The Journal of Nutrition
Nutritional Epidemiology

Supplementation with α-Tocopherol or β-Carotene Reduces Serum Concentrations of Vascular Endothelial Growth Factor-D, but Not -A or -C, in Male Smokers[1,2]

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Abstract

Evidence from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study suggests that vitamin E and β-carotene supplement use may influence the risk of several cancers. Vascular endothelial growth factors (VEGF) are proteins involved in angiogenesis, an important requirement for tumor growth and metastasis. Thus, vitamin E and β-carotene may influence cancer risk through one or more VEGF. The ATBC Study was a randomized, double-blind, placebo-controlled, primary cancer prevention trial in which participants were assigned to 1 of 4 supplementation groups based on a 2 × 2 factorial design: *1*) α-tocopherol (vitamin E); *2*) β-carotene; *3*) both; or *4*) placebo. For the present study, 100 cancer-free participants with follow-up serum available were randomly selected from each intervention group. VEGF-A, -C, and -D concentrations were measured by ELISA in serum obtained at baseline and after at least 2 y of supplementation. Differences in change in VEGF levels from baseline to follow-up between intervention groups were assessed using the ANOVA test. Change in VEGF-A and VEGF-C concentrations between baseline and follow-up did not differ by intervention group ($P = 0.45$ and 0.29, respectively). The decrease in the serum VEGF-D concentration was greater in the men supplemented with α-tocopherol (−9.7 ± 2.5%) or β-carotene (−8.5 ± 2.7%) and tended to be greater in those supplemented with both (−6.8 ± 2.4%) compared to the placebo group, in which there was no change (−0.4 ± 3.0%) ($P = 0.03$). In this population of male smokers, supplementation with α-tocopherol or β-carotene was associated with a decrease in VEGF-D levels over time. Although the mechanism through which these supplements affect cancer etiolog remains unclear, our results support the hypothesis that vitamin E and β-carotene may influence cancer progression through VEGF-mediated lymphangiogenesis. J. Nutr. 141: 2030–2034, 2011.

Introduction

Evidence from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study intervention suggests that vitamin E and beta-carotene supplement use may influence the risk of several cancers. For example, the ATBC Study reported that vitamin E supplement use was associated with a decreased risk of prostate cancer, particularly more aggressive disease (1), although 2 recent trials did not confirm that finding (2,3), and that β-carotene supplement use increased lung and possibly prostate cancer incidence (1,4). However, the mechanisms through which these micronutrients might exert their effects remain unclear. The vascular endothelial growth factors (VEGF) are a family of proteins involved in normal and pathological vascularization. VEGF-A, commonly referred to in the literature as VEGF, is primarily involved in angiogenesis and VEGF-C and VEGF-D have both angiogenic and lymphangiogenic functions (5,6). Given the importance of angiogenesis in tumor growth and metastasis (7), it is possible that a biological impact of vitamin E and β-carotene on one or more VEGF is responsible for their influence on the risks of prostate and lung cancers.

Animal and cell culture experiments indicate that α-tocopherol and other vitamin E compounds inhibit VEGF-A–mediated angiogenesis (8–15). Fewer studies have examined β-carotene, with 2 studies suggesting that β-carotene also reduces VEGF ex-

[1] Supported by U.S. Public Health Service contracts from the National Cancer Institute, Department of Health and Human Services (N01-CN-45165, N01-RC-45035, N01-RC-37004, and HHSN261201000006C, and by funding from the Intramural Research Program of the National Cancer Institute. The laboratory assays were funded in whole or in part with federal funds from the National Cancer Institute, NIH, under contract no. HSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government.
[2] Author disclosures: A. M. Mondul, H. C. Rager, W. Kopp, J. Virtamo, and D. Albanes, no conflicts of interest
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Manuscript received April 25, 2011. Initial review completed June 6, 2011. Revision accepted August 23, 2011.
First published online September 21, 2011; doi:10.3945/jn.111.143669.

pression (16,17) and others showing increased VEGF-mediated angiogenesis (18,19). An earlier analysis from the ATBC Study found that VEGF-A levels decreased during the intervention in men randomized to receive the trial α-tocopherol supplement (i.e. the intervention group that experienced lower prostate cancer incidence) compared to those who received the placebo (the impact of β-carotene was not examined) (20). The influence of vitamin E and β-carotene on the other members of the VEGF family has not been studied despite a growing understanding of angiogenesis in cancer growth, metastasis, and treatment (5). To address this, we examined the effect of supplementation with vitamin E (α-tocopherol, 50 mg) and β-carotene (20 mg) during the trial on circulating VEGF-A, -C and -D concentrations in the ATBC Study.

Materials and Methods

Study population. The ATBC Study was a randomized, double-blind, placebo-controlled, primary prevention trial conducted to determine the effects of supplementation with α-tocopherol and β-carotene on cancer incidence (21). Male smokers (n = 29,133) were recruited between 1985 and 1988 in southwestern Finland. As part of the enrollment criteria, men were between 50–69 y old at baseline and smoked at least 5 cigarettes/d and were ineligible if they previously had cancer or another serious illness at enrollment or if they reported current use of supplements containing vitamin E (>20 mg), vitamin A (>20.9 μmol), or β-carotene (>6 mg). Trial participants were assigned to 1 of 4 groups based on a 2 × 2 factorial design: *1*) α-tocopherol (dl-α-tocopheryl acetate, 50 mg/d); *2*) β-carotene (20 mg/d); *3*) both supplements; or *4*) placebo. Supplementation was ongoing for 5–8 y until the trial ended on April 30, 1993. Although the intervention trial was completed, follow-up continues through the Finnish Cancer Registry and the Register of Causes of Death. Written informed consent was obtained from all trial participants and the ATBC Study was approved by institutional review boards at both the US National Cancer Institute and the Finnish National Public Health Institute. For the present analysis, 100 cancer-free participants were randomly selected from each trial arm from among those who had both a baseline and a follow-up blood sample available and who had been taking supplements for at least 2 y (range: 2–8 y) at the time the follow-up specimen was obtained.

Data collection. At enrollment, ATBC Study participants completed questionnaires about general risk factors, smoking, and medical history, as well as a FFQ. Participants also underwent a physical examination by registered nurses to measure their height and weight and to collect an overnight fasting blood sample. Starting in the second year of the trial, follow-up serum samples were collected annually from a random sample (n = 800) of the trial participants. All samples were stored at –70°C.

Serum VEGF-A, -C, and -D concentrations were measured for the 400 participants with follow-up serum samples selected for the present analysis by SAIC-Frederick using ELISA kits according to the manufacturer's instructions (VEGF-A: Thermo Scientific/Pierce Biotechnology; VEGF-C and VEGF-D: R&D Systems). Duplicates of each sample were analyzed and the average of the 2 values was used for data analysis. Each batch contained quality control serum samples from the ATBC Study and 4 healthy male donors who were recruited through the NCI-Frederick Research Donor Program. This program stores blood samples from employees of the NCI-Frederick and Fort Detrick communities for research purposes; the program requirements are similar to those in place for the American Red Cross Blood Bank (22). Two batches were excluded because of technical errors and 6 study participants (8 for VEGF-C) were excluded because their duplicate values deviated by >20%. The median quality control CV percents for the 5 QC samples were 21.5, 15.8, and 11.5% for VEGF-A, -C, and -D, respectively. The final analytic data set included 354 participants (352 with VEGF-C).

Statistical analysis. Differences in change in VEGF levels from baseline to follow-up between intervention groups were assessed using ANOVA. In addition to the ANOVA approach, we conducted pair-wise comparisons between each trial arm and placebo using the t test. As expected based on the randomized study design, multivariable linear regression showed no evidence of confounding by any of the following factors: age, BMI, serum total cholesterol, baseline serum α-tocopherol, baseline serum β-carotene, number of cigarettes smoked per day or years smoked, physical activity, or intake of total energy, fruits, vegetables, red meat, or alcohol. Sensitivity analyses were conducted using multivariable linear regression to adjust for baseline values. Secondary analyses were conducted stratifying by baseline age at randomization (<60 vs. ≥60 y), BMI (<26 vs. ≥26 kg/m^2), cigarettes smoked per day (<20 vs. ≥20), and serum total cholesterol (<6.18 and ≥6.18 mmol/L) as well as by time between baseline and follow-up blood collections (<4 vs. ≥4 y). Statistical interaction was assessed using the likelihood ratio test. All analyses were conducted using SAS v. 9.1.

Results

Characteristics of the analytic cohort according to low and high baseline concentrations of the 3 measured VEGF are shown in **Table 1**. Overall, baseline characteristics varied little by VEGF-A, -C, or -D. There were no differences by VEGF-A, whereas those with higher VEGF-C had a lower baseline serum β-carotene concentration (P = 0.045), were more physically active (P = 0.10), and ate slightly more fruit (P = 0.05) than did those with lower VEGF-C concentrations (Table 1). Men with higher VEGF-D concentrations smoked for longer than men with lower VEGF-D (P = 0.03) (Table 1).

There were no significant differences in change between baseline and follow-up concentrations for either VEGF-A or VEGF-C across the 4 trial intervention groups (**Table 2**). By contrast, the change between baseline and follow-up VEGF-D concentrations differed among the 4 groups (Table 2). Men randomized to either α-tocopherol alone or β-carotene alone had a significantly greater reduction in VEGF-D during supplementation compared to men who received placebo, among whom there was no change in VEGF-D concentration (Table 2). Men randomized to receive both supplements tended to have a greater reduction (P = 0.07) in the VEGF-D during the intervention compared to men who received the placebo (Table 2). These findings were unchanged when we adjusted for baseline levels using a linear modeling strategy; the estimated mean change in serum VEGF-D (ng/L) was greater in the men who received α-tocopherol alone (-35.2 ± 16.1; P = 0.004), β-carotene alone (-28.3 ± 16.4; P = 0.02), or both supplements (-23.1 ± 16.4; P = 0.06) compared to those who received the placebo (-1.1 ± 16.5). Similarly, we found no relation between change in serum concentration of either α-tocopherol or β-carotene and change in VEGF-A or -C concentrations. There tended to be an inverse association between the change in serum α-tocopherol and VEGF-D ($\beta = -1.88$; P = 0.08) but not between the change in serum β-carotene and VEGF-D ($\beta = -0.002$; P = 0.44).

There were no significant interactions between changes in VEGF during the intervention and age, BMI, number of cigarettes smoked per day, baseline serum total cholesterol, or time between baseline and follow-up blood collections (all P-interaction ≥ 0.30).

Discussion

In this analysis, we found that men supplemented with either α-tocopherol or β-carotene (or both) for 2–8 y had a greater reduction in circulating VEGF-D concentrations compared to men who were randomized to receive placebo, in whom there was no change. There was no difference across supplementation groups in changes in serum VEGF-A or -C during the intervention, however.

TABLE 1 Selected characteristics of the men by baseline serum VEGF -A, -C, and -D concentrations greater or less than the median of each[1]

	VEGF-A, *ng/L*		VEGF-C, *ng/L*		VEGF-D, *ng/L*	
Characteristics[2]	<108	≥108	<4184	≥4184	<317	≥317
n	177	177	176	176	177	177
Age, *y*	57 ± 0.4	57 ± 0.3	57 ± 0.4	57 ± 0.3	57 ± 0.4	57 ± 0.3
Height, *cm*	174 ± 0.5	174 ± 0.4	174 ± 0.5	173 ± 0.4	174 ± 0.5	174 ± 0.4
Weight, *kg*	78.6 ± 1.0	79.7 ± 1.0	78.6 ± 0.9	79.4 ± 1.0	80.3 ± 1.1	78.2 ± 0.9
BMI, kg/m^2	26.0 ± 0.3	26.3 ± 0.3	25.8 ± 0.3	26.5 ± 0.3	26.5 ± 0.3	25.9 ± 0.3
Cigarettes per day	19.5 ± 0.6	19.3 ± 0.6	19.9 ± 0.6	18.8 ± 0.6	19.2 ± 0.6	19.5 ± 0.6
Years smoked	35.2 ± 0.7	35.0 ± 0.6	35.5 ± 0.6	34.8 ± 0.6	34.1 ± 0.7	36.0 ± 0.6*
Years between blood collections	4.0 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.0 ± 0.1
Physically active, *n (%)*	40 (22.6)	42 (23.7)	34 (19.3)	47 (26.7)	43 (24.3)	38 (21.5)
Intake, unit/d						
Total energy, *kcal*	2740 ± 63	2650 ± 64	2660 ± 63	2710 ± 63	2720 ± 67	2670 ± 60
Fruit, *g*	222 ± 18	258 ± 17	214 ± 16	262 ± 19	260 ± 18	221 ± 17
Vegetables, *g*	298 ± 9	304 ± 9	297 ± 9	298 ± 8	308 ± 10	295 ± 8
Red meat, *g*	73.4 ± 2.5	74.5 ± 2.9	73.2 ± 2.6	74.2 ± 2.8	76.7 ± 2.7	71.2 ± 2.7
Alcohol, *g*	16.0 ± 1.5	16.1 ± 1.3	17.3 ± 1.6	14.7 ± 1.2	15.4 ± 1.4	16.7 ± 1.4
Serum concentrations						
Total cholesterol, *mmol/L*						
Baseline	6.4 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
Follow-up	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
α-Tocopherol, *mg/L*						
Baseline	12.5 ± 0.3	12.4 ± 0.2	12.5 ± 0.3	12.5 ± 0.2	12.2 ± 0.2	12.7 ± 0.3
Follow-up[3]	13.0 ± 0.4/18.6 ± 0.6	13.4 ± 0.4/17.9 ± 0.4	13.2 ± 0.4/18.3 ± 0.6	13.3 ± 0.5/18.3 ± 0.5	12.9 ± 0.4/18.3 ± 0.5	13.5 ± 0.5/18.3 ± 0.6
β-Carotene, $\mu g/L$						
Baseline	263 ± 30	225 ± 13	276 ± 30	211 ± 11*	252 ± 14	235 ± 29
Follow-up[4]	262 ± 32/3160 ± 183	287 ± 38/3240 ± 189	303 ± 42/3220 ± 177	254 ± 30/3190 ± 202	263 ± 23/3190 ± 187	285 ± 44/3180 ± 187
Retinol, $\mu g/L$						
Baseline	592 ± 10	577 ± 10	576 ± 10	592 ± 10	589 ± 9.3	580 ± 10
Follow-up	599 ± 11	601 ± 11	588 ± 10	612 ± 11	608 ± 10	594 ± 11

[1] Values are mean ± SE unless otherwise noted. *Different from corresponding < median group, $P < 0.05$.
[2] Measured at baseline unless otherwise noted.
[3] Data for α-tocopherol supplemented and non-α-tocopherol–supplemented groups are presented separately, $n = 82–95$.
[4] Data for β-carotene supplemented and non-β-carotene–supplemented groups are presented separately, $n = 75–101$.

TABLE 2 Serum VEGF-A, -C, and -D concentrations in male smokers supplemented with placebo, α-tocopherol, β-carotene, or both for at least 2 y[1]

	Intervention group				
	Placebo	α-Tocopherol	β-Carotene	α-Tocopherol and β-carotene	*P* value[2]
VEGF-A, *ng/L*					
n	86	89	90	90	
Baseline	136 ± 13	132 ± 10	134 ± 11	120 ± 10	
Follow-up	127 ± 12	127 ± 10	120 ± 9	120 ± 11	
Change	−8.8 ± 6.4	−5.2 ± 5.2	−13.5 ± 6.9	0.6 ± 6.7	0.45
% Change	−6.5	−3.9	−10.1	0.1	
VEGF-C, *ng/L*					
n	85	90	87	90	
Baseline	4,300 ± 157	4,450 ± 157	4,160 ± 140	3,920 ± 130	
Follow-up	4,180 ± 165	4,080 ± 138	4,100 ± 121	3,920 ± 148	
Change	−120 ± 169	−370 ± 117	−60.0 ± 137	0.0 ± 149	0.29
% Change	−2.8	−8.3	−1.4	0.0	
VEGF-D, *ng/L*					
n	87	91	88	88	
Baseline	314 ± 12	364 ± 14	331 ± 14	337 ± 13	
Follow-up	313 ± 13	329 ± 14	303 ± 14	314 ± 12	
Change	−1.1 ± 9.4	−35.2 ± 8.3**	−28.3 ± 8.3*	−23.0 ± 7.5	0.03
% Change	−0.4	−9.7	−8.5	−6.8	

[1] Values are mean ± SE. Asterisks indicate the change differed from that in the placebo group: *$P < 0.05$, **$P < 0.01$.
[2] *P* value from ANOVA. VEGF, vascular endothelial growth factors.

A great deal of research has focused on factors influencing VEGF-A levels, including members of the vitamin E family and β-carotene (8–20). By contrast, relatively little is known about modifiers of VEGF-D. One study demonstrated that inhibition of NF-κB, a DNA transcription factor, decreased production of VEGF-D (23), and there is evidence that both vitamin E and β-carotene inhibit NF-κB (24,25). Thus, inhibition of NF-κB is one mechanism through which vitamin E and β-carotene supplementation may influence VEGF-D concentrations.

The present findings are consistent with the ATBC Study intervention finding of men receiving α-tocopherol supplements having lower prostate cancer incidence, particularly more aggressive prostate cancer (1). Our data also contradict the increased risk of lung and prostate cancers observed among men who received the trial β-carotene supplement, however (1,4). One possible explanation may have to do with the association between serum VEGF-D and lymph-node metastasis being organ specific. For example, several studies show that greater expression of VEGF-D or its receptor by the tumor cells is associated with increased prostate cancer lymph node metastasis (26–28). By contrast, the association between VEGF-D and lung cancer is less clear, with some studies showing that higher VEGF-D levels in lung tumor tissue were correlated with increased lymph node metastasis and poorer prognosis (29–31) and others finding the opposite (32–35). One hypothesis regarding how VEGF-D might have diverse effects across tumor types involves the degree to which it undergoes postsecretion proteolytic processing in specific tumors (5). Of course, vitamin E and β-carotene exert biologic effects on many pathways other than those related to VEGF; the way in which these effects balance out and interact with one another is likely to influence the ultimate cancer outcome. Thus, although our findings may not be consistent with all of the trial findings, they likely represent one component of the complex system that contributes to cancer etiology. Alternatively, it is possible that our findings are due to chance.

Our study has many strengths, including the randomized, placebo-controlled, double-blind design and the >2-y duration of supplementation. Although we did not replicate the findings from an earlier analysis conducted in this population that found reduced VEGF-A levels in response to supplemental α-tocopherol, this discrepancy may be explained by use of a different VEGF-A kit in the prior study that may have influenced performance, including, e.g., the fact that the 2 antibodies were raised differently (36). Also, the kit used in the present analysis is known to have ~20% cross-reactivity with human VEGF/placental growth factor heterodimer (37), whereas the kit used previously did not cross-react with this heterodimer (38). This could have led to measurement error, preventing us from replicating our previous result, which was consistent with experimental data supporting a role for vitamin E in reducing VEGF-mediated angiogenesis (8–15). Future studies comparing the results from different measurement techniques may shed light on the most sensitive method of detecting VEGF in serum and whether they robustly reflect local tissue concentrations. One weakness of our present analysis was the relatively high CV percent for VEGF-A (21.5%), which could have prevented us from observing a true association for this isoform. However, it seems unlikely that this could explain the discrepancy with the earlier ATBC report, because the CV percent was actually higher for VEGF-A in that analysis (34%) (20). Another consideration in comparing these 2 analyses is that the storage time for the samples was longer for the current analysis than for the previous one. Given that the VEGF isoforms we measured have nearly identical molecular structure and weight, however, we would expect storage time to affect all isoforms to a similar degree. Thus, our observation of an association for VEGF-D argues against storage time-dependent degradation of our serum specimens.

In this population of male smokers, supplementation with either α-tocopherol or β-carotene was associated with a significant decrease in serum VEGF-D concentrations. Although the mechanism through which these supplements may affect cancer etiology remains unclear, our results support the hypothesis that vitamin E and β-carotene may influence cancer progression through VEGF-mediated lymphangiogenesis.

Acknowledgments

We thank Yanyu Wang for her contributions to the laboratory analyses. J.V. and D.A. designed research; H.C.R. and W.K. conducted research; A.M.M. analyzed data; A.M.M., J.V., and D.A. wrote the paper; and A.M.M. had primary responsibility for the final content. All authors read and approved the final manuscript.

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