00 | (0.29, 1.73) |
| 2 | 20/82 | 1.92 | (0.58, 3.75) | 10/80 | 0.71 | (0.67, 3.20) |
| 3 | 20/84 | 1.48 | | 25/85 | 1.46 | |
| $P_{trend}$ | | *0.17* | | | *0.17* | |
| Left colon[d] | | | | | | |
| 1 | 51/83 | 1.00 | (0.64, 1.94) | 45/85 | 1.00 | (0.41, 1.36) |
| 2 | 51/82 | 1.12 | (0.27, 1.02) | 33/80 | 0.75 | (0.67, 2.18) |
| 3 | 27/84 | 0.52 | | 51/85 | 1.21 | |
| $P_{trend}$ | | *0.03* | | | *0.61* | |
| Multiplicity | | | | | | |
| Multiple adenomas | | | | | | |
| 1 | 18/83 | 1.00 | (1.23, 5.45) | 22/85 | 1.00 | (0.56, 2.44) |
| 2 | 35/82 | 2.59 | (0.48, 2.65) | 22/80 | 1.17 | (0.71, 3.05) |
| 3 | 21/84 | 1.13 | | 30/85 | 1.47 | |
| $P_{trend}$ | | *0.73* | | | *0.32* | |
| Single Adenoma | | | | | | |
| 1 | 44/83 | 1.00 | (0.47, 1.50) | 39/85 | 1.00 | (0.29, 1.08) |
| 2 | 36/82 | 0.84 | (0.29, 1.14) | 21/80 | 0.56 | (0.66, 2.21) |
| 3 | 26/84 | 0.58 | | 46/85 | 1.21 | |
| $P_{trend}$ | | *0.07* | | | *0.50* | |
| Size[e] | | | | | | |
| Large adenoma ≥ 1 cm | | | | | | |
| 1 | 6/83 | 1.00 | (0.54, 5.12) | 8/85 | 1.00 | (0.19, 2.13) |
| 2 | 12/82 | 1.67 | (0.23, 2.95) | 5/80 | 0.63 | (0.44, 3.52) |
| 3 | 9/84 | 0.52 | | 14/85 | 1.24 | |
| $P_{trend}$ | | *0.52* | | | *0.19* | |
| Small adenoma < 1 cm | | | | | | |
| 1 | 47/83 | 1.00 | (0.70, 2.14) | 45/85 | 1.00 | (0.46, 1.50) |
| 2 | 49/82 | 1.23 | (0.36, 1.36) | 34/80 | 0.83 | (0.68, 2.16) |
| 3 | 31/84 | 0.14 | | 48/85 | 1.21 | |
| $P_{trend}$ | | *0.37* | | | *0.80* | |
| Shape | | | | | | |
| Pedunculated | | | | | | |
| 1 | 13/83 | 1.00 | (0.70, 3.59) | 13/85 | 1.00 | (0.30, 1.91) |
| 2 | 21/82 | 1.59 | (0.33, 2.24) | 10/80 | 0.75 | (0.70, 3.79) |
| 3 | 12/84 | 0.86 | | 23/85 | 1.63 | |
| $P_{trend}$ | | *0.84* | | | *0.10* | |
| Sessile | | | | | | |
| 1 | 46/83 | 1.00 | (0.72, 2.26) | 46/85 | 1.00 | (0.44, 1.46) |
| 2 | 50/82 | 1.28 | (0.36, 1.35) | 33/80 | 0.80 | (0.68, 2.13) |
| 3 | 35/84 | 0.69 | | 52/85 | 1.20 | |
| $P_{trend}$ | | *0.31* | | | *0.61* | |
| Histologic type of worst adenoma | | | | | | |
| Villous or tubulovillous | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 8/83 | 1.00 | (0.39, 3.27) | 6/85 | 1.00 | (0.13, 2.50) |
| 2 | 9/82 | 1.13 | (0.07, 1.56) | 3/80 | 0.56 | (0.54, 5.54) |
| 3 | 3/84 | 0.32 | | 11/85 | 1.72 | |
| $P_{trend}$ | | *0.18* | | | *0.20* | |
| Tubular | | | | | | |
| 1 | 54/83 | 1.00 | (0.78, 2.30) | 55/85 | 1.00 | (0.45, 1.39) |
| 2 | 62/82 | 1.34 | (0.41, 1.40) | 40/80 | 0.79 | (0.74, 2.22) |
| 3 | 44/84 | 0.76 | | 65/85 | 1.28 | |
| $P_{trend}$ | | *0.41* | | | *0.46* | |
| Degree of atypia of the worst adenoma | | | | | | |
| Mild | | | | | | |
| 1 | 24/83 | 1.00 | (0.43, 2.01) | 27/85 | 1.00 | (0.36, 1.67) |
| 2 | 20/82 | 0.93 | (0.53, 2.87) | 19/80 | 0.78 | (0.40, 1.97) |
| 3 | 26/84 | 1.24 | | 24/85 | 0.89 | |
| $P_{trend}$ | | *0.83* | | | *0.70* | |
| Moderate/severe | | | | | | |
| 1 | 38/83 | 1.00 | (0.78, 2.44) | 34/85 | 1.00 | (0.40, 1.46) |
| 2 | 21/82 | 1.38 | (0.23, 0.95) | 24/80 | 0.77 | (0.86, 2.82) |
| 3 | 50/84 | 0.46 | | 51/85 | 1.56 | |
| $P_{trend}$ | | *0.06* | (0.82, 4.48) | | *0.11* | |

Abbreviations: CI, confidence interval; MAP, Markers of Adenomatous Polyps; OR, odds ratio; NSAIDs: Non-steroidal anti-inflammatory drug. Study-specific tertiles of plasma α-Tocopherol (mg/dL): MAP I (T1: ≤ 0.918, T2: 0.918 – 1.213, T3: ≥ 1.213), MAP II (T1: ≤ 0.883, T2: 0.883 – 1.422, T3: ≥ 1.213). Study-specific tertiles of plasma γ-Tocopherol (mg/dL): MAPI (T1: ≤ 0.918, T2: 0.918 – 1.213, T3: ≥ 1.213), MAP II (T1: ≤ 0.883, T2: 0.883 – 1.422, T3: ≥ 1.213).

[a] Odds ratios with 95% confidence intervals were adjusted for age (continuous), sex, plasma cholesterol, and study. The stratification variable was not included in the model.

[b] Odds ratios with 95% confidence intervals were adjusted for age (continuous), sex, plasma cholesterol, α-carotene, and β-carotene level and study. The stratification variable was not included in the model.

[c] Largest adenoma in the right colon, which includes the cecum, ascending colon, hepatic flexure, and transverse colon.

[d] Largest adenoma in the left colon, which includes the splenic flexure, descending colon, sigmoid colon and rectum.

[e] Adenoma size from *in vivo* comparison of maximum diameter to fully opened endoscope forceps.

# Chapter 3: Summary

In summary, we observed no clear patterns of associations between plasma α-tocopherol or γ-tocopherol and incident, sporadic colorectal adenomas in our study. Although our results suggested that cases in the highest relative to those in the lowest tertile of plasma α-tocopherol were at an estimated 20% lower risk, and those in the highest relative to the lowest tertile of plasma γ-tocopherol were at an estimated 24% higher risk, neither of the associations were statistically significant nor was there a dose-response pattern. The sample size for stratified analyses in our study was limited; only one of the multiple comparisons across the various strata of demographic, lifestyle, and dietary risk factors, or according to adenoma characteristics suggested an association among a particular sub-group of individuals: among those who regularly took NSAIDs, there was a stronger inverse association of α-T with adenomas.

To our knowledge, our study was one of few studies conducted in humans to assess associations of tocopherols with colorectal adenoma. Although our findings were not statistically significant, they were consistent with those of previous studies and their implications for chemoprevention remain unclear. To fully understand these associations, future studies should assess interactions of dietary/circulating α-tocopherol and γ-tocopherol, while taking other risk factors related to colorectal cancer and tocopherols, especially inflammation, into consideration.