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Reduced Risk of Colon Cancer with High Intake of Vitamin E: The Iowa Women's Health Study[1]

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ABSTRACT

Antioxidant micronutrients, including vitamin E, vitamin C, the carotenoids, and selenium, defend the body against free radicals and reactive oxygen molecules, suggesting a potential for these dietary components in cancer prevention. To investigate whether high intakes of antioxidant micronutrients protect against colon cancer in humans, we analyzed data from a prospective cohort study of 35,215 Iowa women aged 55–69 years and without a history of cancer who completed a dietary questionnaire in 1986. Through 1990, 212 incident cases of colon cancer were documented. Adjusted for age, total vitamin E intake was inversely associated with the risk of colon cancer (P for trend < 0.0001); the relative risk for the highest compared to the lowest quintile was 0.32 [95% confidence interval (95% CI) 0.19, 0.54]. Further adjustment for total energy intake and other risk factors in proportional hazards regression had little effect on these estimates. The association was not uniform across age groups: the multivariate relative risk of colon cancer for the highest compared to the lowest quintile of total vitamin E intake was 0.16 (95% CI 0.04, 0.70) for those 55–59 years old, 0.37 (95% CI 0.12, 1.16) for those 60–64 years old, and 0.93 (95% CI 0.27, 3.25) for those 65–69 years old. Multivariate-adjusted relative risks among women with higher total intakes of vitamins A and C and β-carotene, and among users of selenium supplements, were not significantly different from 1.0. These prospective data provide evidence that a high intake of vitamin E may decrease the risk of colon cancer, especially in persons under 65 years of age.

INTRODUCTION

Colorectal cancer is now the second most common cause of cancer deaths in the United States (1). Mortality from colon cancer has not changed substantially over the past 50 years (2), suggesting that prevention may offer the best opportunity to control the disease. Ecological and migration studies indicate the importance of environmental factors (3). Diet appears to have a strong link (4, 5) and thus offers promise for intervention.

Antioxidant micronutrients, including vitamin E, vitamin C, the carotenoids, selenium, zinc, copper, iron, and manganese, are part of the body's primary defenses against free radicals and reactive oxygen molecules (6). Sources of free radicals and reactive oxygen molecules include endogenous production from normal metabolic reactions as well as exogenous sources (6). These compounds can initiate cell damage by reacting with unsaturated bonds in membrane lipids, denaturing proteins, and altering nucleic acids (6). Vitamins E and C and carotenoids trap free radicals and reactive oxygen molecules, whereas selenium, zinc, copper, iron, and manganese are essential components of antioxidant enzymes (6).

Antioxidant micronutrients may also have other anticarcinogenic effects. Vitamin E is the major lipid-soluble antioxidant found in cell membranes, where it protects against lipid peroxidation (6). In addition, like carotenoids and water-soluble vitamin C, it can also stimulate the immune system and may protect against the development of cancer by enhancing immune surveillance (7–9). Vitamins E and C reduce nitrite, inhibiting the production of nitrosamines and nitrosamides (9), compounds that induce tumors in experimental animals and possibly in humans. Selenium, an essential constituent of the enzyme glutathione peroxidase (which reduces peroxides and thus prevents damage to intracellular membranes) (6), may stimulate the immune system and inhibit DNA synthesis and cell proliferation (10).

Relatively few epidemiological studies have addressed dietary antioxidant intake and the risk of colon cancer. Since the vitamin E content of many foods had not been determined until recently, epidemiological studies that have investigated the association of dietary vitamin E with cancer are few; fewer still have focused on colon cancer (8). Several studies have investigated intake of vitamin C and carotenoids in relation to colon cancer, but findings have been inconsistent (8). Because it is not usually possible to estimate the selenium content of individual diets in epidemiological studies, there are almost no data on selenium intake and the development of colon cancer (8). The present prospective cohort study addresses several of these issues by providing new information on the association of colon cancer with vitamin E intake and selenium supplement use in a well-defined population from a well-defined, geographically uniform area of the United States. In addition, new data are provided to add to the growing but, as yet, inconsistent body of data on the association of colon cancer with vitamin C and the carotenoids.

MATERIALS AND METHODS

The Iowa Women's Health Study Cohort. The methodology of the Iowa Women's Health Study has been described previously (11, 12). Briefly, in 1986, 41,837 women 55–69 years of age who had a valid Iowa driver's license in 1985 returned a mailed questionnaire on known and suspected risk factors for cancer.

Data Collection. The mailed questionnaire included a semiquantitative food frequency questionnaire that was virtually identical to that used in the 1984 survey of the Nurses' Health Study (13). The food frequency questionnaire covered usual food intake and vitamin and mineral supplement use. The rationale for use of a food frequency questionnaire to assess dietary habits and nutrient intake in a large-scale cohort study has been described elsewhere (14–16). The reliability and accuracy of this questionnaire among members of this cohort (17) are comparable to those observed in the Nurses' Health Study (14). Information solicited on vitamin and mineral supplement use included the dose per day of specific nutrient supplements, the name of multivitamin/mineral pills used, and the number of multivitamin/mineral pills taken per week. A nutrient data base was maintained and used to calculate the amount of each antioxidant micronutrient from the named multivitamin/minerals. Duration of vitamin and mineral use was not assessed.

Data on body measurements were self-reported. BMI,[3] defined as weight divided by the square of the height (kg/m^2), was used as a measure of relative weight. A paper tape measure and written instructions were enclosed with the questionnaire so that a friend could measure the circumference of the waist

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[3] The abbreviations used are: BMI, body-mass index; RR, relative risk; 95% CI, 95% confidence interval.

(1 in. above the umbilicus) and hips (maximal protrusion). From these measures a waist-to-hip ratio was calculated (in./in.) for each respondent.

Information on current address was collected through follow-up questionnaires and the National Change of Address Service. Deaths among nonrespondents were determined through the National Death Index. The number of questionnaires returned at the 2-year follow-up was 37,579, and at the 4-year follow-up, 35,438. At the 4-year follow-up, 1,182 (2.8%) of the original cohort were known dead, 1,208 (2.9%) had moved from Iowa, and 35,109 (83.9%) were verified alive in Iowa. There was no evidence of an out-of-state move for the remaining 4,328 (10.3%) who were presumed alive in Iowa.

Identification of Cases of Colon Cancer. Cancer incidence was ascertained through the State Health Registry of Iowa, a part of the National Cancer Institute's Surveillance, Epidemiology, and End Results Program (18). The definition of colon cancer used in this study included *International Classification of Diseases, Oncology* codes 153.1–153.4 and 153.6–153.9; all stages of the disease were included [including *in situ* colon cancer (n = 13)]. Those developing rectal cancer (78 cases) or cancer of the appendix (1 case) were not included as colon cancer cases. As migration from Iowa was less than 1%/year, a computer match was performed annually between the list of study participants and the record of Iowans with incident cancer in the Health Registry. Combinations of first and last names, maiden names, zip codes, birth dates, and social security numbers were used for matching, with possible matches confirmed visually.

Population Analysis. Before beginning hypothesis testing, we excluded women who reported a history of cancer at baseline (nonmelanoma skin cancer patients were not excluded) (n = 3,519), those who left 30 or more food items blank on the food frequency questionnaire (n = 2,299), and those who measured having implausibly high or low total daily energy intake (<600 or >5000 kcal/day) (n = 291). An additional 512 women had 2 or more of these exclusions. Thus, the baseline population at risk was 35,216.

Statistical Analyses. Age-adjusted mean baseline characteristics were computed for cases and noncases and compared using analysis of covariance. Women were categorized according to quintiles of intake of various foods, nutrients, and other characteristics as computed from the 1986 questionnaire. For nutritional supplement items, it was necessary to establish categories of intake based on the distribution of use. Dietary intake of selenium was not examined. Supplemental intake of selenium was investigated only by comparing users *versus* nonusers. All categories were determined prior to hypothesis testing.

For cases, length of follow-up was calculated for each individual as the number of days elapsed since completion of the baseline questionnaire until date of diagnosis of colon cancer. For noncases, different termination dates based on the following prioritization were used: (*a*) date of death for deaths in Iowa; (*b*) date moved out of Iowa if date of move known; (*c*) midpoint between date of last contact in Iowa and first known date out of Iowa or end of follow-up period if moved from Iowa at an unknown date; or (*d*) the midpoint between date of last contact in Iowa and date of death for non-Iowa deaths. Noncases for whom these criteria did not apply contributed follow-up through December 31, 1990.

Person-time for each exposure was accumulated, and an incidence rate was calculated by dividing the number of first events by the person-years of follow-up. The RR, defined as the incidence rate in a particular category of exposure divided by the corresponding rate in the comparison category, was used as a measure of strength of association. Age-adjusted rates were calculated using 5-year categories. The Mantel extension test (19) was used to evaluate trends across categories of variables stratified according to age. Analyses to control for simultaneous effects of multiple variables were conducted using proportional hazards methods (20, 21). The multivariate-adjusted RR for a given category of an exposure variable was determined by exponentiating its regression coefficient. The test for trend after multivariate adjustment for covariates was determined by calculating the Wald χ^2 statistic across the vector of indicator variables for the exposure of interest. For all relative risks, 95% CI were calculated (22).

Proportional hazards regression models were built by adding and/or deleting hypothesized colon cancer risk factors, their interactions, and hypothesized confounding variables one at a time. Decisions on which covariates to include in the final reported models were based on: (*a*) biological plausibility; (*b*) whether the covariates entered the model at the 0.10 level of significance; and (*c*) whether the covariates acted as confounders of the primary association of interest (confounding was considered to be present if the regression coefficient of the primary independent variable changed ≥10% after adding the potential confounding variable to the model). Although several other hypothesized associations were confirmed or refuted in these analyses, this paper focuses only on antioxidant micronutrients in relation to colon cancer risk.

Proportional hazards assumptions were examined by use of Schoenfeld (23) residuals and Harrell's (24) z test for linear trend in the residuals. There were no departures from proportional hazards assumptions for any covariate included in the reported final models.

RESULTS

Descriptive Analyses. During 167,447 person-years of follow-up over a 5-year period, 212 cases of colon cancer were documented. Selected age-adjusted mean baseline characteristics for cases and noncases are presented in Table 1. Those who developed colon cancer were, on average, 8–9 months older than those not developing colon cancer and had significantly lower mean daily intakes of dietary, supplemental, and total vitamin E (dietary and supplemental vitamin E combined) and of supplemental vitamin A. Cases and noncases did not differ at $P \leq 0.05$ in mean height, BMI, waist-to-hip ratio, or intakes of total energy, total vitamin A, total β-carotene, or vitamin C. They also did not differ significantly in the proportion using any type of vitamin or mineral supplement combined (a measure of general supplement pill taking behavior) but did differ in using a selenium-containing supplement (3% of cases and 8% of noncases used them) (Table 2).

Data on vitamin intake were skewed and multimodal. For example, for vitamin E, peaks were displayed at 6–10 IU (generally unsupplemented daily dietary intakes), 30 IU (vitamin E supplements through multivitamins), and 100–400 IU (specific vitamin E supplements). At the descriptive level, therefore, differences in vitamin intake are more appropriately examined using proportions (Table 2). Twenty-five % of cases were taking vitamin E-containing supplements compared to 37% of noncases (P = 0.001), 25% of cases were taking vitamin A-containing supplements compared to 34% of noncases (P = 0.006), and 37% of cases were taking vitamin C-containing supplements compared to 45% of noncases (P = 0.02).

Selected baseline characteristics of users and nonusers of supplemental antioxidant micronutrients (from multivitamins/minerals and/or specific nutrient pills) are compared in Table 3. Users of any of the 4 micronutrients did not differ materially from nonusers in mean age or alcohol intake. Compared to nonusers, nutrient supplement

Table 1 *Selected age-adjusted mean (± SEM) baseline characteristics in relation to incidence of colon cancer in Iowa women, 1986–1990*

Characteristic	Colon cancer (n = 212)	No colon cancer (n = 35,004)	P value[a]
Age (yrs)	62.2 ± 0.3	61.5 ± 0.02	0.02
Height (in)	64.4 ± 0.2	64.1 ± 0.0	0.06
BMI (kg/m^2)	27.4 ± 0.4	27.0 ± 0.03	0.22
Waist-to-hip ratio	0.843 ± 0.006	0.837 ± 0.000[b]	0.27
Total energy intake (kcal/day)	1,727 ± 42	1,802 ± 3	0.06
Vitamin E intake (IU/day)			
Total[c]	36 ± 10	66 ± 0.08	0.004
Dietary	7 ± 0.3	8 ± 0.02	0.03
Supplemental	29 ± 10	58 ± 0.8	0.005
Vitamin A intake (IU/day)			
Total[c]	13,472 ± 736	14,754 ± 56	0.09
Dietary	12,046 ± 632	12,412 ± 49	0.56
Supplemental	1,463 ± 333	2,342 ± 26	0.009
Total β-carotene intake[c] (IU/day)	9,241 ± 551	9,572 ± 43	0.55
Vitamin C intake (mg/day)			
Total[c]	274 ± 21	297 ± 2	0.28
Dietary	151 ± 6	152 ± 0.04	0.74
Supplemental	124 ± 20	145 ± 2	0.29

[a] F test for difference.
[b] SEM <0.0005.
[c] Total intake = dietary sources plus supplemental sources.

Table 2 *Distributions of intakes of selected nutritional supplements in relation to incidence of colon cancer in Iowa women, 1986–1990*

Characteristic	Colon cancer (n = 212)		No colon cancer (n = 35,004)		χ^2 (P value)
	n	%	n	%	
Vitamin/mineral supplement user	123	58	22,148	63	2.50 (0.11)
Use of vitamin E supplement					
None	158	75	22,109	63	11.71 (0.001)
Via multivitamins only	40	19	7,990	23	1.88 (0.17)
Via specific vitamin E pills	14	7	4,905	14	9.63 (0.002)
Use of vitamin A supplement					
None	159	75	23,133	66	7.48 (0.006)
Via multivitamins only	42	20	9,317	27	5.02 (0.03)
Via specific vitamin A pills	11	5	2,554	7	1.39 (0.24)
Use of vitamin C supplement					
None	133	63	19,200	55	5.29 (0.02)
Via multivitamins only	30	14	5,940	17	1.19 (0.28)
Via specific vitamin C pills	49	23	9,864	28	2.67 (0.10)
Selenium supplement user	7	3	2,639	8	5.44 (0.02)

Table 3 *Selected age-adjusted mean (± SEM) baseline characteristics in relation to use of selected nutritional supplements*[a] in Iowa women, 1986–1990

Characteristic	Vitamin E		Vitamin A		Vitamin C		Selenium	
	Nonusers (n =22,267)	Users (n = 12,949)	Nonusers (n = 23,292)	Users (n = 11,924)	Nonusers (n = 19,333)	Users (n = 15,883)	Nonusers (n = 32,570)	Users (n = 2,646)
Age (yrs)	61.5 ± 0.03	61.6 ± 0.04	61.4 ± 0.03	61.6 ± 0.04	61.5 ± 0.03	61.6 ± 0.03	61.5 ± 0.02	61.5 ± 0.08
BMI (kg/m^2)	27.2 ± 0.03	26.6 ± 0.04	27.2 ± 0.03	26.6 ± 0.05	27.3 ± 0.04	26.6 ± 0.04	27.0 ± 0.03	26.8 ± 0.10
Total energy intake (kcal/day)	1,811 ± 4	1,785 ± 5	1,810 ± 4	1,786 ± 6	1,813 ± 4	1,787 ± 5	1,804 ± 3	1,770 ± 12
Total fat (g/day)	69.7 ± 0.2	66.9 ± 0.2	69.6 ± 0.2	66.9 ± 0.2	69.9 ± 0.2	67.2 ± 0.2	69.0 ± 0.2	65.8 ± 0.5
Animal fat (g/day)	40.3 ± 0.1	38.1 ± 0.2	40.2 ± 0.1	38.1 ± 0.2	40.6 ± 0.1	38.2 ± 0.1	39.7 ± 0.1	37.2 ± 0.4
Dietary fiber (g/day)	20.1 ± 0.1	20.9 ± 0.1	20.1 ± 0.1	20.9 ± 0.1	19.9 ± 0.1	20.8 ± 0.1	20.3 ± 0.05	21.3 ± 0.2
Alcohol (g/day)	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	3.7 ± 0.2

[a] Through the use of multivitamins and/or specific nutrient pills.

users, on average, had a 0.6-kg/m^2 lower BMI, and consumed about 25–30 kcal/day less energy, 3 g/day less total fat, 2 g/day less animal fat, and 0.8 g/day more dietary fiber.

Eighty-four % of vitamin E supplement users also used a supplement containing vitamin A, vitamin C, and/or selenium, *versus* 21% among those not using vitamin E supplements. However, there were no substantial or statistical differences in this distribution between cases and noncases (data not shown). Pearson correlations between supplement nutrient scores were: vitamin E and vitamin A, 0.42; vitamin E and vitamin C, 0.49; vitamin E and selenium, 0.28; vitamin A and vitamin C, 0.50; vitamin A and selenium, 0.29; and vitamin C and selenium, 0.25.

Age-adjusted Associations. Age-adjusted relative risks of colon cancer according to categories of intake of various dietary components are presented in Table 4. There was no significant trend across categories of total energy intake; however, the relative risks for colon cancer in the third and fifth quintile groups of total energy intake were significantly less than 1.0. There were significant inverse trends in relative risks for total ($P < 0.0001$) and supplemental ($P = 0.0002$) vitamin E and for supplemental vitamin A ($P = 0.002$) and vitamin C ($P = 0.01$). Furthermore, the risk for women in the highest quintile of total vitamin E intake was about one-third that of those in the lowest quintile. For those in the highest category of supplemental vitamin E intake, the risk of colon cancer was less than half that of those in the lowest category of intake and for those in the middle category the risk was intermediate. For those in the highest category of supplemental vitamin A intake, the risk was also less than half that of those in the lowest category of intake, and for those in the highest category of supplemental vitamin C the risk was reduced by one-third. Similarly, the risk for users of selenium supplements was less than half that of those not using selenium supplements. Although relative risks tended to be lower for those with higher levels of intake of total vitamins A and C, β-carotene, and dietary vitamin E compared to those with lowest intakes, no significant trends were observed, and only women with higher total vitamin A intakes had significantly lower relative risks (second, fourth, and fifth quintiles).

Multivariate-adjusted Associations. To assess main effects of each antioxidant, the following covariates were included in final multivariate models: age, total energy intake, height, parity, and a summary low-fat meat variable (intake of seafood and skinless poultry). In final models not evaluating a vitamin E variable, total vitamin E intake and a total vitamin E by age interaction term were also included. In final models not evaluating a vitamin A variable, vitamin A supplement intake was also included. Examples of omitted covariates that did not confound associations or did not fit the final models at the 0.10 level of significance include education, occupation, BMI, waist-to-hip ratio, physical activity, an indicator variable for use of any vitamin or mineral supplement(s) (*i.e.*, multivitamin/mineral supplements and/or nutrient-specific supplements, even if contained the nutrient of interest), an indicator variable for use of any nutrient-specific vitamin or mineral supplement(s) (*i.e.*, calcium only, folate only, etc.), and intakes of fat, fiber, vegetables and fruit, red meat, alcohol, calcium, vitamin D, and other antioxidant micronutrients (zinc, copper, iron, and manganese). After adjustment, the risk for women in the highest quintile of total vitamin E intake was approximately two-fifths that of those in the lowest quintile (Table 5). The reduced risk was largely associated with the use of supplemental vitamin E. Although after multivariate adjustment relatively high intakes of vitamin A supplements were significantly associated with reduced risk, the tendency for lower relative risks among women with higher total intakes of vitamins A and C and β-carotene was no longer observed. Multivariate adjustment moderately attenuated the estimated relative risk of colon cancer in women using selenium supplements compared to those not using them, but the estimate was no longer statistically significant.

In a single multivariate model with a single reference cell, a significant interaction ($P = 0.026$) was observed between age and total vitamin E intake (Table 6). The relative risks in the highest quintiles of intake across the age categories were striking: 0.16 for those 55 to 59 years old, 0.37 for those 60 to 64 years old, and 0.93 for those 65 to 69 years old. No other significant interactions were noted, including total vitamin E intake by intake of each of the other antioxidants,

Table 4 *Age-adjusted relative risks of colon cancer according to categories of intake of various dietary components, Iowa women, 1986–1990*

Variable	Categories[a] 1	2	3	4	5	χ^2 for trend (*P* value)
Total energy (kcal/day)	<1,301	1,301–1,591	1,592–1,865	1,866–2,238	>2,238	
Cases	55	43	33	47	34	
Person-yrs	33,440	33,404	33,485	33,488	33,640	
Relative risk (95% CI)	1.00	0.78 (0.53, 1.17)	0.60 (0.39, 0.92)	0.85 (0.58, 1.26)	0.62 (0.40, 0.95)	3.42 (0.06)
Total vitamin E[b] (IU/day)	<5.7	5.7–7.8	7.9–12.2	12.3–35.7	>35.7	
Cases	55	46	51	41	18	
Person-yrs	33,589	33,447	33,456	33,567	33,389	
Relative risk (95% CI)	1.00	0.82 (0.56, 1.21)	0.91 (0.62, 1.33)	0.73(0.49, 1.09)	0.32 (0.19, 0.54)	15.67 (<0.0001)
Dietary vitamin E (IU/day)	<5	5–6.3	6.4–7.7	7.8–9.7	>9.7	
Cases	47	46	46	40	33	
Person-yrs	33,500	33,555	33,475	33,468	33,449	
Relative risk (95% CI)	1.00	0.97 (0.65, 1.46)	0.97 (0.65, 1.46)	0.84 (0.55, 1.29)	0.70 (0.45, 1.09)	2.82 (0.10)
Supplemental vitamin E (IU/day)	0	1–30	>30			
Cases	158	35	19			
Person-yrs	105,917	32,946	28,583			
Relative risk (95% CI)	1.00	0.71 (0.49, 1.02)	0.44 (0.28, 0.71)			13.72 (0.0002)
Total vitamin A[b] (IU/day)	<7,262	7,262–10,737	10,738–14,590	14,591–20,335	>20,335	
Cases	55	32	52	37	36	
Person-yrs	33,485	33,508	33,460	33,488	35,505	
Relative risk (95% CI)	1.00	0.58 (0.37, 0.90)	0.93 (0.64, 1.36)	0.66 (0.43, 1.00)	0.63 (0.41, 0.96)	2.90 (0.09)
Dietary vitamin A (IU/day)	<6,278	6,278–8,930	8,931–12,215	12,216–17,040	>17,040	
Cases	40	51	40	44	37	
Person-yrs	33,547	33,425	33,476	33,425	33,575	
Relative risk (95% CI)	1.00	1.26 (0.84, 1.91)	1.00 (0.65, 1.55)	1.09(0.71, 1.67)	0.90 (0.58, 1.41)	0.52 (0.47)
Supplemental vitamin A (IU/day)	0	1–5,000	≥5,000			
Cases	159	38	15			
Person-yrs	110,817	34,379	22,251			
Relative risk (95% CI)	1.00	0.77 (0.54, 1.09)	0.47 (0.27, 0.79)			9.55 (0.002)
Total β-carotene[b,c] (IU/day)	<4,449	4,449–6,132	6,133–8,926	8,927–13,502	>13,502	
Cases	48	35	46	44	39	
Person-yrs	33,486	33,506	33,511	33,408	33,586	
Relative risk (95% CI)	1.00	0.73 (0.47, 1.12)	0.95 (0.63, 1.42)	0.90 (0.60, 1.36)	0.80 (0.52, 1.22)	0.27 (0.60)
Total vitamin C[b] (mg/day)	<112	112–162	163–227	228–392	>392	
Cases	50	46	42	39	35	
Person-yrs	33,602	33,581	33,494	33,450	33,320	
Relative risk (95% CI)	1.00	0.90 (0.61, 1.35)	0.83 (0.55, 1.25)	0.77 (0.51, 1.18)	0.70 (0.45, 1.07)	3.34 (0.07)
Dietary vitamin C (mg/day)	<91	91–124	125–155	156–201	>201	
Cases	47	41	48	31	45	
Person-yrs	33,546	33,564	33,414	33,498	33,425	
Relative risk (95% CI)	1.00	0.86 (0.56, 1.31)	1.00 (0.67, 1.50)	0.65 (0.41, 1.03)	0.94 (0.62, 1.41)	0.58 (0.44)
Supplemental vitamin C (mg/day)	0	1–60	>60			
Cases	133	25	54			
Person-yrs	92,063	19,922	55,462			
Relative risk (95% CI)	1.00	0.87 (0.57, 1.33)	0.67(0.49, 0.92)			6.17 (0.01)
Selenium supplementation						
Cases	205	7				
Person-yrs	154,900	12,547				
Relative risk (95% CI)	1.00	0.42 (0.20, 0.90)				

[a] Categories of all variables are based on quintiles except those for supplemental vitamins E and A, where 1 = no supplement use and categories 2 and 3 are based on a low-high median split; for supplemental vitamin C, where 1 = no supplement use and categories 2–4 are based on tertiles; and for supplemental selenium, where 1 = no selenium-containing supplement use and 2 = take selenium-containing supplement.

[b] Total intake = dietary sources plus supplemental sources.

[c] Partition by dietary and supplement sources not provided by food frequency questionnaire program.

polyunsaturated fat, or nitrites, or of age by intake of any other antioxidant. To clarify whether an inverse association between total vitamin E intake and colon cancer was absent or merely attenuated at older ages, separate multivariate models were fit for each age stratum. The relative risks in the highest quintile groups of intake across the age categories were: 0.16 (95% CI 0.03, 0.81) for those 55 to 59 years old, 0.27 (95% CI 0.08, 0.85) for those 60 to 64 years old, and 0.92 (95% CI 0.37, 2.30) for those 65 to 69 years old. To examine further whether these findings were driven by residual confounding by age with the supplemental vitamin E intake component of the total vitamin E score, the following were calculated: (*a*) the Pearson correlation coefficient for age and vitamin E supplement use for the entire cohort ($r = 0.009$; $P = 0.09$) and limited to only those among the cohort reporting supplemental vitamin E intake ($r = 0.0001$; $P = 0.99$); and (*b*) frequency distributions of supplemental vitamin E intake by age (no substantial differences revealed).

It was hypothesized that because some women consuming low amounts of dietary vitamin E could have been consuming high amounts of supplemental vitamin E and *vice versa*, the observed associations between dietary vitamin E intake and colon cancer shown in Tables 4 and 5 could have been attenuated. To clarify this and whether dietary vitamin E was substantially contributing to the observed association between total vitamin E intake and colon cancer, further analyses were conducted. First, multivariate-adjusted RRs of colon cancer according to quantiles of dietary vitamin E intake were stratified by supplemental intake using a single multivariate model with a single reference cell (Table 7). Because there was so little spread in dietary intakes (98% of the at-risk cohort consumed between 3 and 14 IU daily), the dietary vitamin E variable was dichotomized on a median split. The *p* value for the dietary vitamin E by supplemental vitamin E interaction was 0.72. Within each dietary stratum, the RR decreased with each higher category of supplement intake. Within supplement categories, a pattern of decreasing risk with increased dietary vitamin E intake was not observed in the no-supplement category, but was suggested in the 2-supplement categories. Second, a 3-way interaction among age, dietary vitamin E intake, and supplemental vitamin E intake was tested (data not shown) but probably because of small numbers, did not provide additional clarification.

Table 5 *Multivariate-adjusted relative risks of colon cancer according to categories of intake of various dietary compounds, Iowa women, 1986–1990*

Variable	Categories[a] 1	2	3	4	5	χ^2 for trend (*P* value)
Total vitamin E[b,c]	1.00	0.94 (0.62,1.41)	1.16 (0.74,1.81)	0.94 (0.57,1.53)	0.42 (0.22,0.78)	5.66 (0.02)
Dietary vitamin E[c]	1.00	1.08 (0.71,1.64)	1.16 (0.74,1.82)	1.09 (0.65,1.80)	1.02 (0.55,1.88)	0.01 (0.94)
Supplemental vitamin E[c]	1.00	0.78 (0.50,1.21)	0.50 (0.28,0.87)			5.98 (0.01)
Total vitamin A[b,d]	1.00	0.63 (0.50,0.97)	1.08 (0.73,1.60)	0.82 (0.53,1.28)	0.92 (0.57,1.47)	0.03 (0.86)
Dietary vitamin A[d]	1.00	1.38 (0.91,2.10)	1.13 (0.72,1.78)	1.34 (0.85,2.11)	1.25 (0.76,2.06)	0.53 (0.47)
Supplemental vitamin A[d]	1.00	0.85 (0.59,1.22)	0.57 (0.33,0.98)			4.09 (0.04)
Total β-carotene[b,d,e]	1.00	0.79 (0.51,1.22)	1.09 (0.72,1.66)	1.10 (0.72,1.69)	1.10 (0.70,1.76)	0.94 (0.33)
Total vitamin C[b,f]	1.00	1.00 (0.67,1.51)	1.03 (0.67,1.57)	1.09 (0.69,1.72)	1.23 (0.75,2.02)	0.76 (0.38)
Dietary vitamin C[f]	1.00	0.94 (0.61,1.42)	1.16 (0.77,1.76)	0.80 (0.50,1.29)	1.32 (0.83,2.09)	0.51 (0.47)
Supplemental vitamin C[f]	1.00	0.96 (0.52,1.79)	0.86 (0.56,1.33)	1.11 (0.71,1.74)		0.06 (0.80)
Selenium supplementation[f]	1.00	0.60 (0.27,1.32)				

[a] Categories of all variables are based on quintiles, except those for supplemental vitamins E and A, where 1 = no supplement use and categories 2 and 3 are based on a low-high median split; for supplemental vitamin C, where 1 = no supplement use and categories 2–4 are based on tertiles; and for supplemental selenium, where 1 = no selenium-containing supplement use and 2 = take selenium-containing supplement.

[b] Total intake = dietary sources plus supplemental sources.

[c] Covariates included in final model for reported relative risks: age, caloric intake, height, parity, vitamin A supplement intake, and intake of low-fat meats (seafoods and skinless chicken). For covariates tested and not fitting and/or confounding, see text.

[d] Covariates included in final model for reported relative risks: total vitamin E intake, a total vitamin E by age interaction term, age, caloric intake, height, parity, and intake of low-fat meats (seafoods and skinless chicken). For covariates tested and not fitting and/or confounding, see text.

[e] Partition by dietary and supplement sources not provided by food frequency questionnaire program.

[f] Covariates included in final model for reported relative risks: age, caloric intake, height, parity, vitamin A supplement intake, intake of low-fat meats (seafoods and skinless chicken), total vitamin E intake, and a total vitamin E by age interaction term. For covariates tested and not fitting and/or confounding, see text.

Table 6 *Levels of age and total vitamin E intake in relation to the multivariate-adjusted[a] relative risk of colon cancer, Iowa women, 1986–1990*

	Quintile of total vitamin E intake[b] 1	2	3	4	5
Age (yrs)					
55–59	1.00[c]	0.85 (0.41,1.75)	1.36 (0.68,2.74)	1.09 (0.52,2.31)	0.16 (0.04,0.70)
60–64	1.22 (0.56,2.66)	1.27 (0.57,2.83)	0.95 (0.39,2.29)	0.96 (0.39,2.34)	0.37 (0.12,1.16)
65–69	1.10 (0.35,3.49)	1.05 (0.32,3.43)	1.64 (0.51,5.26)	1.17 (0.35,3.91)	0.93 (0.27,3.25)

[a] A single model with a single reference cell with adjustment for age, total energy intake, height, parity, vitamin A supplement intake, and intake of low-fat meats (seafoods and skinless poultry).

[b] Total intake = dietary sources plus supplemental sources.

[c] Reference category.

To investigate whether vitamin E could reduce risk of colon cancer over a plausible dietary range, multivariate modeling was undertaken using data only from those women with a total vitamin E intake ≤25 IU daily. The at-risk cohort was thus reduced to 26,406, and the number of cases to 183. The final model covariates included age, height, total energy intake, parity, vitamin A supplement intake, and intake of low-fat meat. The RR of colon cancer for those in the highest quintile group of total vitamin E intake compared to those in the lowest quintile was 0.76 (95% CI 0.40, 1.42).

Table 7 *Levels of supplemental vitamin E and dietary vitamin E intakes in relation to the multivariate-adjusted[a] relative risk of colon cancer, Iowa women, 1986–1990*

	Dietary vitamin E intake[b] Low	High
Supplemental vitamin E intake (IU/day)		
0	1.00[c]	1.09 (0.75,1.59)
1–30	0.86 (0.49,1.48)	0.75 (0.40,1.42)
>30	0.58 (0.29,1.14)	0.45 (0.20,1.02)

[a] Single model with single reference cell with adjustment for age, total energy intake, height, parity, vitamin A supplement intake, and intake of low-fat meats (seafoods and skinless poultry). For interaction term, $P = 0.72$.

[b] Dichotomized on median split.

[c] Reference category.

DISCUSSION

These prospective data provide evidence that a higher consumption of vitamin E may decrease the risk of colon cancer—especially in younger women—or, equally plausibly, delay onset. The inverse association between vitamin E intake and colon cancer in these data is strong. Most, if not all, of the association of vitamin E was derived from supplemental vitamin E intake. At least 3 interpretations of the latter are reasonable. First, vitamin E may protect against colon cancer, and supplemental intake is required to expand the range and/or produce sufficiently high intakes to observe an inverse association. The vitamin E content of the diets consumed in this cohort in general was low, comparable to that in United States diets in general but unlike that of hunter-gatherers or primitive agriculturists consuming large amounts of nuts, seeds, whole grains, and other micronutrient-rich wild plant foods. Furthermore, although the correlation between the food frequency questionnaire estimate for vitamin E and that from 5 24-h dietary recalls was $r > 0.6$ (17), implying respectable discriminatory power, the dietary range of vitamin E in this cohort was very narrow, and the ability of the dietary instrument to categorize women properly over this narrow range may still be questionable. The chance afforded of detecting a protective association of dietary vitamin E in our study population, consequently, may also have been questionable. Despite this, and although the confidence intervals for RR estimates overlapped, the association for total vitamin E was more in the direction of protection than that for supplemental vitamin E alone. A second interpretation of the finding is that it is one or more other factors related to taking vitamin E supplements that confer protection rather than vitamin E itself. In support of this is that since the main driving factor in the total vitamin E/colon cancer association was supplemental vitamin E, and since supplemental vitamin A was also associated with reduced risk, it may be that women who were taking specific nutritional supplements had other lifestyle factors responsible for the protective association. Against this interpretation is the failure to detect confounding from several healthy lifestyle factors, use of supplements in general (multivitamins/minerals and/or specific nutrient supplements), or use of specific supplements in general, although residual confounding cannot be ruled out. Also, there was no evidence, despite contributions from specific supplements, that total

intakes of vitamins A or C conferred protection. Finally, a third interpretation is, of course, that the observed association is merely a random occurrence. An additional point regarding the vitamin E findings in this study is that they are consistent with a protective association with relatively high intakes of vitamin E within a plausible (but not usual) dietary range; however, the statistical power to adequately address this question in this study was insufficient. Further study is indicated in light of our strong findings.

The present study has several strengths and weaknesses. It is limited by the lack of information on duration of supplemental vitamin and mineral use: intake at baseline is assumed to reflect past and subsequent use. It also is limited by the relatively short follow-up (5 years), and by the lack of information on family history of colon cancer, aspirin, and other nonsteroidal anti-inflammatory drug use, colon cancer screening practices, and methods of cooking meat. On balance, however, the study has several advantages over most previous epidemiological studies investigating diet and colon cancer including the prospective design, the use of a well-defined cohort derived from a general population, the validated dietary methodology, and the relatively large number of cases.

The suggestion of a delayed onset of colon cancer with a higher consumption of vitamin E is supported by both epidemiological and animal experimental data. In a Finnish male cohort study, prediagnostic serum vitamin E levels were associated with a decreased risk of all cancers combined, but the inverse association was limited to those younger than 70 years of age (25). In rats given a chemical carcinogen, vitamin E delayed the appearance, but ultimately did not affect the incidence or multiplicity, of colon tumors (26). Dietary vitamin E also delayed the appearance and decreased the incidence of oral tumors in hamsters treated with chemical carcinogens (27).

Evidence from other animal studies for a protective effect of vitamin E, irrespective of age, against colon cancer, or cancers in general, is mixed. In mice supplemented with vitamin E, both inhibition (28) and enhancement (29) of chemically induced colon tumors have been reported. Topical vitamin E decreased the incidence of chemically induced skin tumors in mice (30), but dietary vitamin E had no effect (31). The incidence of chemically induced mammary tumors was decreased in mice fed diets high in vitamin E (32), but the incidence of forestomach tumors was not (33).

Evidence from epidemiological studies that vitamin E protects against colon cancer in humans, irrespective of age, is also mixed. Findings in 5 prospective studies suggested that the level of α-tocopherol, the predominant form of vitamin E in the blood, was lower in subjects who subsequently developed colorectal cancer than in control subjects (34–39). Differences were not significant in any one of the 5 studies. The original data from the 5 studies were pooled and analyzed (40). The odds ratio for the highest quartile of serum α-tocopherol concentration compared to the lowest was 0.6 (95% CI 0.4–1.0). Adjustment for serum cholesterol level attenuated the odds ratio to 0.7 (95% CI 0.4–1.1). Vitamin E intake and colon cancer were not associated in the only other prospective cohort study of women (Nurses' Health Study) using a virtually identical semiquantitative food frequency questionnaire as the present study; however, no mention was made of whether an interaction with age was investigated (41). Results of earlier studies of vitamin E and risk of other cancers in humans are inconsistent as well (8).

The findings of this study also suggest that further investigation of selenium and colon cancer is warranted. Dietary intake of selenium cannot be measured accurately in studies of this kind; selenium content of food varies with soil and growing conditions, rendering dietary values meaningless. Dietary intake is generally estimated at 150–200 μg/day, whereas the mean selenium supplement intake in this study was 66 μg/day, an amount that may have been small relative to mean actual dietary intakes. Thus, analyses involving selenium should be considered exploratory at best. The number of participants taking selenium-containing supplements was small, so that even though a strong inverse association was suggested, the 95% confidence interval around the RR in the final model was wide and included 1.0. The incidence and multiplicity of chemically induced colon tumors in rats have been shown to decrease with selenium supplementation (42, 43). Based on per capita food consumption, dietary selenium in 27 countries was inversely correlated with age-adjusted mortality from colon cancer (44). Within the United States, local plant selenium levels were inversely correlated with age-adjusted mortality from colon cancer (45), and blood selenium concentrations were inversely correlated with age-adjusted mortality from colon cancer both within the United States and internationally (44). In 3 cohort studies measuring serum selenium levels, no association with colon cancer was seen in one (46); a marginal association was observed in another (47); and, in the third study, lower mean levels of selenium were found in individuals who developed colon cancer, but no difference was detected in the relative odds of colon cancer for individuals in the highest and lowest quintiles of selenium intake (37). In a prospective cohort study, toenail selenium levels were marginally but not statistically significantly associated with risk of colon cancer (48).

This study provides little support for an association of vitamins A or C or of β-carotene with colon cancer risk. Although supplemental vitamin A intake was inversely associated with the risk of colon cancer in these data, total vitamin A intake was not, casting serious doubt on the likelihood of a causal relationship between the nutrient itself (vitamin A) and colon cancer. In addition, the results of both animal and human studies on the carotenoids and vitamin C and colon cancer have been mixed and thus provide little evidence for a protective effect of higher intakes of these nutrients against colon cancer (8).

Possible mechanisms of an interaction of vitamin E intake and age on risk of colon cancer are proposed. First, evidence exists that an increased level of epithelial cell turnover in the colon is associated with an increased risk of colon cancer (49). Increased colonic epithelial cell proliferation is seen in the presence of cancer-promoting factors (49). Both colonic epithelial cell proliferation and risk of colon cancer rise with age (50). If colon cancer risk is a product of rates of initiation and levels of colonic epithelial cell proliferation, factors that could lower rates of initiation would delay clinical presentation to later ages. If, by its antioxidant capacity, vitamin E protects colon epithelial cells against DNA damage, then in the presence of high vitamin E intake, rates of cell initiation would be reduced. If one assumes a relatively constant lifetime intake of both initiating agents and vitamin E (and therefore a constant rate of initiation) as well as a relatively constant exposure to dietary or other exogenous cancer-promoting factors, age at presentation will be determined by the level of vitamin E intake. Under this scenario, at older ages the influence of high levels of colonic epithelial cell proliferation will finally outweigh the influence of rates of cell initiation. This could occur either because cells no longer have time to repair DNA damage before it can be further destabilized and/or propagated, or because of the sheer increase in probability that among the large number of rapidly proliferating cells the right combination of initiation and promotion or other conditions for an abnormal cell line to advance will occur.

Second, vitamin E is known to protect cell membranes via its antioxidant activity and therefore may protect against premature cell death and loss. This in turn may reduce compensatory colonic epithelial cell proliferation. If initiation rates rise with age because of declining ability to repair or eliminate abnormal cells, vitamin E would delay onset of colon cancer to older ages.

Third, vitamin E may reduce both initiation and colonic epithelial cell proliferation. This is not inconsistent with the fact that supplementation (especially with vitamin E) is a relatively recent population behavior. While vitamin E may be able to delay onset of late stages of carcinogenesis and even possibly prevent new DNA damage, it may be unable to reverse progression of cells in which mutations took place before vitamin supplementation began.

Some evidence exists to support the proposed anti-initiation role, but little evidence is available for or against the anti-promotion role. Vitamin E is known to reduce mutation rates (51) and nitrosamine formation (9), and may potentiate the immune response (7–9). A significant decrease in epithelial cell proliferation in the upper 40% of colon crypts (thought to be associated with lower risk) (49) was found in a small controlled clinical trial assessing the effect of vitamin A, C, and E supplementation in patients with colorectal adenomas (52). Otherwise, there are currently few data regarding the effect of vitamin E on colonic epithelial cell proliferation, particularly in humans. We believe that further epidemiological investigations to confirm our findings are indicated as are further investigations into whether or not vitamin E can reduce colonic epithelial cell proliferation in humans.

In summary, our data show a strong reduction of colon cancer risk associated with high intakes of vitamin E in women under the age of 65 years. These results are congruent with other data and plausible explanatory mechanisms exist. The results of our investigation provide little evidence for a decreased risk of colon cancer associated with high intakes of carotenoids or vitamin C, but do provide suggestive evidence that high intakes of selenium may protect against colon cancer.

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