

Association Between Serum α -Tocopherol and Serum Androgens and Estrogens in Older Men

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Abstract: *There is evidence supporting a role for sex hormones in the etiology of prostate cancer. Supplementation with α -tocopherol reduced prostate cancer in the α -Tocopherol, β -Carotene Prevention Study (ATBC Study). The objective of this study was to assess the relation of baseline levels of serum α -tocopherol and serum sex hormones in older men. A cross-sectional analysis of serum α -tocopherol and sex hormone concentrations was conducted within a subset of the ATBC Study. Serum was collected in the morning after an overnight fast at baseline from 204 men ages 50–69 years participating in the ATBC Study and free of prostate cancer. Hormones were measured by radioimmunoassay, and α -tocopherol was measured by high-performance liquid chromatography by standard procedures. Multivariate linear regression was used to evaluate the association of serum α -tocopherol with nine androgens and estrogens after controlling for age, body mass index, hormone assay batch, and serum cholesterol. Serum α -tocopherol was significantly inversely associated with serum androstenedione, testosterone, sex hormone-binding globulin, and estrone. The difference in hormone concentration per milligram of α -tocopherol was 1.8–2.6% for these four hormones. These results indicated that α -tocopherol is related to concentrations of several sex hormones in older men and may have implications for the observed protective effect of supplemental vitamin E in relation to prostate cancer in the ATBC Study.*

Introduction

Although prostate cancer is one of the most common cancers afflicting men in the United States and Western Europe (1), few risk factors other than age, race, and family history have been identified. In the α -Tocopherol β -Carotene Cancer Prevention (ATBC) Study, supplementation with α -tocopherol (vitamin E) resulted in an unexpected 32% reduction in the incidence of prostate cancer (2,3) and a 41% reduction in mortality from the disease. The relation of α -

tocopherol to sex hormones is of interest because of evidence supporting a role for sex hormones in the etiology of prostate cancer. Although results of epidemiological studies of serum hormones in relation to prostate cancer are inconsistent (4–18), in the largest prospective analysis to date, Gann and colleagues (17) reported a significant positive association for testosterone and a significant inverse association for estradiol with prostate cancer risk. Furthermore, suppression of androgen production by the testis and adrenal cortex dehydroepiandrosterone sulfate is an effective pharmacological therapy for prostate cancer (19). In animal models, prostate tumors can be induced by prolonged administration of testosterone, and this effect is enhanced by estrogens (20).

We conducted a cross-sectional analysis of baseline serum sex hormone and serum α -tocopherol concentrations within a subset of the ATBC Study population who were free of prostate cancer to assess whether there is a relationship between serum sex hormones and serum α -tocopherol and, thus, to evaluate the potential for serum α -tocopherol to modulate serum sex hormone concentrations.

Subjects and Methods

Sample Population

The sample population for this cross-sectional analysis consisted of men who participated in the ATBC Study and were selected as controls for a nested case-control study of serum sex hormones and subsequent development of prostate cancer (21). The ATBC Study was a large, randomized, double-blind, placebo-controlled prevention trial to determine whether daily supplementation with α -tocopherol and/or β -carotene would reduce the incidence of lung or other cancers. The study was conducted in Finland between 1985 and 1993 as a joint project between the National Public Health Institute in Finland and the US National Cancer Institute.

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The overall design and initial results of the ATBC Study have been published (2,22). Briefly, 29,133 male smokers between 50 and 69 years of age were recruited from southwestern Finland between 1985 and 1988 and randomly assigned to one of four groups based on a 2×2 factorial design. Participants received 50 mg/day α -tocopherol (as *dl*- α -tocopheryl acetate), 20 mg/day β -carotene, both α -tocopherol and β -carotene, or placebo. Active follow-up continued for five to eight years until death or 30 April 1993. During this time, 246 prostate cancers were diagnosed. Of these, 116 were randomly selected for inclusion in a prospective nested case-control study of serum sex hormones and risk of prostate cancer (21). Two controls were selected for each case in the nested case-control study. Controls were alive and the same age (± 1 yr) as the case on their date of diagnosis and were free of any known cancer other than possible nonmelanoma skin cancer for the duration of follow-up. Controls were also matched to cases by date of baseline blood collection (± 28 days), intervention group, and study center. With use of incidence density sampling, a total of 231 men were chosen to be controls in the study of serum sex hormones and prostate cancer risk. The present study includes 204 of these men who had fasted eight or more hours before the time of their baseline blood collection.

Data Collection

At baseline, study participants completed a demographic and general medical history questionnaire and a food frequency (use) questionnaire, and height and weight were measured. Blood was collected in the morning after an overnight fast. Ninety-five percent of baseline blood samples for the cohort were collected before 10 AM. Serum was separated into 1-ml aliquots and stored in glass vials at -70°C for future analysis.

Serum α -tocopherol was determined by high-performance liquid chromatography (HPLC) (23) in the chemistry laboratory of the National Public Health Institute (Helsinki, Finland) with a between-run coefficient of variation of 2.2%. Androgens and sex hormone-binding globulin (SHBG) were measured by Endocrine Sciences (Calabassas Hills, CA), and estrogens were measured by Corning-Nichols Institute (San Juan Capistrano, CA) by standard procedures. Testosterone and dihydrotestosterone (DHT) were extracted with hexane and ethyl acetate and separated by aluminum oxide chromatography, then measured by radioimmunoassay (RIA). SHBG was measured by an immunoradiometric assay. The non-SHBG-bound testosterone, comprised of free and albumin-bound testosterone, was determined by ammonium sulfate precipitation, and the quantity of testosterone that was non-SHBG bound was then calculated as the product of the total testosterone concentration and the percentage that was non-SHBG bound. Androstenedione was measured by RIA after extraction with hexane and ethyl acetate and separation from the aqueous phase by centrifugation. Dehydroepiandrosterone sulfate (DHEAS) was subjected to enzyme hydrolysis, and

released dehydroepiandrosterone was measured directly by RIA. Androstenediol glucuronide was extracted with a polar solvent and subjected to enzymolysis. Released androstenediol was extracted with hexane ethyl acetate, purified using HPLC, and quantitated using RIA. Estradiol in serum was measured by RIA after extraction with an organic solvent and celite-ethylene glycol chromatography. Estrone was extracted with an organic solvent and separated by celite chromatography and double antibody-polyethylene glycol precipitation before quantification by RIA. Blind quality control samples were randomly positioned within each batch. The overall coefficients of variation for the sex hormones for these quality control samples were as follows: testosterone, 5.4%; non-SHBG-bound testosterone, 5.8%; DHT, 11.3%; androstenedione, 14.8%; DHEAS, 12.9%; androstenediol glucuronide, 15.7%; estradiol, 13.8%; estrone, 14.2%; and SHBG, 7.0%.

The hormone concentrations observed in this study were within the adult male normal ranges for the laboratories performing the assays. SHBG concentrations in this study were above the normal range reported by the laboratory that performed this assay, as previously noted (21). This is believed to have been the result of a laboratory error that affected the absolute but not the relative serum concentration of SHBG reported. Problems associated with the SHBG assay do not affect the non-SHBG-bound testosterone results, since SHBG concentrations were not used to estimate non-SHBG-bound testosterone concentrations. The serum α -tocopherol levels for the men in this study were also within the normal limits for men in this age range. Only 13% of the men reported using a low-dose α -tocopherol supplement at baseline; thus serum α -tocopherol levels are largely attributable to dietary intake.

Statistical Analysis

Statistical analyses were performed using Statistical Analysis Systems software (24,25). The serum hormones were logarithmically transformed to normalize their distributions before analysis. Because α -tocopherol is carried on serum lipids, serum α -tocopherol was adjusted for total serum cholesterol via the residual method (26). Residuals were generated from a regression model, with serum α -tocopherol as the response variable and serum total cholesterol as the explanatory variable. The mean serum α -tocopherol concentration was then added to the residual to generate a meaningful value for cholesterol-adjusted serum α -tocopherol concentration. Multiple linear regression was used to predict serum hormone concentrations from (cholesterol) adjusted α -tocopherol concentration. For each hormone, a model was defined with logarithmic hormone concentration as the dependent variable and α -tocopherol as the independent variable. Age, body mass index (BMI, kg/m^2), and hormone assay batch were included in models as covariates. Smoking status, time of day of blood collection, and dietary intake variables, including energy, fat, cholesterol, fiber, alcohol,

vitamin C, and α -tocopherol intake, did not modify the associations between α -tocopherol and sex hormones and were not included in the models. Percent difference in hormone concentration per unit in α -tocopherol was calculated by using the parameter estimates (*b*) for α -tocopherol modeled as a continuous variable [the formula $(e^b - 1) \times 100$]. We also evaluated the relation between α -tocopherol and serum sex hormones by categorizing the men into quartiles of α -tocopherol concentration and including three indicator variables for the three highest quartiles in the models. Analysis of covariance was used to generate the geometric means of hormone concentrations by quartile of serum α -tocopherol after adjusting for covariates. Effect modification by age and BMI was assessed by including factors and their cross-product terms with the continuous serum α -tocopherol variable in the models.

Results

Table 1 presents the characteristics of study participants. The participants in this study were all Caucasian men who were smokers. The mean age of participants was 60.5 years. On average, participants consumed 12.7 mg/day α -tocopherol from dietary and supplemental sources. Only 13% of the men reported consuming a supplement containing α -tocopherol.

Mean serum hormone concentrations (with 95% confidence intervals) adjusted for age, BMI, serum cholesterol, and hormone assay batch are reported by quartile of serum α -tocopherol in Table 2. In multiple regression analyses, serum α -tocopherol was a significant ($p < 0.05$) negative predictor of testosterone, androstenedione, estrone, and SHBG after controlling for covariates. The difference in hormone concentration per milligram of α -tocopherol was 1.8–2.6% for these four hormones. Serum concentrations of testosterone, androstenedione, estrone, and SHBG decreased monotonically ($p < 0.05$) with increasing quartile of serum α -tocopherol. Serum concentration of DHT also decreased with increasing α -tocopherol concentration, but the trend was not statistically significant ($p = 0.11$). Serum α -tocopherol, together with the other covariates, predicted 15–34% of the variability in hormone concentrations for androgens and SHBG, but only 4–6% of the variability in concentration for estradiol and estrone. Overall, there were very low concentrations of estrogens in the men's serum and only a limited range of values. We did not observe effect modification of the α -tocopherol-hormone associations by age or BMI in these data.

Discussion

After accounting for the potential confounding effects of age, BMI, hormone assay batch, and serum cholesterol, serum α -tocopherol concentration was significantly inversely associated with serum androstenedione, estrone, testosterone, and SHBG in this group of older Caucasian men.

Table 1. Characteristics of Study Participants^a

Characteristics	Mean ^b	SD
Age, yr	60.5	5.1
Height, cm	174	6.3
Weight, kg	79.0	12.7
BMI, kg/m ²	26.2	3.7
Cigarettes, no./day	18.9	8.3
Dietary intake		
Energy, kJ	11,042	862
Fat, g	113	12.5
Fiber, g	22.7	4.1
Alcohol, g	5.6	23.5
Total vitamin E, mg	12.7	5.6
Dietary vitamin E, mg	11.0	2.4
Laboratory values		
Testosterone, nM	20.64	2.06
Non-SHBG-bound testosterone, nM	5.11	0.45
Dihydrotestosterone, nM	1.87	0.36
Androstenedione, nM	4.76	0.37
DHEAS, μ M	3.16	0.97
Androstenediol glucuronide, nM	5.89	1.90
Estradiol, nM	0.10	0.01
Estrone, nM	0.16	0.01
SHBG, nM	83.93	10.61
Total cholesterol, mM	6.13	0.18
HDL cholesterol, mM	1.17	0.08
α -Tocopherol, mg/l	11.75	0.47

a: $n = 204$. BMI, body mass index; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; HDL, high-density lipoprotein.

b: Geometric means and their standard deviations are given for dietary and serum variables.

The cross-sectional nature of the data used for this study limits the inferences that can be made from the results. We cannot determine whether α -tocopherol influenced sex hormone concentrations, whether the reverse may have occurred, or whether some other factor influenced both in the observed directions. However, data from other studies suggest that α -tocopherol could influence serum sex hormone concentration.

There is evidence from *in vitro* (27,28), animal (29–31), and clinical studies (32–35) that α -tocopherol may play a role in hormone metabolism. There are high amounts of α -tocopherol in the testis and adrenal cortex, the primary sites of androgen production in men. High levels of α -tocopherol have also been reported in the prostate, and α -tocopherol binding sites have been found in adrenal cell membranes (36).

In animals (29–31) and in cultured cells (27,28), supplementation with α -tocopherol beyond levels required for adequate nutrition inhibits prostaglandin synthesis. Prostaglandins stimulate secretion of several hypothalamic, gonadotropic, and pituitary hormones and their releasing factors, including luteinizing hormone, follicle-stimulating hormone, and adrenocorticotrophic hormone (37–39), which in turn stimulate androgen production by the testis and adrenal cortex. Consequently, higher serum α -tocopherol could lead to lower levels of serum androgens.

In a controlled feeding trial, Bhatena and colleagues (32) administered 15 g/day of fish oil and fish oil supple-

Table 2. Geometric Means and 95% CI of Serum Hormone Concentration by Quartile of Serum α -Tocopherol After Adjustment for Covariates^{a-c}

	Mean	95% CI	P Value (for Trend)
Testosterone, nM			
Q1	22.38	20.61–24.31	0.03
Q2	20.33	18.71–22.08	
Q3	20.62	19.06–22.30	
Q4	19.61	18.06–21.30	
Non-SHBG-bound testosterone, nM			
Q1	5.10	4.69–5.55	0.88
Q2	5.19	4.77–5.64	
Q3	5.07	4.68–5.49	
Q4	5.19	4.77–5.64	
Dihydrotestosterone, nM			
Q1	1.96	1.79–2.14	0.11
Q2	1.91	1.75–2.08	
Q3	1.86	1.71–2.02	
Q4	1.76	1.61–1.92	
Androstenedione, nM			
Q1	5.32	4.96–5.71	0.007
Q2	4.96	4.63–5.31	
Q3	4.49	4.20–4.80	
Q4	4.35	4.06–4.65	
DHEAS, μM			
Q1	3.49	3.03–4.03	0.11
Q2	3.37	2.92–3.89	
Q3	2.85	2.48–3.27	
Q4	3.00	2.60–3.47	
Androstenediol glucuronide, nM			
Q1	5.46	4.60–6.50	0.92
Q2	6.81	5.78–8.02	
Q3	5.31	4.54–6.21	
Q4	5.81	4.96–6.81	
Estradiol, nM			
Q1	0.104	0.095–0.113	0.27
Q2	0.103	0.094–0.113	
Q3	0.105	0.096–0.114	
Q4	0.102	0.094–0.111	
Estrone, nM			
Q1	0.158	0.145–0.171	0.02
Q2	0.159	0.147–0.172	
Q3	0.147	0.136–0.158	
Q4	0.150	0.139–0.163	
SHBG, nM			
Q1	92.11	84.65–100.23	0.02
Q2	80.98	74.40–88.15	
Q3	85.24	78.56–92.48	
Q4	78.37	72.05–85.26	

a: $n = 204$. Values were adjusted for BMI, age, batch, and serum cholesterol. CI, confidence interval; Q1–Q4, Quartiles 1–4.

b: Serum α -tocopherol adjusted for serum cholesterol via residual method, then mean was readded.

c: Quartile cut points of adjusted serum α -tocopherol (mg/l) are as follows: Q1 ≤ 10.2 ; Q2 = 10.3–11.7; Q3 = 11.8–13.3; Q4 ≥ 13.4 .

mented with 200 mg vitamin E/day to each of 40 men for 10-week periods. Decreases in insulin, DHEAS, and growth hormone were observed during the vitamin E supplementation period. In our study, we observed a nonsignificant inverse association between serum α -tocopherol and DHEAS. In contrast to our results, in a hospital-based study, researchers supplemented 11 men 30–69 years of age with 438 mg/day α -tocopherol and reported a significant increase in plasma testosterone levels (33). Unfortunately, be-

cause no control group was included in this study, it is difficult to rule out the possibility that other factors may have contributed to these results. In another trial, 200 subjects given 600 IU/day *dl*- α -tocopheryl acetate showed significant decreases in serum thyroid hormone levels (34). No sex hormone concentrations were measured in this study; however, administration of triiodothyronine in another clinical study significantly increased serum levels of testosterone (35).

Another potential mechanism for our findings is that men with higher steroid levels utilized more α -tocopherol to counteract the effects of steroid-induced oxidation. The process of steroid biosynthesis uses molecular oxygen, and all interactions of the cellular cytochrome *P*-450 enzymes with cholesterol and its metabolites are major sources of free radical formation (40,41).

In conclusion, we identified significant inverse associations between baseline serum α -tocopherol and several sex hormones in this population. These results may have implications for the observed protective effect of supplemental α -tocopherol in relation to prostate cancer in the ATBC Study (3). Studies are underway to further examine the effect of supplemental α -tocopherol on sex hormone concentrations in this study.

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