Serum Metabolomic Response to Low- and High-Dose Vitamin E Supplementation in Two Randomized Controlled Trials

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ABSTRACT

Background: Vitamin E is an essential micronutrient and critical human antioxidant previously tested for cancer preventative effects with conflicting clinical trial results that have yet to be explained biologically.

Methods: We examined baseline and on-trial serum samples for 154 men randomly assigned to receive 400 IU vitamin E (as alpha-tocopheryl acetate; ATA) or placebo daily in the Vitamin E Atherosclerosis Prevention Study (VEAPS), and for 100 men administered 50 IU ATA or placebo daily in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC). Over 970 metabolites were identified using ultrahigh-performance LC/MS-MS. Linear regression models estimated the change in serum metabolites of men supplemented with vitamin E versus those receiving placebo in VEAPS as compared with ATBC.

Results: Serum alpha-carboxyethyl hydrochroman (CEHC) sulfate, alpha-tocopherol, and beta/gamma-tocopherol were significantly altered by ATA supplementation in both trials (all P values $\leq 5.1 \times 10^{-5}$, the Bonferroni multiple comparisons corrected statistical threshold). Serum C_{22} lactone sulfate was significantly decreased in response to the high-dose vitamin E in VEAPS ($\beta = -0.70$, $P = 8.1 \times 10^{-6}$), but not altered by the low dose in ATBC ($\beta = -0.17$, $P = 0.4$). In addition, changes in androgenic steroid metabolites were strongly correlated with the vitamin E supplement–associated change in C_{22} lactone sulfate only in the VEAPS trial.

Conclusions: We found evidence of a dose-dependent vitamin E supplementation effect on a novel C_{22} lactone sulfate compound that was correlated with several androgenic steroids.

Impact: Our data add information on a differential hormonal response based on vitamin E dose that could have direct relevance to opposing prostate cancer incidence results from previous large controlled trials.

Introduction

The lipid-soluble, essential nutrient vitamin E is a critical cellular antioxidant that inhibits lipid peroxidation, platelet aggregation, and inflammation, and vitamin E supplementation has been tested for cancer and cardiovascular disease prevention for decades with mixed results (1–4). Prostate cancer has received substantial attention in this regard, with several large randomized controlled trials (RCT) examining the hypothesis. The first of these reported significant 32% and 40% reductions in prostate cancer incidence and mortality, respectively, in response to a modest daily dose of 50 IU alpha-tocopheryl acetate (ATA), albeit the findings were secondary hypotheses in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), which was focused primarily on lung cancer (4–6). On the basis of these and other basic and observational data, two additional RCTs subsequently tested vitamin E for prostate cancer prevention. The Physicians' Health Study–II (PHS-II) reported no effect of 400 IU every other day (2), while the Selenium and Vitamin E Cancer Prevention Trial (SELECT) found 17% increased prostate cancer incidence following 7–12 years of supplementation with 400 IU ATA daily (3). In comparison with the two supplementation dosages, the current recommended U.S. dietary allowance for vitamin E is 22.4 IU daily, and the majority of multivitamin supplements provide 30 IU (7). The biological basis and molecular responses for the qualitative difference between ATBC (50 IU daily) and SELECT (400 IU daily) has not been elucidated. In addition, a previous meta-analysis showed that high-dose vitamin E supplementation (≥400 IU daily) may increase all-cause mortality, whereas low doses appeared to have no effect on mortality (8).

The present investigation measured the serum biochemical changes in men receiving 400 IU ATA in a controlled clinical trial, the Vitamin E Atherosclerosis Prevention Study (VEAPS; ref. 9), and an additional set of men supplemented with 50 IU ATA in ATBC to gain biological insight into vitamin E dosage–related effects potentially relevant to the divergent prostate cancer findings in previous RCTs.

Materials and Methods

Study populations

VEAPS was a randomized, double-blinded, placebo-controlled trial primarily designed to test whether vitamin E supplementation

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Clinical trial registration: ClinicalTrials.gov Identifier: NCT00114387 for the VEAPS Study (https://clinicaltrials.gov/ct2/show/NCT00114387?term=VEAPS&draw=2&rank=1) and ClinicalTrials.gov Identifier: NCT00342992 for the ATBC Study (https://clinicaltrials.gov/ct2/show/NCT00342992?term=ATBC&draw=2&rank=1).

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prevents progression of subclinical atherosclerosis in healthy individuals (9). Trial participants ($n = 353$ men and women ages 40 and older without clinical evidence of diabetes or cardiovascular disease) were randomly assigned to receive (i) placebo ($n = 176$), or (ii) alpha-tocopheryl acetate ($n = 177$; ATA, 400 IU) daily for two to three years and followed through study clinic visits every 6 months during which fasting blood samples were collected and stored at −80°C. This analysis included 154 men (81 placebo and 73 ATA) with available baseline and on-study fasting serum samples obtained at the 1-year clinic visit (participant flowchart in Supplementary Fig. S1). All study participants submitted written informed consent, the study protocol was approved by the University of Southern California Institutional Review Board, and the trial was registered on clinicaltrials.gov (NCT00114387; investigators of SELECT were invited to collaborate for a similar metabolomic profiling analysis of their trial population to compare to ATBC, but they declined to do so).

ATBC was a randomized, double-blinded, placebo-controlled primary prevention trial conducted to evaluate the effects of supplementation with vitamin E and beta-carotene on the incidence of lung and other cancers (10). The trial recruited 50–69 year old male smokers ($n = 29{,}133$) from 1985 to 1988 in southwestern Finland. The trial participants were randomly assigned to receive one of four supplements daily: (i) ATA (50 IU), (ii) beta-carotene (20 mg), (iii) both vitamins, or (iv) placebo for 5–8 years (a median of 6.1 years). Fasting serum was collected and stored at −70°C for all trial participants at baseline and for a random sample during the trial. For this analysis, 100 men with serum at baseline and after an average of 1.7 years of supplementation (range: 49–332 weeks) with ATA alone ($n = 50$) or placebo ($n = 50$) and not previously examined for metabolite profiles were selected (participant flowchart in Supplementary Fig. S2). All trial participants provided written informed consent, ATBC was approved by Institutional Review Boards in both the Finnish National Public Health Institute and the NCI. The trial was registered on clinicaltrials.gov (NCT00342992).

Serum metabolite assays

The high-resolution accurate mass (HRAM) platform (global "HD4") of ultrahigh-performance LC/MS-MS of Metabolon, Inc. was used to measure 1,217 baseline and follow-up serum metabolites in both VEAPS and ATBC. After excluding unknown compounds and those with values below the limit of detection in more than 90% of participants, 974 metabolites were included in the final analysis. Metabolites were batch normalized by dividing by the batch mean of all nonmissing values, missing values were imputed to one-half of the minimum detectable metabolite value, and metabolites were classified according to eight chemical classes: amino acids, carbohydrates, cofactors and vitamins, energy metabolites, lipids, nucleotides, peptides, or xenobiotics, where the categorization was based on the databases from Kyoto Encyclopedia of Genes and Genomics (KEGG) and Human Metabolome Database (HMDB). Samples from VEAPS and ATBC were measured at the same time in the same platform runs but in separate batches. The paired baseline and follow-up samples from each study were tested within the same batches. Coefficients of variations (CV) and intraclass correlation coefficients (ICC) were calculated for each metabolite using replicate quality control samples placed in each batch from pooled ATBC serum (32 total quality control samples in 16 batches). The median ICC and CV for all measured metabolites were 0.96 (interquartile range = 0.88 to 0.99) and 0.12 (interquartile range = 0.07 to 0.24) in VEAPS, and 0.96 (interquartile range = 0.87 to 0.99) and 0.11 (interquartile range = 0.06 to 0.24) in ATBC, respectively.

Statistical analysis

All batch-normalized metabolites were log-transformed and standardized (mean = 0 and variance = 1) in the VEAPS Study and ATBC Study, separately. In the main analysis, we investigated the association between change in log-metabolite concentrations and trial assignment (i.e., ATA or no ATA) using linear regression (using PROC GLM in SAS) in each study separately. Here, change is defined as the log-level at follow-up minus the log-level at baseline. In both studies, the threshold for statistical significance of the primary analysis was 5.1×10^{-5} according to Bonferroni correction for 974 tests to get a family-wise error rate of 0.05. In a sensitivity analysis, we further adjusted for potential confounding factors obtained at baseline, including age, height, weight, smoking status, smoking years, serum high-density and total lipoprotein cholesterol, and alcohol consumption. In the secondary analyses, we modeled the association between change in log-metabolite concentrations and change in log serum C_{22} lactone sulfate ("X-12063") by linear regression in VEAPS. For the metabolites significantly correlated with C_{22} lactone sulfate at the Bonferroni correction threshold, we further examined their changes in response to ATA supplementation in linear regression in VEAPS and ATBC. A sensitivity analysis was restricted to ATBC men with on-study serum obtained within 1,000 days of randomization ($n = 62$). All analyses were conducted using SAS version 9.4 (SAS Institute), and all statistical tests are two-sided.

Results

Characteristics of the study participants at trial entry in VEAPS and ATBC according to ATA supplement assignment are presented in **Table 1**. With the exception of significant increases in serum alpha-tocopherol concentrations (measured previously by reversed-phase HPLC with UV detection) during vitamin E supplementation in ATBC and VEAPS, there were no participant differences by intervention assignment in either trial.

On the basis of the 974 metabolites identified in both the VEAPS and ATBC trials, only one, C_{22} lactone sulfate (X-12063), was significantly decreased by ATA supplementation after multiple comparisons correction in VEAPS (beta = −0.70, $P = 8.1 \times 10^{-6}$) but not in ATBC (beta = −0.17, $P = 0.4$; **Table 2**). In contrast, three metabolites were significantly altered in both VEAPS and ATBC: alpha-CEHC sulfate ($\beta = 1.56$ and 1.44, $P = 10^{-33}$ and 10^{-17}, respectively), beta/gamma-tocopherol ($\beta = -1.31$ and −0.97, $P = 10^{-20}$ and 10^{-7}, respectively), and alpha-tocopherol ($\beta = 1.04$ and 0.98, $P = 10^{-12}$ and 10^{-7}, respectively; **Table 2**). The findings were not changed after further multivariable adjustment of the vitamin E supplement-metabolite change associations for the possible biological influences of age, weight, height, body mass index, smoking status, smoking years, serum high-density and low-density lipoprotein cholesterol, dietary vitamin E intake, and alcohol consumption (Supplementary Table S1). Our findings were not essentially changed when we restricted analyses to men who were supplemented with vitamin E for less than 1,000 days in ATBC (Supplementary Table S2). The correlations between changes of all measured metabolites and response to vitamin E supplementation in VEAPS and ATBC are presented in Supplementary Tables S3 and S4.

Among 147 metabolites significantly correlated with C_{22} lactone sulfate in VEAPS after Bonferroni correction, 10 were positively correlated with P values $<10^{-10}$, including six androgenic steroids, the carbohydrate 1,5-anhydroglucitol (1,5-AG), the amino acid 2,3-dihydroxy-5-methylthio-4-pentenoate, the cofactor/vitamin

Table 1. Participant characteristics according to vitamin E supplementation group in VEAPS and ATBC[a].

	VEAPS			ATBC		
	Placebo	ATA (400 IU/day)	*P*	Placebo	ATA (50 IU/day)	*P*
N	81	73		50	50	
Age (years)	55.4 (8.6)	54.3 (8.9)	0.43	57.2 (5.3)	57.8 (5.0)	0.59
Height (cm)	176.6 (6.9)	176.3 (7.5)	0.79	173.9 (4.7)	173.7 (5.5)	0.85
Weight (kg)	86.1 (12.4)	86.3 (15.3)	0.91	79.8 (10.0)	79.1 (11.4)	0.77
BMI (kg/m^2)	27.6 (3.4)	27.7 (4.2)	0.83	26.4 (3.0)	26.2 (3.3)	0.80
Cigarettes per day	7.0 (12.9)	7.9 (13.6)	0.68	20.0 (11.1)	20.7 (9.1)	0.73
Years of smoking	7.7 (11.7)	5.9 (9.7)	0.32	35.0 (8.9)	36.6 (9.2)	0.38
Smoking status (%)			0.62			n.a.
Never	50 (61.7)	44 (60.3)		0	0	
Former	28 (34.6)	28 (38.4)		0	0	
Current	3 (3.7)	1 (1.4)		50 (100)	50 (100)	
Physically active (%)	46.9	44.6	0.77	24.0	26.0	0.82
Education[b] (%)	96.3	98.6	0.36	38.0	28.0	0.29
Dietary intake per day						
Total energy (kcal)	2,160 (837)	2,014 (716)	0.25	2,809 (834)	2,550 (598)	0.09
Fruit (g)	n.a.	n.a.		129 (100)	121 (86)	0.71
Vegetables (g)	n.a.	n.a.		131 (80)	134 (91)	0.85
Red meat (g)	n.a.	n.a.		78.4 (35.0)	74.9 (29.1)	0.61
Alcohol (ethanol, g)	14.3 (21.7)	9.3 (12.4)	0.09	14.3 (17.5)	16.7 (15.8)	0.50
Serum biomarkers						
Total cholesterol (mmol/L)						
Baseline	5.8 (0.79)	5.8 (0.75)	0.96	6.6 (1.1)	6.2 (1.1)	0.15
Follow-up	5.7 (0.77)	5.9 (0.73)	0.11	6.0 (0.98)	6.3 (1.2)	0.29
Alpha-tocopherol (mg/L)						
Baseline	10.6 (3.7)	10.1 (3.6)	0.37	12.6 (3.1)	12.5 (4.3)	0.91
Follow-up	13.5 (3.3)	24.1 (6.7)	<0.0001	13.3 (2.8)	20.0 (8.6)	<0.0001
Retinol (μg/L)						
Baseline	349 (174)	388 (162)	0.15	611 (107)	586 (132)	0.31
Follow-up	300 (1,056)	354 (142)	0.67	614 (96)	586 (141)	0.28

Abbreviations: BMI, body mass index; n.a., not available.
[a]All variables are from baseline information unless indicated otherwise. All values are means (standard deviation) unless indicated otherwise.
[b]Education: >9th grade in VEAPS; >elementary school in ATBC.

2-O-methylascorbic acid, and the xenobiotic O-sulfo-L-tyrosine (**Table 3**).

In secondary analyses, we examined the response to vitamin E supplementation in VEAPS and ATBC of the androgenic steroids positively and significantly correlated with C_{22} lactone sulfate in VEAPS (**Table 4**). Five of six androgen metabolites in **Table 3** were decreased by the 400 IU ATA supplement in VEAPS ($P < 0.05$, although not achieving Bonferroni significance), but not in response to the 50 IU supplement in ATBC (weaker, nonsignificant reductions were suggested for androstenediol (3alpha, 17alpha) monosulfate; *P* values >0.05; **Table 4**). Four additional androgen metabolites correlated with C_{22} lactone sulfate in VEAPS at Bonferroni significance (not presented in **Table 3**) were also decreased in VEAPS ($P < 0.05$) but not in ATBC (**Table 4**).

Discussion

In this RCT-based serum metabolomic analysis testing the biochemical effects of supplementation with either 50 IU or 400 IU ATA daily, in addition to the anticipated significant increase in alpha-CEHC sulfate and alpha-tocopherol (and

Table 2. Serum metabolites that were significantly altered at the Bonferroni correction threshold in response to vitamin E supplementation in VEAPS and ATBC.

	VEAPS ($n = 154$) (ATA, 400 IU/day)			ATBC ($n = 100$) (ATA, 50 IU/day)		
Metabolite	Effect size (β)[a]	SE	*P*	Effect size (β)[a]	SE	*P*
Alpha-CEHC sulfate	1.56	0.10	4.8×10^{-33}	1.44	0.14	1.9×10^{-17}
Beta-tocopherol/gamma-tocopherol	−1.31	0.12	2.3×10^{-20}	−0.97	0.17	2.4×10^{-7}
Alpha-tocopherol	1.04	0.14	4.3×10^{-12}	0.98	0.18	1.8×10^{-7}
C_{22} lactone sulfate	−0.70	0.15	8.1×10^{-6}	−0.17	0.20	0.4

[a]The effect size indicates the change in log-metabolite concentration for the ATA group versus placebo. Linear regression models were used to estimate the effect sizes and *P* values.

Table 3. Top 10 metabolites significantly correlated with C_{22} lactone sulfate at the Bonferroni correction threshold among 154 men in VEAPS.

Metabolite	Chemical class	Chemical subclass	r	SE	*P* value
Androsterone sulfate	Lipid	Androgenic steroid	0.57	0.066	8.0×10^{-15}
Androstenediol (3alpha, 17alpha) monosulfate	Lipid	Androgenic steroid	0.57	0.067	1.9×10^{-14}
5Alpha-androstan-3alpha,17beta-diol monosulfate	Lipid	Androgenic steroid	0.56	0.067	4.5×10^{-14}
Epiandrosterone sulfate	Lipid	Androgenic steroid	0.51	0.070	1.4×10^{-11}
Dehydroepiandrosterone sulfate (DHEA-S)	Lipid	Androgenic steroid	0.51	0.070	1.7×10^{-11}
5Alpha-androstan-3beta,17beta-diol monosulfate	Lipid	Androgenic steroid	0.50	0.070	2.5×10^{-11}
1,5-Anhydroglucitol (1,5-AG)	Carbohydrate	Glycolysis, gluconeogenesis, and pyruvate metabolism	0.55	0.067	8.6×10^{-14}
2,3-Dihydroxy-5-methylthio-4-pentenoate (DMTPA)	Amino acid	Methionine, cysteine, SAM, and taurine metabolism	0.54	0.068	5.8×10^{-13}
2-O-methylascorbic acid	Cofactor and vitamin	Ascorbate and aldarate metabolism	0.54	0.068	7.0×10^{-13}
O-sulfo-L-tyrosine	Xenobiotic	Chemical	0.51	0.070	9.2×10^{-12}

decrease in beta/gamma-tocopherol) in both trials, a novel C_{22} lactone sulfate compound was significantly decreased only in the high-dose VEAPS trial. In addition, most of the androgenic steroid metabolites directly correlated with serum C_{22} lactone sulfate were significantly reduced by ATA supplementation only in VEAPS. Our investigation points to a direct impact of high-dose vitamin E supplementation on this lactone-containing metabolite that correlated with androgen metabolites potentially relevant to elevated prostate cancer incidence in the SELECT controlled supplementation trial (3).

Although the molecular structure of C_{22} lactone sulfate (**Fig. 1**) suggests possible involvement in the lanosterol synthase pathway, how it is produced and the precise biological pathways the molecule may interact with following high-dose ATA supplementation are unknown. Experimental evidence does support inhibitory effects of lactone-containing metabolites (e.g., sesquiterpene lactones) and lactone-based derivatives on prostate and other cancer cell line growth, mouse xenograft tumor formation, and NFB and STAT3 in the prostate carcinogenic *src* pathway (11–13). Lactone-based derivatives have also demonstrated androgen receptor antagonism in the presence of dihydrotestosterone or R1881 (13). Beyond such potential direct effects of the C_{22} lactone on prostate cancer risk, the observed changes in several androgen metabolites, including DHEA-S, that were strongly positively correlated with the compound in VEAPS suggest an

Table 4. Androgenic steroids significantly correlated with C_{22} lactone sulfate at Bonferroni correction threshold in VEAPS and their change in response to ATA supplementation in VEAPS and ATBC.

					Response to ATA supplementation			
					VEAPS (400 IU/day)		ATBC (50 IU/day)	
Metabolite	Chemical class	Chemical subclass	Metabolite × C_{22} lactone sulfate correlation estimate (r)	*P*	Effect size (β)[a]	*P*	Effect size (β)[a]	*P*
Androsterone sulfate	Lipid	Androgenic steroid	0.57	8.0×10^{-15}	−0.39	0.016	−0.23	0.24
Androstenediol (3alpha, 17alpha) monosulfate	Lipid	Androgenic steroid	0.57	1.9×10^{-14}	−0.30	0.064	−0.01	0.95
5Alpha-androstan-3alpha,17beta-diol monosulfate	Lipid	Androgenic steroid	0.56	4.5×10^{-14}	−0.40	0.014	−0.05	0.80
Epiandrosterone sulfate	Lipid	Androgenic steroid	0.51	1.4×10^{-11}	−0.33	0.043	−0.28	0.16
Dehydroepiandrosterone sulfate (DHEA-S)	Lipid	Androgenic steroid	0.51	1.7×10^{-11}	−0.34	0.034	−0.08	0.68
5Alpha-androstan-3beta,17beta-diol monosulfate	Lipid	Androgenic steroid	0.50	2.5×10^{-11}	−0.49	0.002	−0.07	0.72
Androstenediol (3beta,17beta) monosulfate	Lipid	Androgenic steroid	0.49	1.5×10^{-10}	−0.42	0.009	−0.003	0.99
Androstenediol (3beta,17beta) disulfate	Lipid	Androgenic steroid	0.48	2.9×10^{-10}	−0.32	0.049	−0.16	0.44
5Alpha-androstan-3beta,17beta-diol disulfate	Lipid	Androgenic steroid	0.48	3.0×10^{-10}	−0.36	0.026	−0.39	0.05
Androstenediol (3beta,17beta) monosulfate	Lipid	Androgenic steroid	0.38	1.3×10^{-6}	−0.36	0.024	−0.32	0.11

[a]The effect size indicates the change in log-metabolite concentration for the ATA arm versus no ATA arm. Linear regression models were used to estimate the effect size and *P* value.

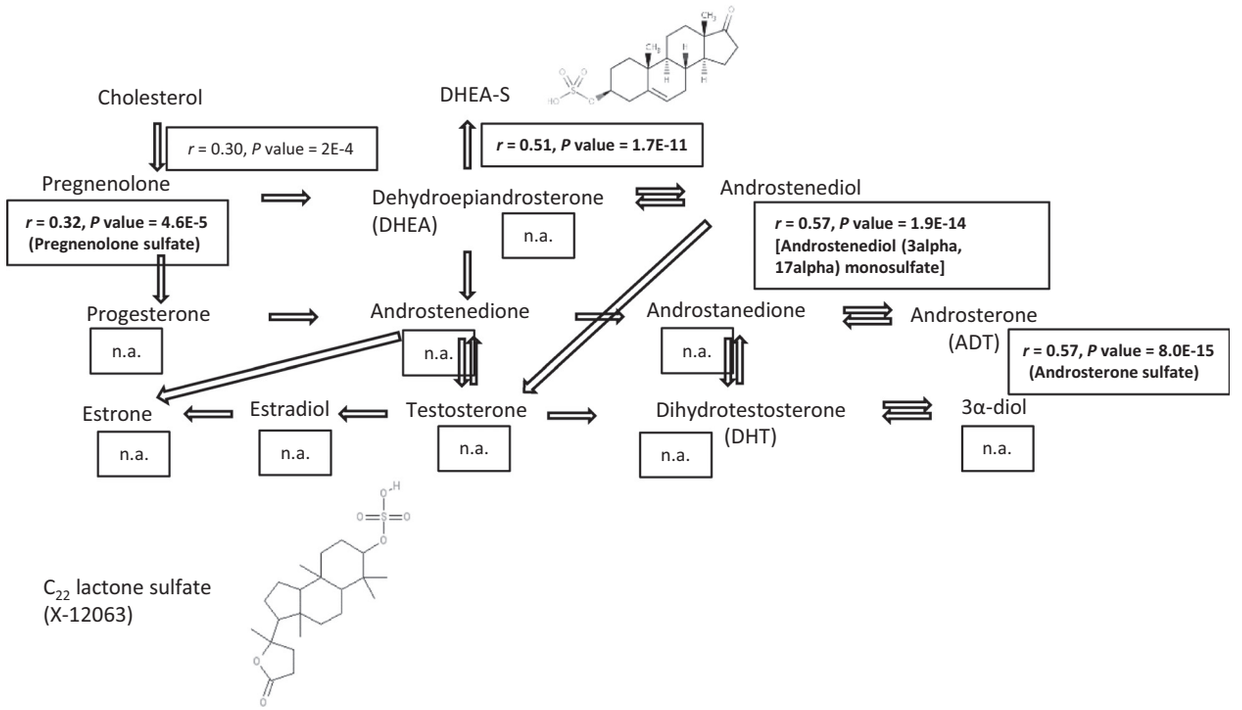

Figure 1.

Diagram of sex steroid metabolism and hormone metabolite correlations with C_{22} lactone sulfate (X-12063). Bold values indicate statistical significance of the correlation reaching the Bonferroni multiple comparisons corrected statistical threshold. n.a., not measured.

impact of high-dose vitamin E on sex steroid metabolism. For example, the decreased profile of these androgen metabolites may indicate higher concentrations of functional androgens upstream in the sex steroid pathway resulting from C_{22} lactone sulfate alteration of specific enzymatic activity that inhibits androgen metabolism and clearance. Functional studies of this compound, with experiments that address how high-dose vitamin E might alter sex steroid metabolism, are needed to elucidate the precise biochemical actions and pathways involved.

Serum metabolite changes observed in both VEAPS and ATBC included the expected increases in alpha-tocopherol and its primary terminal metabolite alpha-CEHC sulfate, and decreases in beta/gamma-tocopherol, consistent with earlier findings (14). Alpha-CEHC sulfate is produced following serial ω-oxidation and β-oxidation reactions of alpha-tocopherol and ATA catalyzed by cytochrome p450 enzymes (notably, CYP4F2; ref. 15). Although alpha-CEHC and its sulfated metabolite have not been thoroughly studied with respect to prostate cancer risk, experimental data demonstrate anti-proliferative, anti-inflammatory, and antioxidative activity for alpha-CEHC (16–18). Circulating beta- and gamma-tocopherol are decreased by alpha-tocopherol supplementation because of the affinity-based reduction in hepatic uptake and lipoprotein transfer by alpha-tocopherol transfer protein of the former tocopherols (19). Accumulating evidence demonstrates unique antioxidant and anti-inflammatory properties for gamma-tocopherol relevant to chronic disease prevention (20, 21). However, neither the large increase in alpha-CEHC, nor the substantial decrease in beta/gamma-tocopherol, in response to ATA supplementation can explain the divergent prostate cancer findings of ATBC and SELECT given the relatively similar magnitudes of their serum changes observed here and previously.

This is likely the first investigation of metabolomic responses to supplementation with a low- and high-dose of vitamin E in two randomized, placebo-controlled clinical trials. The untargeted metabolomic platform exhibited high laboratory validity and reproducibility and identified more than 970 known metabolites reflecting a broad array of biochemicals and biological pathways. Application of the stringent Bonferroni statistical threshold for the large number of compounds still permitted discovery of several individual metabolites directly related to the vitamin E dosages of the two trials. The relatively homogenous male smoker, European ancestry population of ATBC is a limitation. In addition, based on available follow-up serum samples in ATBC, men received the trial vitamin E supplementation for up to 6 years as compared with the one-year timepoint selected in VEAPS to maintain sample size; however, our findings remained essentially unchanged when only the first three follow-up years were included for ATBC. Also, despite the large number of measured compounds, there are possibly other unmeasured metabolites or biochemical pathways related to low- and high-dose vitamin E supplementation still to be identified.

In conclusion, high-dose (400 IU/day), but not low-dose (50 IU/day), vitamin E supplementation resulted in a significant reduction in serum C_{22} lactone sulfate that was highly correlated with alterations of androgenic steroid metabolites. Our study provides evidence of distinct steroid hormone pathway responses based on vitamin E dosages that could have direct relevance to opposing prostate cancer incidence results from two large controlled trials, ATBC and SELECT. Reexamination of the observed responses to vitamin E supplementation in other populations, and further elucidation of the interrelationships among C_{22}

lactone sulfate, androgenic steroid hormones, and prostate cancer risk are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J. Huang, S.J. Weinstein, D. Albanes
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Huang, H.N. Hodis, W.J. Mack, A.M. Mondul, D. Albanes
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Huang, S.J. Weinstein, J.N. Sampson, D. Albanes
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