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A Nutrient Approach to Prostate Cancer Prevention: The Selenium and Vitamin E Cancer Prevention Trial (SELECT)

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The Selenium and Vitamin E Cancer Prevention Trial (SELECT) randomized 35,533 healthy men, >55 yr old (>50 yr if African American), with normal digital rectal exams and prostate specific antigens <4 ng/ml to 1) 200 μg/day l-selenomethionine, 2) 400 IU/day all-rac-alpha-tocopheryl acetate (vitamin E), 3) both supplements, or 4) placebo for 7 to 12 yr. The hypotheses underlying SELECT, that selenium and vitamin E individually and together decrease prostate cancer incidence, derived from epidemiologic and laboratory evidence and significant secondary endpoints in the Nutritional Prevention of Cancer (selenium) and Alpha-Tocopherol Beta-Carotene (vitamin E) trials. In SELECT, prostate cancer incidence did not differ among the 4 arms: hazard ratios [99% confidence intervals (CIs)] for prostate cancer were 1.13 (99% CI = 0.95–1.35, $P = 0.06$; $n = 473$) for vitamin E, 1.04 (99% CI = 0.87–1.24, $P = 0.62$; $n = 432$) for selenium, and 1.05 (99% CI = 0.88–1.25, $P = 0.52$; $n = 437$) for selenium + vitamin E vs. 1.00 ($n = 416$) for placebo. Statistically nonsignificant increased risks of prostate cancer with vitamin E alone [relative risk (RR) = 1.13, $P = 0.06$) and newly diagnosed Type 2 diabetes mellitus with selenium alone (RR = 1.07, $P = 0.16$) were observed. SELECT data show that neither selenium nor vitamin E, alone or together, in the doses and formulations used, prevented prostate cancer in this heterogeneous population of healthy men.

INTRODUCTION

Nutrition, in terms of both diet and individual nutrients, has long been a subject of investigation in relation to cancer risk, causation, prevention, and treatment (1,2), specifically regarding risk of prostate cancer (3–5). The intersection of nutrition and cancer prevention is vividly demonstrated in the Study of Vitamin E and Selenium Cancer Prevention Trial (SELECT), a large prostate cancer prevention trial sponsored by the National Cancer Institute (NCI) and designed and implemented by the Southwest Oncology Group (SWOG). The need for preventive interventions in this disease is brought home by the fact that prostate cancer is the most common (217,730 estimated new cases in 2010) and the second most deadly (32,050 estimated deaths in 2010) cancer in men in the United States (6). Early stage disease, although effectively treated with either surgery or radiation, often results in adverse effects on quality of life (7), whereas therapies for advanced or recurrent disease are at best palliative. Together, these challenges to cancer treatment have encouraged investigations of preventive approaches to prostate cancer.

In view of the well-established contribution of androgens to prostate carcinogenesis (8,9), these hormones have been targeted in treatment of existing disease and in the first large, Phase 3 prevention trial for prostate cancer, the NCI-sponsored, SWOG-conducted Prostate Cancer Prevention Trial (PCPT). This trial used as an intervention the benign prostatic hyperplasia drug finasteride, which exerts its antiandrogenic effects by inhibiting 5-alpha-reductase, the enzyme that converts testosterone to its more potent form, dihydrotestosterone. In the PCPT, 18,882 men aged 55 yr or older without evidence of disease based on a normal digital rectal exam (DRE) and prostate-specific antigen (PSA) were randomized to finasteride (5 mg/day) or placebo for 7 yr (10). Although finasteride reduced the 7-yr prevalence of prostate cancer by 25% compared to placebo, this favorable outcome was accompanied by an apparent 1.3% increase in the prevalence of high-grade prostate cancer. Initial concern about this finding has been largely allayed by subsequent analyses that traced this result to the effect of finasteride on prostate volume and selective inhibition of low-grade cancer (11). The NCI cooperative group system, which completed accrual in less than the anticipated timeframe, offered an infrastructure that remained intact at the end of study and was thus available for SELECT when that trial was initiated.

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NUTRITIONAL SUPPLEMENTATION AS A PREVENTIVE INTERVENTION FOR PROSTATE CANCER: SELECT, A STUDY OF SELENIUM AND VITAMIN E CHEMOPREVENTION TRIAL

Exploration of additional promising agents for chemoprevention of prostate cancer led investigators to selenium and vitamin E, each of which had been studied extensively in the laboratory and in humans in a variety of cancer types and in relation to cancer risk and prevention. The following survey describes the experimental and epidemiologic/clinical study data leading to the hypotheses that served as the foundation for SELECT (12). In addition, we offer an updated review of the evidence for the anticancer and antiprostate cancer properties of selenium and vitamin E, providing ongoing validation of their selection as interventions in this large, Phase 3, clinical prevention trial.

Selenium

Selenium is a nutritionally essential nonmetallic trace element. In normal physiology, selenium is well documented as a cofactor to antioxidant enzymes, particularly glutathione peroxidase. The biological effects that have been observed in experimental studies reflect the varied activities of a wide variety of selenium compounds (Fig. 1, Table 1), including selenium metabolites. These biological activities determine its clinical efficacy, including its chemopreventive effects (5,13–16). The main form in mammalian proteins is selenocysteine, sometimes viewed as the "21st" amino acid (17). Selenite or selenomethionine are the two essential micronutrient formulations used in animal diets. The selenium compound that is present in key staple foods has been suggested to be the most desirable form (18). This major dietary form is L(+)-selenomethionine, but other compounds, specifically Se-methylselenocysteine and selenocystathionine, are present in plants such as broccoli, garlic, and onions, which selectively accumulate this trace element (18). The documentation of the essential nature of selenium led to establishment of a recommended daily allowance in the United States of 0.87 μg/kg, or 70 ug/day for men, and 55 ug/day for women, of which 98% of an oral dose is absorbed (19). The typical U.S. dietary intake is 80 to 150 μg daily. Normal blood selenium values vary geographically depending on the selenium content in the soil, with ranges as wide as 10 to 34 ug/100 ml being reported.

Selenium: Background laboratory research. Evidence for the anticancer effect of selenium comes from laboratory as well as epidemiologic studies. Selenium suppresses in vitro proliferation of cells including DU-145 human prostate carcinoma cells (13,20,21). The in vitro growth suppression of prostate cancer cells is accompanied by a decrease in proliferating cell nuclear antigen (PCNA) (22). Selenium inhibits protein kinase C and protein kinase A activities (16,23). Nuclear factor kappa B (NF-κB) activation, but not its synthesis, is also suppressed by selenium (16,24,25). Other basic cellular activities influenced by selenium include alteration of carcinogen metabolism (16), cytotoxicity due to selenium metabolites formed as a result of high selenium concentration (13), enhancement of immune function (26–28) and mediation of inflammation (17,29), and influencing testosterone metabolism (5,30,31). Selenium also induces apoptosis in prostate cancer cell lines and mouse xenograft models (22,23,32,33), an activity that is associated with increases in the proapoptotic proteins Bax, Bak, and Bid and decreases in

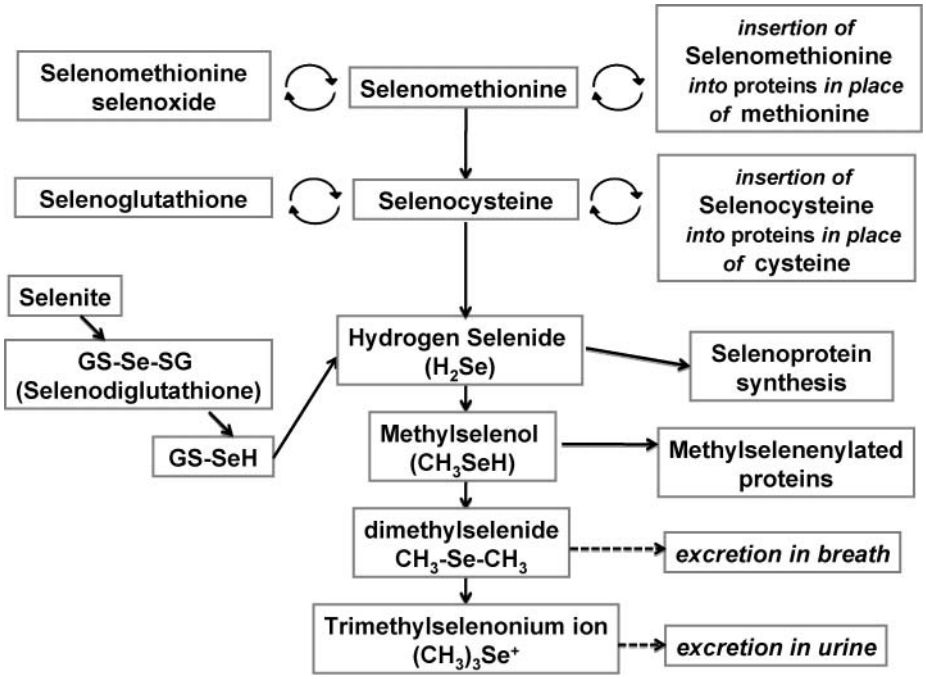


FIG. 1. Selenium metabolites.

TABLE 1
Selenium and vitamin E: Antitumor mechanisms and molecular targets[a]

	A. Cellular Mechanisms Vitamin E		
Selenium	α-tocopherol (or other form of tocopherol or mixed tocopherols)	γ-tocopherol relative to α-tocopherol	Selenium+ Vitamin E
Antioxidant/decreases ROS (24)	Antioxidant/free radical scavenger (30,58–60)	γ-T stronger antioxidant vs. α-T (64) γ-T complements α-T in antioxidant activity (68)	
Induces apoptosis (22,23,31,33) Induces PC cell apoptosis (31,38)[b]	Induces apoptosis (62,76,77) Induces PC cell apoptosis (77)[b]	γ-T stronger induction of apoptosis vs. α-T in colon cancer cell lines (149)	Induces PC cell apoptosis more than vitamin E or Se alone (22,32,91)[b]
Inhibits: Cell cycle progression, cell proliferation (22,23,31), PC cell proliferation (31,37,38)[b]	Inhibits: Cell proliferation (22), carcinogenesis—aberrant crypt foci (134) Mammary tumorigenesis (148) PC cell proliferation (74–79)[b] Inhibits cell invasiveness—human PC cells (80)[b]	γ-T stronger inhibition of proliferation vs. α-T—colon cancer cell lines (149)	Inhibits PC cell viability, growth more than vitamon E or Se alone (22,32,91)[b]
Enhances immune response (17) Mediates inflammatory response	Anti-inflammatory (134)	γ-T stronger anti-inflammation vs. α-T (136); γ-T anti-inflammatory in colon (64)	
Maintains cellular respiration Detoxifies environmental mutagens, carcinogens	Mixed tocotrienols and δ-tocotrienol inhibit angiogenesis (61)		
	B. Molecular Targets		
Inhibits: NF-κB activation (24,25) Cyclin D1 (33) AP-1 binding to DNA Cdk2 COX LOX Protein kinase C isoenzymes (23) Expression of AR, PSA, androgen responsive genes	Vitamin E inhibits: NF-κB (62,72) Kinases: MAPK, ERK1/2, p38 AR function (73,74)[b] PSA expression (73,74)[b] VEGF expression (61) ICAM-1 (62,72) Cell surface gp130 (62) Vitamin E activates AP-1 (62)	Traps ROS species: γ−T more than α-T (64) Increases PPARγ expression: γ−T more than α-T (64)	

(Continued on next page)

TABLE 1
Selenium and vitamin E: Antitumor mechanisms and molecular targets[a] *(Continued)*

B. Molelecular Targets			
	Vitamin E		
Selenium	α-tocopherol (or other form of tocopherol or mixed tocopherols)	γ-tocopherol relative to α-tocopherol	Selenium+ Vitamin E
Se targets in apoptosis: Endoplasmic reticulum stress/cytokine signaling pathway (32) PARP cleavage (31,32) Caspases 3, 6, 7, 1, 12 activation (34) TRAIL-mediated apoptosis: PC Cells (34)[b] Inhibits TRAIL-mediated BAD phosphorylation (34)[b] Increases proapoptotic proteins Bax, Bak, Bid-dog prostate (22)[b]	Vitamin E targets in apoptosis: Increased proapoptotic proteins Bax, Bak, Bid (22) Inhibits pro-survival proteins XIAP, Bcl-X_L, Bcl-2 (62,75,76)	γ-T targets in apoptosis: PARP cleavage Caspase 3, 7, 8 (149)	Se + vitamin E targets in apoptosis: Increased pro-apoptotic proteins Bax, Bak, Bid-PC cells (22)[b] Decreased Bcl-2 (22)[b] Increased Bax/Bcl-2 ratio Mitochondrial+Endoplasmic reticulum stress/cytokine signaling pathways (91)[b] Caspases 3, 6, 7, 1, 12, 9 activation (91)[b] Augmented apoptotic response vs. Se or vitamin E alone (91,92)[b]
Se mechanisms to inhibit the cell cycle and proliferation: Decreases PCNA protein levels (22) Induces DNA single-strand breaks (35) Potential inhibition of cell cycle progression: GADD153, CHK2, CDK1, $p21^{WAF1}$, cyclin A, DHFR (38) Cell cycle arrest in G2-M phase (31) Decreases AR, PSA expression in PC xenograft (37)[b] PC cells: gene expression changes are inverse to androgen response (39)[b]	Vitamin E mechanisms to inhibit the cell cycle and proliferation: Inhibits DNA synthesis (78) PCNA protein levels decrease (22,79) Inhibits cdk4 kinase activity, Rb protein phosphorylation (78) Decreases expression: Cyclins D1, D3, E; Cdks 2, 4 (78) Decreases AR, PSA expression (74)[b] Cell cycle arrest in G1/S phase (78)	γ-T mechanisms to inhibit cell cycle and proliferation: Decreases PCNA	Se + vitamin E mechanisms to inhibit cell cycle and proliferation: Decreases PCNA protein levels in more cell lines than vitamin E or Se alone (22)
Thyroid hormone metabolism (17)	Vitamin E targets in cell invasiveness: Reduces MMP-9 secretion and activity (80) Vitamin E mechanisms in angiogenesis: δ-tocotrienol: suppresses GF-dependent PI3K/PDK/Akt signaling (61) α-tocopherol: inhibits VEGF expression in PC cells (62)[b]		

(Continued on next page)

Se targets in immune response:
Cell-mediated immunity:
T-cells (27)—CD8+, CD4+
Macrophages, NK cells
IL-2R induction (26,27)
TH1/TH2 balance (26)
Protection against viruses, other pathogens
Pattern of cytokines, chemokines (149)
Se targets in inflammatory response:
Influences production of proinflammatory cytokines IL-6, TNFα, IL-1β (28) Example: low Se leads to increased IL-6 (149)

Vitamin E targets in immune response:
Downregulates IL-6, IL-8 in PC cells (62)[b]
Inhibits neutrophil migration via down-modulating chemotaxis and reducing IL-8 secretion (62)
Inhibits ICAM-1 expression and results in decreased monocyte adhesion (62)

γ−T targets in inflammatory response:
Inhibits COX:
γ−T inhibits COX more than α-T (64,134)
γ−T, γ−CEHC inhibit PGE_2 synthesis more than α-T (135,136)
γ-CEHC reduces PGE_2 (133,136)
Inhibits LOX:
γ−T inhibits LOX
γ−T inhibits proinflammatory eicosanoid formation (64) more than α-T (136)
γ−T inhibits LTB_4 (136) γ-CEHC, γ−T inhibit LTB_4 more than α-T (136)
Inhibits neutrophil infiltration (136):
γ-CEHC, γ−T inhibit neutrophil infiltration more than α-T
Inhibits
TNF-α (136): γ−T inhibits TNF-α more than α-T
Attenuates inflammation-mediated damage (136)
Inhibits iNOS expression: γ−T $\sim$ α-T (134)

[a]Abbreviations are as follows: γ-T, gamma-tocopherol; α-T, alpha-tocopherol; ROS, reactive oxygen species; PC, prostate cancer; NF-κB, nuclear factor kappa B; AP-1, activating protein-1; Cdk, cyclin-dependent kinase; COX, cyclooxygenanse; LOX, lipooxygenase; AR, androgen receptor; PSA, prostate specific antigen; MAPK, mitogen-activated protein kinase; ERK1/2, extracellular-regulated kinase 1/2; VEGF, vascular endothelial growth factor; ICAM-1, intercellular adhesion molecule-1; PPARγ, peroxisome proliferator-activated receptor-γ; PARP, polyADP-ribose polymerase; Se, Selenium; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand-mediated mechanism; BAD, Bcl-2-associated death promoter gene; Bax, B-cell-2-associated x protein; Bad, Bcl-2-associated death promoter gene protein; PCNA, proliferating cell nuclear antigen; MMP-9, matrix metalloproteinase-9; PI3K, phosphotidylinositol-3 kinase; PDK, phosphoinositide; IL, interleukin; TH1, TH2, T-helper cell type 1, type 2; TNF-α, tumor necrosis factor alpha; CEHC, carboxyethyl hydroxychroman; PGE_2, prostaglandin E_2; LTB, leukotriene B; iNOS, inducible nitric oxide synthase.

[b]Activity demonstrated in prostate cancer cells and/or xenografts.

antiapoptotic Bcl-2, leading to an increased Bax/Bcl-2 ratio (22). Sensitization of cells to apoptosis has been reported to occur in response to selenium via a tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated mechanism (34). Some forms of selenium are genotoxic; and for selenite and selenide, the ability to induce DNA single-strand breaks has been linked to cell growth inhibition (35). Conversely, selenomethionine has been shown to protect cells from DNA damage by activating the p53 pathway (36). Selenium inhibits human prostate cancer xenograft growth, which is accompanied by a decrease in expression of the androgen receptor (AR) and PSA (37). Investigations into the molecular basis of selenium activity in human prostate cancer cells using mRNA expression arrays have reinforced and expanded upon earlier reports of individual gene changes (16). Expression arrays have revealed a large number of selenium-responsive genes, including a cluster of genes involved in cell cycle regulation: GADD153, CHK2, $p24^{WAF1}$, cyclin 1, DHFR, CDK1, CDK2, CDK4, PCNA, and cyclin E2 (methylseleninic acid) (15,38), including a group of 35 established androgen-responsive genes (selenomethionine) (39).

Selenium administered at levels higher than those required for nutritional needs has been shown to suppress carcinogenesis in a number of animal models, including both transplantable and virally induced models, and at many organ sites, including breast, liver, and skin (13,14,20,40). Yet, the in vivo data for prostate cancer have been inconsistent. Selenium did not decrease the incidence of prostate cancer in male rats pretreated with the chemical carcinogen 3,2′-dimethyl-4-aminobiphenyl (41). In another in vivo model, however, pharmacologic doses of monomethylated selenium were shown to inhibit growth in mice of xenografts derived from lymph node carcinoma of the prostate (LNCaP) human prostate cancer cells (37).

Selenium: Background epidemiologic and clinical research. The initial epidemiological evidence for an anticancer effect of selenium came from ecological and correlational studies that have demonstrated lower human cancer mortalities in high- vs. low-selenium regions of the United States (42,43). Case-control studies that have compared selenium levels in blood, serum, or other indicator organs of healthy subjects to those of cancer cases showed overall an inverse association with cancer, with some studies suggesting that low prediagnostic selenium levels serve as a predictor of cancer risk (43). In a matched case-control study nested within the prospective Health Professionals Follow-Up Study, higher selenium levels in toenails were associated with a reduced risk of advanced prostate cancer [odds ratio (OR) = 0.49, highest to lowest quintile] (44). In contrast, two recently published case-control studies nested respectively within the Multiethnic Cohort and the European Prospective Investigation in Cancer and Nutrition (EPIC) revealed no such association between selenium levels and risk of prostate cancer (45,46). The only evidence for an inverse association of selenium levels with prostate cancer risk was noted among African-American participants (P trend = 0.02) in the Multiethnic Cohort.

Additional support for such an inverse association between selenium and cancer comes from several large randomized trials (43). In Qidong, a region in China with a high incidence of primary liver cancer (PLC), an intervention trial showed that using salt fortified with 15 ppm of sodium selenite, providing about 30 to 50 ug of selenium/day for 8 yr, led to a decrease in the incidence of PLC relative to surrounding areas maintained on ordinary salt (47). Subsequent withdrawal of the supplemental selenium resulted in an increase in PLC incidence. The Nutrition Intervention Trials included over 29,000 individuals 40 to 69 yr old from the general population of Linxian, a region of China with among the highest rates of esophageal/gastric cardia cancer in the world (48). The results showed that supplementation with 50 μg selenium, 30 mg vitamin E, and 15 mg β-carotene daily was associated with a 13% decrease in mortality from cancer at all sites and a 21% decrease in mortality from stomach cancer. The second trial, also in Linxian, tested a multivitamin/mineral supplement, including 50 μg selenium plus 15 mg β-carotene daily, in 3,000 individuals with esophageal dysplasia (49). Total cancer mortality was 7% lower, cumulative esophageal/gastric cardia death rates were 8% lower, and esophageal cancer deaths were 16% lower in the supplemented group. None of these mortality reductions was significant in the short-term follow-up of this study, and cumulative cancer incidence rates were comparable in the 2 groups. Specific attention to the impact of selenium in preventing prostate cancer emerged from a clinical trial that was designed to address the effect of this nutrient on skin cancer, the Nutritional Prevention of Cancer (NPC) Trial (50) In this trial, 1,312 patients with a history of skin cancer, randomized to receive 200 μg elemental selenium/day in the form of selenized yeast or placebo, were followed an average of 4.5 yr. The primary endpoints were occurrence of new basal or squamous cell carcinomas of the skin. Secondary endpoints included all-cause mortality and total cancer mortality; total cancer incidence; and the incidences of lung, prostate, and colorectal cancers, the most common cancers in this cohort (51,52). Although the original secondary analyses of results through December 31, 1993, showed no difference in the rate of skin cancers, inverse associations were observed between selenium supplementation and the incidence of total, lung, prostate, and colorectal cancer and total cancer mortality (50). Prostate cancer incidence was decreased by two-thirds in men in the selenium supplemented group [relative risk (RR) = 0.37; 95% confidence interval (CI) = 0.18–.71, $P = 0.002$]. Stratified analysis of a small number of cases suggested that the decrease in prostate cancer was greater in men with low baseline selenium, men younger than 65 yr, and those with low serum PSA (51,53–55). A subsequent report presenting analyses of results through February 1, 1996, the end of blinded treatment, revealed continued reduction of prostate cancer incidence [hazard ratio (HR) = 0.48, 95% CI = 0.28–0.80, $P = 0.005$] but not lung and colorectal cancer incidence (53,55–57). Interest in testing the potential benefit of selenium in decreasing prostate cancer risk as a primary endpoint in a clinical trial was sparked by the inverse association

observed in these secondary subset analyses, contributing to the hypotheses underlying the next prostate cancer prevention trial, SELECT.

Vitamin E (α-Tocopherol and Other Forms)

The vitamin E family has essential, vitamin compounds that make up the major lipid-soluble antioxidants in cell membranes, acting as peroxyl and alkoxyl free radical scavengers that inhibit lipid peroxidation in vivo (31,58–60). Naturally occurring vitamin E comprises 8 different forms: the $\alpha-$, $\beta-$, $\gamma-$, and δ-isomers of both tocopherol and tocotrienol, with tocopherol having an unsaturated isoprenoid tail and tocotrienol having a completely saturated phytyl side-chain (Fig. 2A) (61). Although γ-tocopherol is the predominant form in the human diet (62), it has only 20% of the activity according to the standard fertility restoration assay (63,64) of α-tocopherol (64), which is the most active form (62,65,66). This increased activity, in the face of antioxidant properties that are equivalent for all the vitamin E forms—and possibly higher for γ-tocopherol (67,68)—is due in part to the poor recognition of non-α-tocopherols by the hepatic α-tocopherol transfer protein (α-TTP). α-TTP transfers hepatic α-tocopherol into plasma lipoproteins for extrahepatic delivery and is thus responsible for maintaining plasma α-tocopherol concentrations (60,66,69). The lower affinity of the non-α-tocopherol forms for α-TTP results in their being preferentially metabolized relative to α-tocopherol, a mechanism for regulating hepatic vitamin E concentrations (Fig. 2B) (60,64,69).

In the United States, the average dietary vitamin E intake in men is estimated to be 10 mg/day and in women, 7 mg/day. The recommendation of the National Research Council for a daily dietary allowance, established in 2000, is 15 mg α-tocopherol for both men and women (59,60,70,71). Its oral absorption is 20–50%.

Vitamin E: Background laboratory research. The classic view that the function of α-tocopherol is restricted to its activity as a free radical scavenger/antioxidant in the lipid phase of cell membranes (66) has been challenged in view of a variety of other cellular activities exhibited by 1 or more of the 8 family members (Table 1) (69). α-tocopherol inhibits NF-κB (62,72), AR function, and expression of PSA in LNCaP prostate cancer cells (73,74) and induces apoptosis via the mitochondrial caspase cascade, evident in increased Bax/Bcl-2 ratios (22,75). α-tocopherol decreases VEGF expression in prostate cancer cells (62), and vitamin E tocotrienol inhibits angiogenesis in vivo (61) (Table 1). Anti-inflammatory effects of α-tocopherol include downregulation of the cytokines IL-6 and IL-8 in prostate cancer cells (62). These activities, in addition to the well-established antioxidant properties, are consistent with the anticancer effect seen in a variety of human cancer cell lines on exposure to vitamin E. For example, α-tocopherol inhibits growth of several prostate cancer cell lines, including the androgen-dependent LNCaP and the androgen-insensitive PC-3 and DU145 cell lines (22,73,75,76). This growth inhibitory effect on LNCaP cells is associated with suppression of expression of the AR and PSA, in sharp contrast to the inhibition of LNCaP cell growth by selenium, which is unrelated to the AR/PSA pathway (73). Inhibition of prostate cancer cell growth is reflected in suppression of DNA synthesis (77); decreases in the proliferation markers Ki67 and PCNA; association with cell arrest in G1/S phase; downregulation of the cell cycle proteins cyclins D1 and D3, cdk2, cdk4; as well as decreased Rb phosphorylation and cyclin E expression (78). Vitamin E also inhibits human prostate cancer cell invasiveness, with concomitant reduction in secreted matrix metalloproteinase (MMP)-9, but without alterations in cell survival, the cell cycle, cell adhesion, or cell motility (79). α-tocopherol also inhibits expression of intercellular adhesion molecule-1 (ICAM-1) (62,72,80,81) with concomitant decrease in the metastatic potential of PC-3 prostate cancer cells (62). Additional mechanisms proposed to underlie the anticarcinogenic effect of vitamin E include blocking nitrosamine synthesis; inducing the detoxification enzyme nicotinamide adenine dinucleotide phosphate:quinine reductase; and inhibiting fatty acid metabolism, protein kinase C activity, and arachidonic acid and prostaglandin metabolism (31). The argument favoring nonantioxidant mechanisms is supported by the fact that experiments documenting its anticarcinogenic effects in vitro have frequently used vitamin E succinate, a vitamin E derivative that does not possess antioxidant activity (78).

In animals, vitamin E has been shown to prevent various chemically induced tumors, including some that are hormonally mediated (76,77). Vitamin E slows the growth of prostate cancer in vivo in rats receiving various doses of chemotherapeutic agents.

Vitamin E: Background epidemiologic and clinical research. Vitamin E is present in a wide variety of foods but at low concentrations, leading to flawed estimates of dietary intake (60). As a result, serum or plasma α-tocopherol concentrations are often used to assess vitamin E status. The observational studies that have assessed prostate cancer risk have only inconsistently demonstrated a possible beneficial association between vitamin E status (α-tocopherol circulating levels) or intake and prostate cancer (31). Several positive studies have shown prediagnosis serum or plasma vitamin E concentrations to be lower years prior to prostate cancer diagnosis in cases compared to noncases (82–84). Yet, other observational studies, with both case-control (85) and cohort designs, have reported no association. One cohort analysis, which is nested within the Alpha-Tocopherol Beta-Carotene (ATBC) Cancer Prevention Trial (see below), did not show any association between any baseline measures of vitamin E (serum α-tocopherol, dietary vitamin E) and prostate cancer, except when the analysis was limited to the intervention group (31,86). In a subsequent case-control study nested within the ATBC (100 randomly selected incident prostate cancer cases and 200 matched controls), high baseline serum levels for both α- and γ-tocopherol were associated with decreased prostate cancer risk (highest vs. lowest tertile for α-tocopherol; OR = .49, 95% CI = 0.24–1.01, $P_{\text{trend}} = 0.05$; and for γ-tocopherol,

OR = .57, 95% CI = 0.31–1.06, $P_{trend} = 0.08$), an association that was stronger in the α-tocopherol-supplemented arm (87). However, other observational studies have not supported such associations (82,83,88).

The outcomes of a number of randomized clinical trials testing high-dose (exceeding the 15 mg, or 33 IU *all rac* α-tocopherol, dietary level) vitamin E supplements have suggested benefits for noncancer endpoints, including decreased coronary heart disease risk (60). Regarding prostate cancer, the ATBC trial, conducted in Finland by the National Public Health Institute of Finland and the U.S. National Cancer Institute, offers the most convincing evidence that vitamin E is associated with a decrease in prostate cancer risk. The ATBC study was a randomized, double-blind, placebo-controlled trial of α-tocopherol (50 mg synthetic dl-α-tocopherol acetate) daily and β-carotene (20 mg) daily alone or in combination in 29,133 male smokers 50 to 69 yr old at study entry (89). ATBC was designed to determine whether these nutritional interventions would reduce the risk of lung cancer among participants in this study population (90). Paradoxically, the incidence of lung cancer increased among men receiving β-carotene. Yet, of the 14,564 patients assigned to the α-tocopherol supplementation arms, 99 incident prostate cancers occurred compared with 147 in the 14,569 assigned to the non-α-tocopherol arms. This statistically significant 32% decrease in prostate cancer incidence (95% CI = 12–47, $P = 0.002$) represented a preventive effect that appeared to be stronger in clinically evident cases (stages B–D disease), where participants who received α-tocopherol showed a 40% (95% CI = –20 to –55) decrease in disease. Furthermore, despite being based on fewer events, prostate cancer mortality also showed a statistically significant decrease of 41% (95% CI = –1 to –64) among the 14,564 men taking vitamin E compared to the men not receiving vitamin E (89).

These findings, which were prespecified as a secondary endpoint in the ATBC trial, were hypothesis generating, offering strong support for testing vitamin E in a prospective clinical trial of prostate cancer prevention.

Selenium in Combination With Vitamin E (α-Tocopherol)

Despite their common antiprostate cancer effects, experimental evidence suggests that selenium and vitamin E operate through different sets of cellular processes (73,78). These mechanistic differences are consistent with laboratory observations that have pointed to a substantially more pronounced effect of selenium and vitamin E in combination on some molecular markers of anticancer cellular activity than seen with either nutrient alone (Table 1). Together, they inhibited prostate cancer cell growth by 78% in contrast to 47% with vitamin E alone and 37% with selenium alone (78). Furthermore, the selenium and vitamin E combination induced significantly more apoptosis (37–43%) in prostate cancer cells, accompanied by greater increases in the protein Bax/Bcl-2 ratio, and reduced PCNA protein levels more than either single nutrient (22). The specific apoptotic pathways elicited by selenium and vitamin E appear to differ, however, with the former targeting the endoplasmic reticulum stress/cytokine signaling and the latter targeting the mitochondrial pathway (91). In a novel approach using synthesized conjugates of succinylated tocopherols and tocotrienols with selenium, the phenylselenyl moiety was shown to enhance the proapoptotic and antiproliferative effects of vitamin E succinates in prostate cancer cells (92). These enhanced anticancer effects retrospectively support the inclusion in SELECT of a combined treatment arm (see below and Fig. 2).

Yet, data generated from epidemiologic and clinical studies incorporating selenium and vitamin E together have not always yielded such obvious benefits from the combination. The prospective cohort Vitamins and Lifestyle (VITAL) study did not show an association of vitamin E and selenium with prostate cancer risk overall, although a reduction in clinically relevant advanced disease was associated with greater long-term vitamin E supplementation (93). The Supplementation en Vitamins et Mineraux Antioxidants (SU.VI.MAX) trial randomized 12,741 participants to placebo or to a supplement that included 30 mg of vitamin E and 100 ug of selenium along with nutritional doses of 120 mg of ascorbic acid, 6 mg of β-carotene, and 20 mg of zinc (94). An adjunct study of SU.VI.MAX pointed to an overall moderate, nonsignificant reduction in prostate cancer rate with supplementation vs. placebo, whereas the rate reduction was statistically significant in men with normal PSA (95).

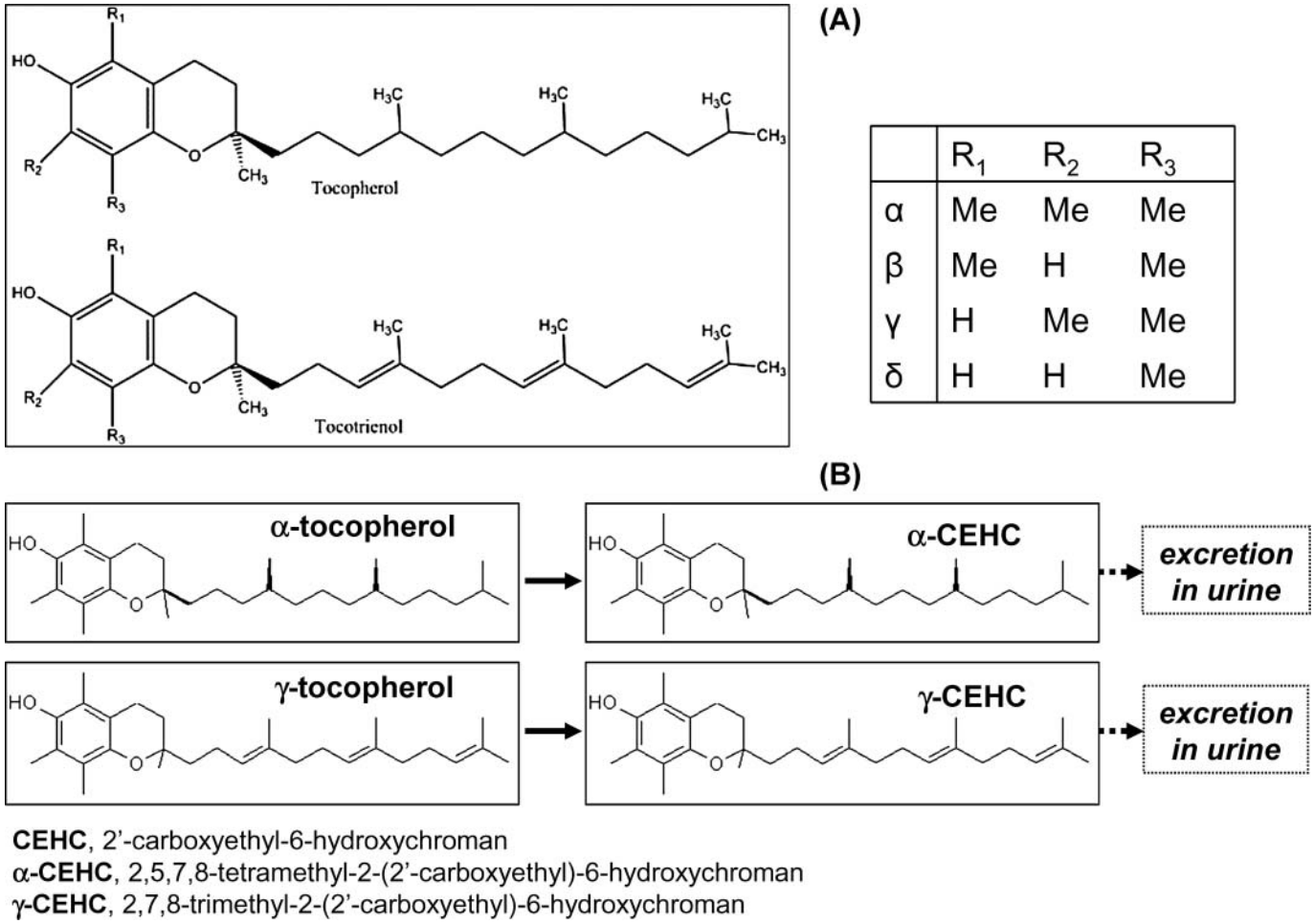
SELECT

Background

Documentation of the anticancer properties of the two nutrients culminated in two key studies, the NPC Study (50) and the ATBC Cancer Prevention Trial (89,90) for selenium and vitamin E respectively, laying the foundation for SELECT. This prostate cancer prevention trial is the second to be designed and implemented by the NCI together with the Southwest Oncology Group (SWOG) as SWOG Protocol S0000, the Selenium and Vitamin E Cancer Prevention Trial (30,31,59,96).

Study Objectives

The hypotheses underlying the SELECT trial, that selenium and vitamin E have activity against prostate cancer, are the basis for its primary objective: to assess the effects of selenium and vitamin E alone and in combination on incidence of prostate cancer. Prespecified secondary endpoints include prostate cancer-free survival, all cause mortality, the incidence and mortality of other cancer types such as lung and colorectal, overall cancer incidence and survival, and disease potentially impacted by chronic administration of selenium and vitamin E. Serious cardiovascular events were also being monitored because of concerns over the safety of vitamin E with regard to the risk of hemorrhagic stroke (31,96). Additional trial objectives included periodic quality of life assessment, serum micronutrient measurement and prostate cancer risk, and the evaluation



	R_1	R_2	R_3
α	Me	Me	Me
β	Me	H	Me
γ	H	Me	Me
δ	H	H	Me

FIG. 2. A: Naturally occurring forms of vitamin E. B: Vitamin E metabolites.

of biological and genetic markers associated with the risk of prostate cancer (97).

Selection of Study Agents

Advice from an NCI-sponsored panel of experts led to selection of l-selenomethionine over selenized yeast for SELECT. Selenized yeast was the form used in the hypothesis-generating NPC trial (50); but the marked batch-to-batch variability in various forms of selenium in the selenized yeast, the lack of commercial availability of the selenized yeast used in the NPC study, and laboratory analysis showing that l-selenomethionine is the predominant selenium species in currently commercially available selenized yeast led to the panel's recommendation of the essential nutrient form. The best dose and formulation of vitamin E were also the subject of some debate. Ultimately, α-tocopherol (all rac (dl)-α-tocopheryl acetate) was selected because of the observed association of long-term supplementation with this form of vitamin E with reduction in prostate cancer incidence in the ATBC trial (86,89). The chosen daily dose of 400 mg was based on its potential benefits for other noncancer diseases (Alzheimer's disease, age-related macular degeneration) as well as its inclusion in widely used vitamin supplements, suggesting its safety (71,98,99).

Study Cohort, Design, and Statistical Methods

Eligibility for SELECT was based on elevated risk of disease due to age: $\geq$55 yr in Caucasian men and $\geq$50 yr in African-American men since 50- to 55-yr-old Black American men have a prostate cancer incidence rate comparable to that of 55- to 60-yr-old White men. Participation also required that a man be healthy, having a DRE not suspicious for cancer and serum total PSA 4.0 ng/ml or less. The complete list of eligibility criteria appears in Table 2. At completion of accrual, 35,533 eligible men had been enrolled onto SELECT, exceeding the goal of 32,400.

SELECT is a prospective randomized, double-blind, placebo-controlled, 2 $\times$ 2 factorial study of selenium and vitamin E alone and in combination in eligible healthy men. Randomization should lead to equal participant distribution among the 4 study arms and to avoidance of hidden sources

TABLE 2
SELECT eligibility criteria[a]

Age ≥55 yr (African-American men ≥50 yr)
Total PSA ≤ 4.0 ng/ml
DRE not suspicious for cancer
No previous prostate cancer or high grade PIN
Normal blood pressure
No current anticoagulation therapy
Willing to restrict off-study supplement use

[a]Abbreviations are as follows: SELECT, Study of Vitamin E and Selenium Cancer Prevention Trial; PSA, prostate-specific antigen; DRE, digital rectal exam; PIN, prostate intraepithelial neoplasia.

of bias in participant characteristics. The study interventions, selenium 200 μg (l-selenomethionine) and vitamin E 400 mg (racemic (dl)-α-tocopheryl acetate) daily, were administered to participants in designated arms (Fig. 3). Study duration was planned to be 12 yr, including the 5-yr uniform accrual period, and a minimum of 7 and maximum of 12 yr of intervention depending on the time of randomization. A predetermined follow-up schedule is shown in Fig. 3.

The planned sample size of 32,400 men was based on estimates of prostate cancer incidence among men in the placebo group using the Surveillance, Epidemiology and End Results (SEER) data from 1991–1995 (100), which in the first 3 yr of the trial would be similar to the rate from the PCPT (10,59). Prostate cancer incidence in SELECT was anticipated to be higher than the relevant SEER age-related incidence for two main reasons. Most men in SELECT were probably receiving annual DRE and PSA screening, and the trial population was expected to include a substantial percentage of African-American men, with a higher rate of disease, due to intensive recruitment (101) (see below).

Secondary analyses of the NPC study (50) and ATBC study (89) showed that selenium and vitamin E were associated with reductions in prostate cancer incidence during the interventions of greater than 60% and greater than 30%, respectively. These observations are the basis for the estimated 25% treatment effect for either nutrient, on which the primary study analysis was designed. This analysis, involving 5 prespecified comparisons [1) vitamin E vs. placebo; 2) selenium vs. placebo; 3) combined vitamin E plus selenium vs. placebo; 4) combined vitamin E plus selenium vs. vitamin E; 5) combined vitamin E plus selenium vs. selenium], would have allowed detection of a 25% decrease in the incidence of prostate cancer for selenium or vitamin E alone, with an additional 25% decrease for combined selenium and vitamin E compared with either agent alone. Additional statistical analyses for vitamin E vs. no vitamin E, selenium vs. no selenium, and interactions of the 2 agents were to be carried out. Prostate cancer was assessed on a recommended routine clinical diagnostic evaluation, including yearly digital rectal examination and serum PSA measurement. Importantly, although the study protocol recommended prostate biopsy for study participants with DRE suspicious for cancer and/or elevated serum PSA, biopsy was performed at the discretion of study physicians according to local community standards (Fig. 3). No end-of-study biopsy was required, as was the case in the PCPT. A histologic diagnosis was made by the Study Site and confirmed by the SELECT Pathology Review committee for all cases. Some of the planned ancillary studies utilizing collected biospecimens (97) appear in Table 3. Omission of the end-of-study biopsy, a tool for assessing prevalent clinically, nonevident disease, shifts the focus of SELECT from screening

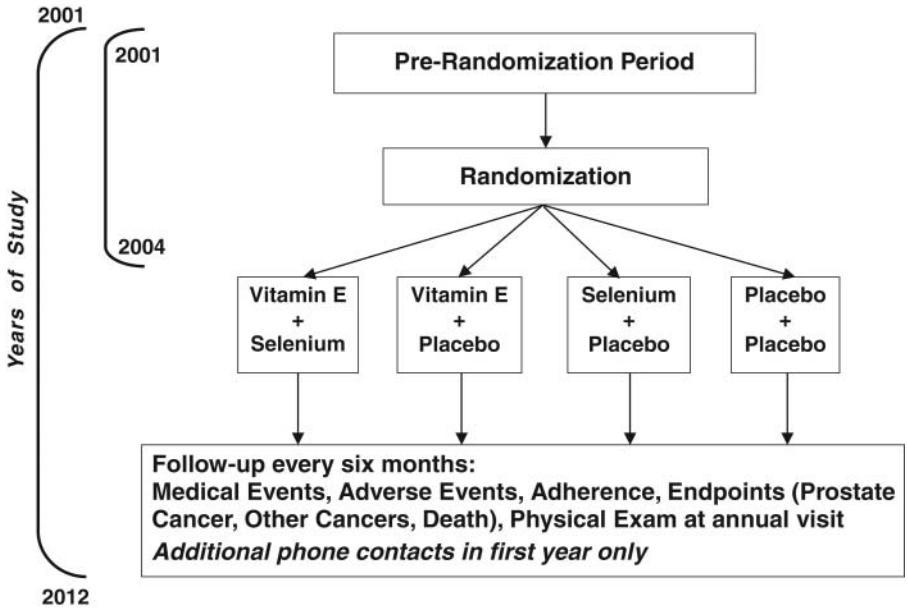


FIG. 3. Study schema and follow-up schedule.

TABLE 3
Baseline characteristics and study adherence of SELECT participants[a]

Baseline Characteristic	Placebo	Vitamin E	Selenium	Selenium + Vitamin E
	No. (%) Participants[b]			
Age, yr				
Median (interquartile range)	62.6 (58.1–67.8)	62.3 (58.0–67.8)	62.6 (58.2–68.0)	62.4 (58.1–67.8))
50–54	355 (4)	402 (5)	337 (4)	385 (4)
55–64	5, 078 (58)	5, 143 (59)	5, 076 (58)	5, 052 (58)
65–74	2, 702 (31)	2, 641 (30)	2, 733 (31)	2, 731 (31)
≥75	561 (6)	551 (6)	606 (7)	535 (6)
Race/ethnicity				
White	6, 863 (79)	6, 890 (79)	6, 942 (79)	6, 874 (79)
African American	1, 078 (12)	1, 107 (3)	1, 053 (12)	1, 076 (12)
Hispanic (non-African American)	492 (6)	477 (5)	481 (5)	484 (6)
Hispanic (African American)	76 (1)	103 (1)	86 (1)	95 (1)
Other[b]	187 (2)	160 (2)	190 (2)	174 (2)
PSA, ng/ml				
0.1–1.0	4, 122 (47)	4, 208 (48)	4, 218 (48)	4, 213 (48)
1.1–2.0	2, 728 (31)	2, 653 (30)	2, 661 (30)	2, 666 (31)
2.1–3.0	1, 168 (13)	1, 228 (14)	1, 211 (140)	1, 149 (13)
3.1–4.0	666 (8)	634 (7)	652 (7)	659 (8)
>4.0	5 (< 1)	3 (< 1)	2 (< 1)	1 (< 1)
Unknown/missing	7 (< 1)	11 (< 1)	8 (< 1)	15 (< 1)
	Baseline Median Serum Levels (Interquartile Range)			
Yr	Placebo	Vitamin E	Selenium	Selenium + Vitamin E
Serum selenium, ug/ml	137.6 (124.7–151.8)	135.9 (122.4–148.4)	135.0 (123.4–145.9)	136.4 (122.9–150.0)
Cholesterol-adjusted α-tocopherol, ug/ml	12.45 (10.70–14.95)	12.79 (10.69–15.37)	12.58 (10.43–14.75)	12.20 (10.12–15.35)
Cholesterol-adjusted γ-tocopherol, ug/ml	1.31 (0.83–2.01)	1.43 (0.89–2.21)	1.50 (0.96–2.21)	1.44 (0.96–2.02)
	Study Adherence: Pill Counts—% of Men Adherent[c] (Range)[d]			
Yr	Placebo	Vitamin E	Selenium	Selenium + Vitamin E
	Selenium/Matching Placebo			
1 ($n = 34,708$)	85 (76–85)	85 (77–85)	84 (76–84)	85 (77–84)
2 ($n = 34,163$)	81 (72–81)	80 (72–81)	79 (71–80)	80 (72–80)
3 ($n = 33,616$)	76 (68–77)	77 (69–77)	75 (68–76)	76 (69–77)
4 ($n = 32,976$)	69 (65–73)	73 (66–74)	71 (64–72)	72 (65–74)
5 ($n = 23,419$)	69 (63–71)	71 (64–73)	69 (62–70)	70 (64–71)
	Vitamin E/Matching Placebo			
1 ($n = 34,708$)	85 (76–85)	85 (77–85)	85 (76–85)	85 (77–85)
2 ($n = 34,163$)	80 (71–80)	80 (71–80)	79 (70–79)	79 (71–80)
3 ($n = 33,616$)	75 (67–75)	75 (67–76)	74 (67–75)	76 (69–77)
4 ($n = 32,976$)	70 (63–72)	70 (63–72)	69 (62–71)	70 (63–72)
5 ($n = 23,419$)	67 (61–69)	69 (62–71)	67 (61–69)	68 (61–70)

[a]Abbreviations are as follows: SELECT, Study of Vitamin E and Selenium Cancer Prevention Trial; PSA, prostate-specific antigen.
[b]Number (%) of participants refers to all entries in this section except those for median age (interquartile range).
[c]Percentage of men adherent defined as taking at least 80% of their study supplements.
[d]These ranges are estimates including those with missing data and assumes those missing were either all not adherent (low estimate) or all adherent (high estimate).

issues, prominent in PCPT (10,102), to molecular epidemiology and risk of prostate and other cancers (97).

Study Implementation, Recruitment Strategies, and Participant Baseline Characteristics

Recruitment strategies. Eligible men from the United States, Canada, and Puerto Rico were enrolled between July 2001 and June 2004—a period 2 yr shorter than projected. Although the accrual target of 32,400 was reached in April 2004, the official closure was delayed to allow men who had begun the enrollment process to be randomized. This resulted in a total of 35,533 participants, 22% of whom were minorities with 15% African Americans, 6% Hispanics, and 11% Asian Americans (103). Not only was SELECT the largest randomized chemoprevention trial ever conducted, but it had the largest percentage of Black participants ever randomized to this type of study (101).

The SELECT leadership recognized that formulating a robust study-specific recruitment plan well before a clinical trial opens, even before protocol finalization, is critical to timely participant accrual (104–106). This trial's plan included a substantial recruitment-and-adherence-focused coordinating center, a clinical design with recruitment feasibility, careful selection of a large number of sites with documented accrual capabilities, and a strong media kickoff. The recruitment management team was composed of a designated recruitment and adherence staff at the central coordinating center (including a minority coordinator) and an advisory committee of experienced research clinicians, the Recruitment and Adherence Committee (RAC). Importantly, the RAC membership was enriched by its Minority and Medically Underserved (MMUS) subcommittee that included national experts and opinion leaders. Prior to study initiation, the central recruitment and adherence staff was an operational unit that refined the recruitment plan, developing specific recruitment and retention strategies and materials while working closely with the RAC (101).

The SELECT media campaign was a highly coordinated effort that was deployed through print, TV, and electronic channels. It included a massive distribution of materials to 800 national and regional print and electronic media outlets that targeted minority, health professional, and advocacy groups as well as the public at large. The direct effect on the number of randomizations is difficult to quantify, but data from the NCI's Communication Information Service, which received 6,400 calls during the first week of the media launch (their highest call volume for 1 wk), suggested that the target audience was reached (Southwest Oncology Group).

The SELECT trial design required baseline blood and toenail samples, a blood sample at 5 yr, clinic visits with a limited physical examination and assessment for adherence and adverse events every 6 mo (annually for those with prostate cancer), and a commitment to refrain from over-the-counter selenium and vitamin E (Fig. 3). Testing for PSA and DRE per local site standard of care was recommended but not required (103). This relatively nondemanding protocol, testing two fairly nontoxic agents, more than likely contributed to participants' willingness to participate (107–110).

The large number of African-American participants is attributed mainly to the lowering of the minimum age of eligibility to 50 for Black men (103), a decision based on the higher age-adjusted prostate cancer risk in Black men. However, although the effectiveness of this change was undeniable, with a full 33% of the SELECT Black cohort being under 55 (the age minimum for other racial/ethnic groups), several other strategies also contributed to the SELECT minority accrual. For example, because the standard eligibility exclusion criterion of prohibiting participants with chronic health conditions is a known obstacle to African-American study enrollment (109,111,112), the SELECT protocol allowed the participation of men with stable comorbidities who are often excluded from clinical trials. In addition, investigators who had previously enrolled large numbers of minority group members were recruited, and budget supplements were provided to sites with a high minority enrollment potential. Of note, many of the strategies that led to rapid overall accrual also enhanced minority accrual. Ironically, the rapid accrual indirectly prevented SELECT from achieving its target minority accrual goal of 24% by reaching the overall goal early (101).

To prevent this effect, researchers would need to be able to better predict ethnic distribution of enrolled patients. Although this would be difficult at this point due to lack of published data on enrollment rate patterns, the thorough reporting of the SELECT recruitment experience in the literature may inform recruitment predictions for future large trials. The total number of evaluable minority participants was further reduced by the elimination of 621 participants from the analysis. Due to inadequate local study site coordination, these participants, 99% of whom were African American, were excluded to avoid jeopardizing their safety and to ensure data integrity. Otherwise, the retention of participants on the study was excellent, with only 24 other men being excluded for either eligibility or informed consent-related issues (103).

Participant adherence can be particularly challenging in long-lasting cancer prevention clinical trials. To give study candidates an opportunity to decide if they were willing to commit to participation and protocol adherence (most significantly, abstaining from vitamins not provided by the study) for the planned 7- to 12-yr study, there was a 28 to 90 day prerandomization period, after which they would return to the clinic if they chose to enroll. To foster continued adherence, staff considered the participants' convenience and comfort by sending visit reminders, assisting with transportation problems, having flexible clinic hours, and maintaining a pleasant clinic experience. Efforts were made to acknowledge the participants' time and dedication to the study by offering materials ranging from certificates of appreciation to items with the study logo such as key chains or post-it notes. In short, the relationship between the study site staff and the

participants is arguably the most important factor in bonding the participant to the study.

Study agent adherence, assessed via pill count (Table 3, Fig. 4A), participant diary, and serum levels (Fig. 4B, Fig. 4C, and Fig. 4D), is described in detail elsewhere (103). Bioadherence, according to levels of selenium and cholesterol-adjusted vitamin E, was measured in a subset of participants: the "adherence cohort." Selenium and vitamin E intervention supplements were discontinued as of October 23, 2008 based on an assessment of the SELECT data as of August 1, 2008 by the data and safety monitoring committee, with a median overall follow-up of 5.46 yr (range = 4.17–7.33 yr) (103). This independent committee concluded that the null hypothesis—that no convincing evidence of benefit existed with either selenium or vitamin E or the two in combination—prevailed according to the SELECT results.

RESULTS

Adherence to Study Supplements

Adherence, assessed both by pill count (Fig. 4A) and in a subset of men by "bioadherence" metrics, that is, serum levels of selenium and vitamin E (Fig. 4B, Fig. 4C, and Fig. 4D), was comparable in all 4 study arms. Importantly, serum selenium and α-tocopherol levels rose only in participants assigned to the selenium- and vitamin E-containing arms, respectively. γ-tocopherol showed reciprocal level changes to those of α-tocopherol, with which it typically varies in inverse fashion (Fig. 4D). These measurements indicate good compliance with assigned study agents and, conversely, minimal "drop-ins" to unassigned supplements by taking over-the-counter selenium and/or vitamin E off study.

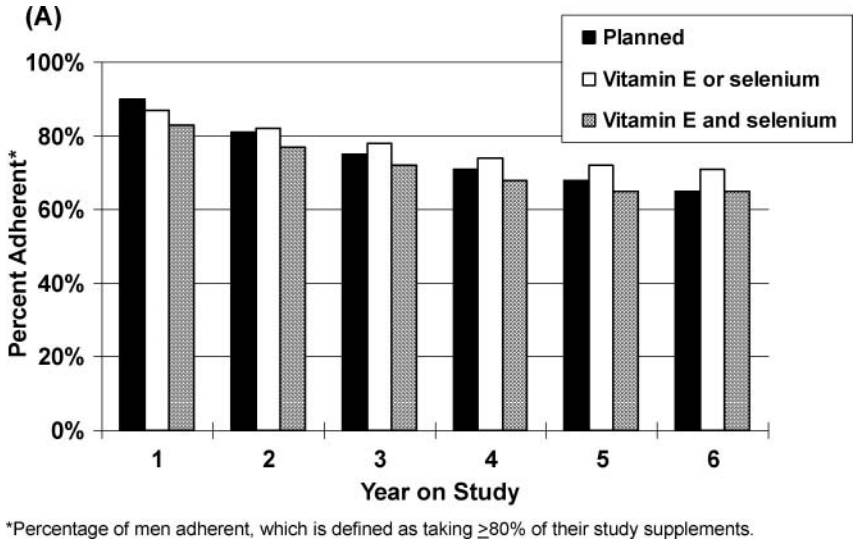


FIG. 4A. Adherence to study supplements according to pill count.

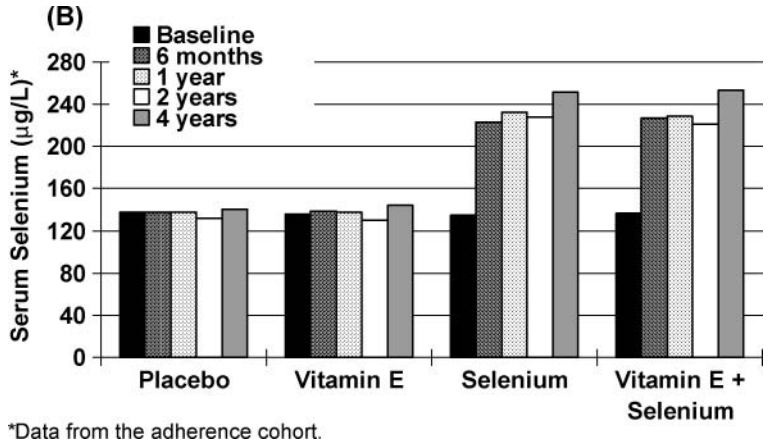


FIG. 4B. Adherence to study supplements according to bioadherence: Serum selenium levels over time.

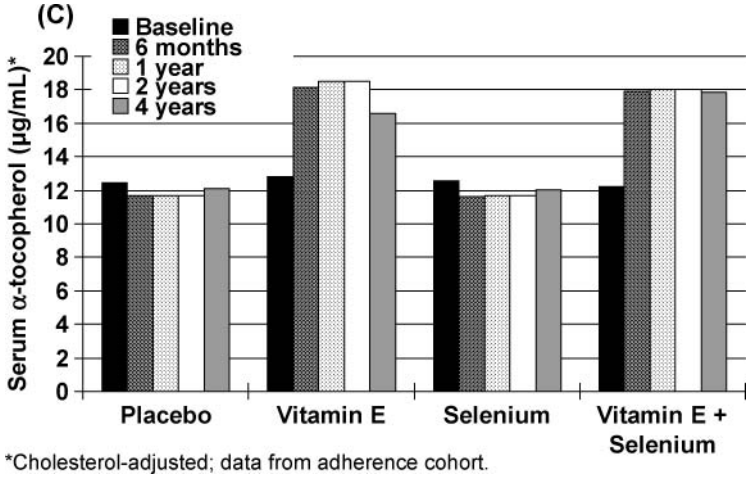


FIG. 4C. Adherence to study supplements according to bioadherence: Serum α-tocopherol levels over time.

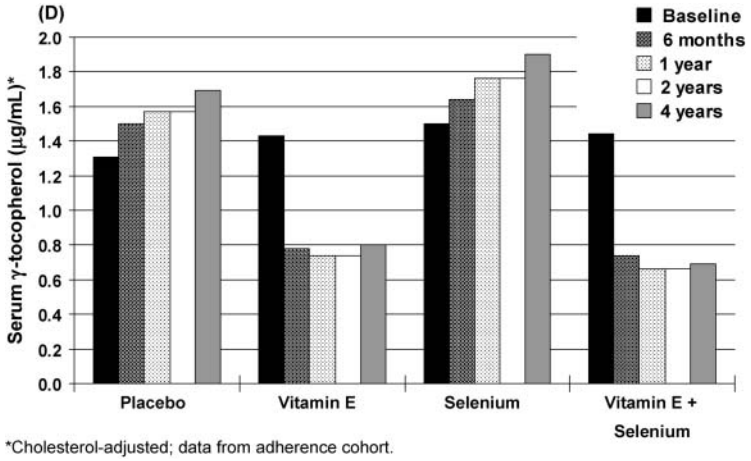


FIG. 4D. Adherence to study supplements according to bioadherence: Serum γ-tocopherol levels over time.

Primary Endpoint: Prostate Cancer

The rates of prostate cancer did not differ statistically among the 4 intervention arms, with HRs for prostate cancer relative to placebo of 1.13 (99% CI = 0.95–1.35, $P = 0.06$) for the vitamin E/α-tocopherol-alone group, 1.05 (99% CI = 0.88–1.25, $P = 0.52$) for the selenium + vitamin E/α-tocopherol group, and 1.04 (99% CI = 0.87–1.24, $P = 0.62$) for the selenium-alone group (Table 4). The graph depicting the cumulative incidence of prostate cancer detected during each study year indicates that the vitamin E/α-tocopherol-alone curve begins to diverge from the placebo and other 2 intervention curves at about 4 yr of follow-up, resulting in a statistically nonsignificant, but somewhat concerning, elevation of prostate cancer incidence (Fig. 5). Most of the prostate cancers were diagnosed by prostate biopsy, constituting histological diagnoses (Table 4). Most were early stage and low Gleason grade, which were similar in all 4 groups (103). The clinical presentation that prompted the biopsy was primarily increased PSA (approximately 2/3 of the cases in each of the 4 groups) or abnormal DRE (11–16% of cases in the 4 groups). Importantly, the proportion of participants undergoing PSA testing and DREs was similar in all groups, obviating any concern that observed outcomes reflected detection bias associated with differential screening.

Secondary Endpoints

Prespecified secondary endpoints included other cancers, especially those influenced by a study supplement in prior nutritional trials (50). None of these cancers differed significantly in rate in any intervention arm compared to the placebo group; all P values were >0.15 (Table 5). Noncancer secondary outcomes included cardiovascular outcomes, none of which showed a significant difference from the reference value in the placebo arm (103). In particular, hemorrhagic stroke—which is a potential concern given the well-known association of vitamin E with bleeding propensity (113) and which was observed among men taking the lower, 50 mg/day dose supplement in the ATBC trial (90)—did not differ among the 4 groups (Table 5). Type 2 diabetes mellitus was of interest because of its increased prevalence

TABLE 4
Clinically diagnosed prostate cancers[a]

	Placebo ($n = 8{,}696$)	Vitamin E ($n = 8{,}737$)	Selenium ($n = 8{,}752$)	Selenium + Vitamin E ($n = 8{,}703$)
Prostate cancers				
Number[b,c]	416	473	432	437
5-yr incidence[c]	4.43%	4.93%	4.56%	4.56%
HR (99% CI)	1.00	1.13(0.95–1.35)	1.04(0.87–1.24)	1.05(0.88–1.25)
P value	—	0.06	0.62	0.52
Diagnosis by prostate biopsy				
Number[c]	404 (97%)	458 (97%)	419 (97%)	420 (97%)
Reason for biopsy (positive biopsies)[c]				
Elevated PSA[c]	259 (64%)	324 (71%)	296 (71%)	263 (63%)
Abnormal DRE[c]	66 (16%)	58 (13%)	46 (11%)	56 (13%)

[a]Abbreviations are as follows: HR, hazard ratio; CI, confidence interval; PSA, prostate-specific antigen; DRE, digital rectal exam.
[b]Total number of prostate cancers diagnosed by the study site.
[c]Number or % of participants per treatment arm.

in association with higher serum selenium levels and its higher incidence following long-term selenium supplementation as reported in earlier studies (114–116). Although an increased risk of Type 2 diabetes, a patient-reported outcome, was observed in SELECT in the selenium-alone arm following randomization (RR = 1.07; 99% CI = 0.94–1.22), this increase was statistically nonsignificant, with a $P = 0.16$. Deaths, total and those due to predesignated causes, also did not differ among the 4 arms (Table 5). The only adverse effects that were significantly increased were alopecia and low-grade dermatitis in the selenium-alone group and halitosis in the selenium-plus-vitamin E group, all previously known side effects of the interventional supplements (Table 5).

DISCUSSION

The results of the SELECT trial, that neither selenium nor vitamin E alone or in combination for a median follow-up time of 5.46 years led to a significant reduction in the clinical incidence of prostate cancer, did not concur with the hypothesis-generating secondary endpoints in the NPC and ATBC trials. Furthermore, the nonsignificant increase in prostate cancer incidence in the vitamin E/a-tocopherol-alone arm has raised concerns that at least this promising nutrient intervention may have the undesirable opposite effect on the outcome of interest. These outcomes of SELECT have been debated extensively, generating a series of potential explanations for the negative results.

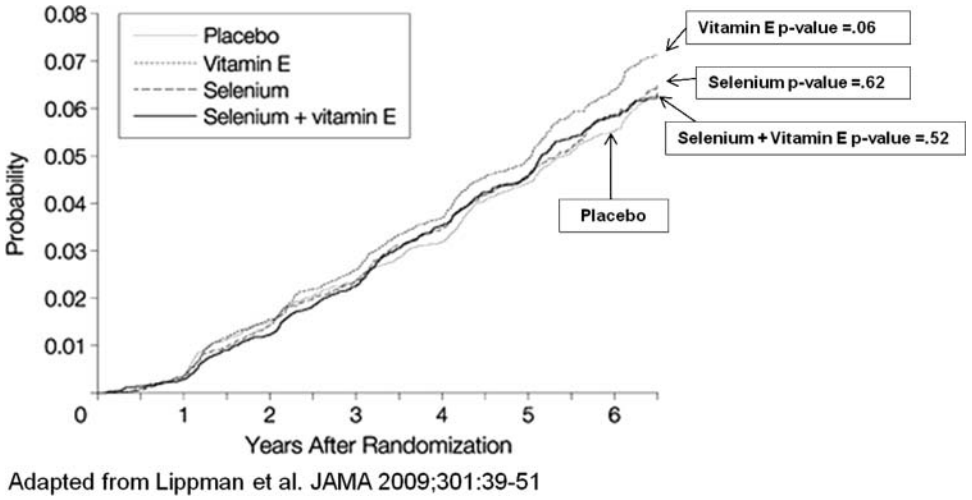


FIG. 5. Cumulative incidence of prostate cancer over time. Adapted from (103).

TABLE 5
Secondary endpoints[a]

	Treatment Arm (No. Participants)			
	Placebo ($n = 8{,}696$) No. HR (99% CI)	Vitamin E ($n = 8{,}737$) No. HR (99% CI)	Selenium ($n = 8{,}752$) No. HR (99% CI)	Selenium + vitamin E ($n = 8{,}703$) No. HR (99% CI)
Cancers				
Any cancer	824	856	837	846
(including prostate)	1 (reference)	1.03 (0.91–1.17)	1.01 (0.89–1.15)	1.02 (0.90–1.16)
Lung	67	67	75	78
	1 (reference)	1.00 (0.64–1.55)	1.12 (0.73–1.72)	1.16 (0.76–1.78)
Colorectal	60	66	63	77
	1 (reference)	1.09 (0.69–1.73)	1.05 (0.66–1.67)	1.28 (0.82–2.00)
Cardiovascular events				
Any (including	1,050	1,034	1,080	1,041
death)	1 (reference)	0.98 (0.88–1.09)	1.02 (0.92–1.13)	0.99 (0.89–1.10)
Hemorrhagic stroke	11	7	11	12
	1 (reference)	0.63 (0.18–2.20)	0.99 (0.33–2.98)	1.09 (0.37–3.19)
Diabetes[b]	669	700	724	660
Deaths	1 (reference)	1.04 (0.91–1.18)	1.07 (0.94–1.22)	0.97 (0.85–1.11)
Total	382	358	378	359
	1 (reference)	0.93 (0.77–1.13)	0.99 (0.82–1.19)	0.94 (0.77–1.13)
All cancers	125	106	128	117
	1 (reference)	0.84 (0.60–1.18)	1.02 (0.74–1.41)	0.93 (0.67–1.30)
Prostate cancer	0	0	1	0
	1 (reference)	N/A	N/A	N/A
Cardiovascular	142	119	129	117
	1 (reference)	0.84 (0.61–1.15)	0.91 (0.66–1.24)	0.82 (0.60–1.13)
Supplement-specific	**No.**	**No. RR**	**No. RR**	**No.**
AEs	**RR (99% CI)**	**(99% CI)**	**(99% CI)**	**RR (99% CI)**
Alopecia	206	220	265[c]	238
	1 (reference)	1.06 (0.83–1.36)	1.28 (1.01–1.62)	1.15 (0.91–1.47)
Dermatitis, Grades	516	591	605	554
1–2	1 (reference)	1.14 (0.98–1.32)	1.17 (1.00–1.35)[c]	1.07 (0.92–1.25)
Halitosis	427	493	503	531
	1 (reference)	1.15 (0.97–1.36)	1.17 (0.99–1.38)	1.24 (1.06–1.46)[c]

[a]Abbreviations are as follows: HR, hazard ratio; CI, confidence interval; N/A, not applicable; AE, adverse event; RR, relative risk. The HRs and RRs given for vitamin E, selenium, and selenium + vitamin E reflect comparisons with the placebo group (reference group).

[b]Based on self-report or reported use of diabetes medication excluding prevalent cases at randomization.

[c]$P < 0.01$.

Why Didn't Selenium Reduce the Clinical Incidence of Prostate Cancer?

Selenium dose and formulation. The dose and, more important, the formulation of selenium used in the SELECT trial have been cited as major contributors to the failure of the selenium-containing arms to exhibit a reduction in prostate cancer incidence. Yet, these features of the selenium intervention were chosen with great care. The chosen selenium dose was the same as the 200 ug/day used in the hypothesis-generating NPC trial. Based on this plus the efficacy and safety data derived from a series of preclinical studies, an expert panel convened in December 1998 concurred that 200 ug would be an appropriate daily dose. Despite this, the optimal dose of selenium supplementation is not established. One idea is that a narrow window exists for the most beneficial dose of dietary selenium. Selenium intake and, more important, the actual selenium concentration in tissues, does not exhibit a linear relationship to DNA damage, the regulation of which is a major mechanism by which selenium is

presumed to serve as a chemopreventive agent in the prostate. Waters et al. (117) demonstrated this phenomenon in terms of a non-linear, U-shaped "dose" (actually toenail selenium concentration) response curve that characterized the relationship between selenium and genotoxic stress in the prostate of dogs. Importantly, this U-shaped relationship between intake or concentration and biological function has more general applicability to trace elements extending beyond selenium (118).

The choice of the formulation of selenium, which exhibits a complex metabolism (13,14,119,120) (Fig. 1), posed a greater challenge. Inorganic forms of selenium, such as selenite, were considered because they have been shown to be more active than organoselenium compounds in suppressing prostate cancer cell growth and inducing apoptosis of prostate cancer cells (32). However, in contrast to the organoselenium compounds, the anticancer properties of inorganic forms are linked to genotoxicity, specifically the rapid induction of DNA single-strand breaks (35). This attribute, especially in view of the prolonged use anticipated in the prevention setting, argued against using an inorganic selenium compound despite the promise of greater efficacy. A similar view confronted the promising compound methylseleninic acid, which exhibited greater potency in vitro and in vivo relative to its organic precursor, Se-methylselenocysteine (120). Methylseleninic acid was new at the time SELECT was being designed; and concern that its toxicity and safety were not well understood, together with its commercial nonavailability, discouraged the panel from considering this form of selenium (59). The remaining options were selenomethionine and selenized yeast (121). Although selenized yeast was the intervention in the NPC trial, concern over large batch-to-batch variation in concentration of specific organoselenium compounds led the panel to reject yeast as the form of intervention. L-selenomethionine was the primary active ingredient in the selenized yeast used in the NPC trial, pointing to this form of selenium as the optimal intervention in SELECT.

Study design difference: SELECT vs. NPC trial design. Study cohort. Differences in the study populations between the SELECT and NPC trials may explain the difference in their prostate cancer outcomes. Unlike SELECT, the NPC trial was conducted in a study population located in east coastal areas of the United States where environmental selenium levels are low (50,122,123). The baseline mean plasma Se levels in both the selenium and placebo arms of this trial were 114 ng/ml. The Se levels rose about 67% in the Se-treated arm, reaching a mean plasma level of 190 ng/ml. Patients with baseline plasma Se levels falling into the lowest (<106.4 ng/ml) and middle (106.4–121.2 ng/ml) tertiles showed a significant reduction in prostate cancer, with RRs of 0.08 ($P = 0.002$) and 0.30 ($P = 0.03$), respectively. In contrast, among those in the highest tertile (>121.2 ng/ml), only a nonsignificant reduction was observed, with an RR of 0.85 ($P = 0.75$) (51). The low baseline selenium levels in the NPC participants appear to have accentuated the beneficial effects of selenium supplementation in reducing prostate as well as total cancer incidence (51,54). Unlike the NPC trial, the men participating in SELECT came from multiple regions all over the United States and Canada and were replete in selenium levels at baseline, with median serum selenium levels of 135 ng/ml (Table 3) compared to the median of 114 ng/ml observed in the NPC trial. In fact, 78% of men participating in SELECT had serum levels above the lower two tertiles of NPC, namely, those serum selenium levels that were associated with a significant benefit from the selenium intervention (103).

In addition to environmental factors feeding into the response of a trial population to the selenium intervention, polymorphisms in genes encoding proteins involved in selenium metabolism and activity contribute to its ability to influence health outcomes. For example, manganese superoxide dismutase (MnSOD), a mitochondrial antioxidant enzyme encoded by the SOD2 gene, participates in processes that depend on selenium (124). In a case-control study nested within the Physicians' Health Study, homozygosity for a functional variant of MnSOD containing an alanine (A) in place of a valine (V) in codon 16 (SNP rs4880, C47T, in SOD2 determines the A16V amino acid polymorphism) was associated with exquisite sensitivity to antioxidant status in the form of prediagnostic plasma selenium levels (124). In analyses stratified by SOD2 genotype, AA homozygotes showed an increased risk of total and clinically aggressive prostate cancer for men with the lowest selenium levels and conversely a decreased risk of total and especially clinically aggressive prostate cancer for men with the highest levels ($P_{\text{interaction}} = 0.05$ for total and 0.01 for clinically aggressive prostate cancer (124,125)). This contrasts with the much weaker inverse associations seen among men with VV and VA genotypes. Genotype with respect SOD2, along with voluntary intake and environmental exposure, is expected to contribute to net selenium balance and in this way to influence selenium-dependent health outcomes. Stratification of SELECT participants according to allelic status in relevant genes such as SOD2 should elicit relationships between selenium supplementation and prostate cancer risk that did not emerge in the trial population as a whole.

Study design difference: SELECT vs. NPC trial design. Statistical issues. Perhaps the most important difference between the 2 cancer prevention trials with respect to their prostate cancer outcomes is the status of the latter as a primary endpoint in SELECT and as a secondary endpoint in NPC. The statistical design ensures that a trial is adequately powered to address the primary endpoint, but this is not necessarily true of secondary endpoints (126). In a clinical trial containing multiple outcomes, prospectively defining a given outcome as the "primary endpoint" protects that endpoint from concerns that the observed result is due to chance due to multiple testing (127). This leaves the "secondary endpoints" at risk of precisely that, representing findings that are due to chance alone. In this manner, the NPC trial was designed to evaluate the effect of selenized yeast on the incidence of nonmelanoma skin cancers, the primary, and hence

the "protected," endpoint. The observations regarding the predetermined secondary endpoints, including other cancers such as prostate cancer, are at risk of being due to chance. In essence, it is as if "all available statistical power had been 'spent' on the primary outcome and the play of chance could have considerable influence even though the secondary outcomes seemed to be statistically significant" (127). The NPC trial was especially vulnerable to the possibility of a chance finding in a secondary endpoint since it was a small trial, with only 1,312 participants.

These statistical concerns regarding interpretation of trial outcomes apply to secondary endpoints irrespective of the significance of the accompanying primary endpoint. However, an even greater degree of skepticism is warranted when the primary outcome is not significant (127–129), as with the NPC trial (50). Furthermore, these concerns are especially pertinent to outcome data relating to interventions being tested for cancer prevention because prevention trials lay the foundation for broad health policy decisions affecting healthy populations. Since health policy must be based on rigorous clinical trial outcomes, satisfying a high level of evidence, the adoption of a cancer preventive intervention based on statistically significant secondary endpoints that emerge alongside a nonsignificant primary endpoint is unacceptable. However, a significant secondary endpoint that coexists with a nonsignificant primary endpoint may generate a hypothesis that, in turn, serves as the basis for the primary endpoint in a derivative clinical trial. This is exactly the role played by prostate cancer incidence in the NPC trial, which laid the groundwork for the selenium intervention incorporated into the factorial design of SELECT. In essence, concerns that prostate cancer incidence was not reduced in SELECT as it was in the hypothesis-generating NPC trial miss the point. The very fact that prostate cancer was merely a secondary endpoint in NPC was precisely why SELECT was implemented (130). The latter trial could only have been justified if equipoise existed regarding the expectation that intervention with selenium would reduce prostate cancer incidence as a primary endpoint.

Why Didn't Vitamin E Reduce the Clinical Incidence of Prostate Cancer?

Vitamin E dose and formulation. Of the 8 naturally occurring forms of vitamin E (Fig. 2A), the selection of α-tocopherol, based not only on its use in the hypothesis-generating ATBC trial but also for its physiological properties (see above section), was prospectively a logical choice. The 400 IU/day dose was chosen for reasons such as its inclusion in vitamin supplements as well as the potential benefit of this high dose for multiple disease endpoints as investigated in the HOPE trial (131), the Physicians' Health Study II (132), and other studies (60). Furthermore, in the ATBC trial, men in the highest quartile of baseline α-tocopherol and total vitamin E serum levels benefited most from the α-tocopherol intervention in terms of reduction in prostate and lung cancer incidence (86,133). This combination of high baseline level and supplementation implicated the total α-tocopherol as being critical in reducing cancer incidence, suggesting a benefit to intervening up front with a higher dose than that used in the ATBC trial (59). Yet, 400 IU/day is 8 times the dose used in the ATBC trial and therefore merits scrutiny as potentially contributing to the failure of α-tocopherol to reduce prostate cancer incidence in either of the two vitamin E-containing arms in SELECT.

One potential contributor to the failure of the high-dose α-tocopherol strategy in SELECT is the reciprocal reduction in γ-tocopherol levels (Fig. 4D) that paralleled the rise in α-tocopherol in the vitamin E intervention arms (Fig. 4C). Although α-tocopherol is generally viewed as the most active form (62,65,66), laboratory evidence for higher antioxidant activity with γ-tocopherol has been discussed (67,68). In addition, the role of γ-tocopherol as an antioxidant has been shown to complement that of α-tocopherol (Table 1) (67). Furthermore, vitamin E homologues have biological activities unrelated to their antioxidant properties; and for some of these functions, γ-tocopherol exhibits greater activity (Table 1) (64). γ-tocopherol inhibits proinflammatory eicosanoid formation. Thus, γ-tocopherol, but not α-tocopherol, inhibits cyclooxygenase enzyme activity resulting in decreased prostaglandin E2 synthesis and neutrophil 5-lipoxygenase, leading to reduced leukotriene B4 formation (134,135). γ-tocopherol has been shown to decrease TNF-α, inflammatory damage, and sphingolipid synthesis more effectively than α-tocopherol (64,136). γ-tocopherol traps reactive nitrogen species and increases peroxisome proliferator-activated receptor (PPAR)-γ expression more than α-tocopherol (64). This constellation of mechanisms in large part underlies the anti-inflammatory activities of γ-tocopherol (68,137,138). These activities undoubtedly contribute to γ-tocopherol's chemopreventive properties, which in some cases have been shown to surpass those of α-tocopherol (64). At the epidemiologic level, several nested case-control studies have shown an inverse relationship between levels of γ-tocopherol, but not α-tocopherol, and prostate cancer (68,139).

The designing of SELECT predated the availability of most of these data, supporting the view of the SELECT planning committee that the decline in serum γ-tocopherol that accompanied high-dose α-tocopherol would not be a major concern. Their decision was additionally based on observations that the decrease in γ-tocopherol levels in response to α-tocopherol supplementation, a well-established relationship (137,138,140,141), occurs at doses as low as 30 mg/day (142) as well as on the inconsistency of dose-response data relating to this inverse relationship between the 2 vitamin E forms (141,143). Together with the fact that α-tocopherol is the predominant form in blood, with biological activity surpassing that of γ-tocopherol despite the latter being the major tocopherol in the Western diet (141), the predicted reduction in γ-tocopherol levels was not anticipated to be harmful to health (59) and are not considered a likely explanation for the absence of prostate cancer risk reduction in the 2 vitamin E arms in SELECT.

Study design difference: SELECT vs. ATBC trial design. Study cohort. As with selenium, the difference in the trial

populations of ATBC and SELECT might explain the discrepancy in prostate cancer outcomes with vitamin E. ATBC, a lung cancer trial, focused on male smokers, whereas only 7.5% of the SELECT participants were tobacco users. This difference in smoking status between the populations in these trials might account for the differing effects of vitamin E on prostate cancer outcomes. Yet, two studies that have explored potential interaction among smoking, vitamin E levels, and prostate cancer risk failed to show any such associations (83,132). In contrast, decreasing risks of advanced prostate cancer (Gleason score >7 or Stage III or IV) were observed in association with increasing dose and duration of supplemental vitamin E among current and recent smokers, although not in the study population as a whole, among men participating in the Prostate, Lung, Colon, and Ovarian Cancer (PLCO) Screening Trial (144). Similarly, an inverse association between vitamin E levels and prostate cancer mortality was seen among smokers in the prospective Basel Study despite the absence of association in the overall study population (84).

Polymorphisms in genes involved in vitamin E-related activities such as vitamin E transport efficiency, rate of metabolism, and levels and structure of plasma lipoproteins might explain the failure of prostate cancer to decrease in response to vitamin E supplementation (145). Homozygosity for the variant G allele of IVS2–2191A>G in TTPA, the gene that encodes the enzyme α-TTP, was recently shown to be inversely associated with the overall risk of prostate cancer in the ATBC study (146). In the same study, higher vitamin E consumption was most protective against advanced prostate cancer in men carrying two copies of an intronic SNP, IVS11_931A>G, in SEC14L2, the gene that encodes α-tocopherol-associated protein (hTAP). A deeper understanding of the factors feeding into the null outcomes in SELECT will require investigations into this type of nutrigenomic interaction between genotype and vitamin E intake/serum levels using the prospectively collected samples.

Finally, the same statistical issues that underlie the skepticism about the effect of selenium on prostate cancer risk in the NPC trial apply to vitamin E in the ATBC trial. The ATBC trial, which was not designed primarily to address the effect of vitamin E on prostate cancer risk, could only provide a hypothesis-generating finding on this point. A definitive trial, SELECT, was necessary to test this hypothesis (130). The fact that the SELECT results support the null hypothesis might be disappointing but serves as testament to the importance of rigorous implementation of a well designed, randomized clinical trial.

CONCLUSION

The absence of positive findings in SELECT for either selenium or vitamin E is surprising in view of the plethora of data, both laboratory and epidemiologic, that support associations between these nutrients and decreased risk of prostate cancer. Future clinical research addressing these associations will likely involve smaller biomarker-intense studies (147). The dose and formulation of each agent, together with selection of a cohort most likely to benefit from intense supplementation, should be the focus of trial design. In the case of selenium, a trial cohort depleted in selenium would seem most likely to benefit from supplementation. Design of a trial incorporating vitamin E will need to carefully balance the relative doses of α-tocopherol and γ-tocopherol, since the two have been shown to be complementary in their anti-inflammatory activity (148) and other anticancer activities (149,150). In general, nutritional agents appear to exhibit an optimal "window" of activity (a U-shaped dose-response curve), below and above which their benefits disappear and toxicity may even ensue. Because unlike purely synthetic drugs, nutrients derive from natural products, the state of endogenous nutritional repletion of an individual participant must be prospectively factored into the trial designs aimed at achieving this optimal level. Similarly, the genotype with respect to a relevant gene, such as SOD2 in the case of selenium, should be studied in these trials. In cases in which prior evidence exists for association of a given allelic state with response to the nutrient in question, prospective stratification according to genotype should be implemented. Where the evidence of a nutrigenomic association is strong (MnSOD A/A genotype inversely associated with prostate cancer risk (124,125)) and the variant allele common (allele frequency for SOD2 variant encoding A is 41–55% in the Caucasian population (124)), consideration should be given to incorporating specific genotypic states as eligibility criteria into the study design in order to enrich the trial for individuals likely to respond to the nutritional intervention. In addition, DNA samples prospectively collected from SELECT participants should be used to test the interaction of implicated genotypes with selenium and/or α-tocopherol supplementation and serum levels with prostate cancer outcomes.

In summary, the very fact that the SELECT outcomes with respect to both interventional nutrients support the null hypotheses strongly argues for the importance of carrying out prospective randomized clinical trials. Massive amounts of observational data support associations between selenium and vitamin E with decreased risk of prostate cancer. Yet, such associations, derived from epidemiologic studies or as secondary endpoints or subset analyses from clinical trials, are not sufficient to infer causal relationships. Only a well-conducted, randomized clinical trial prospectively intervening with selenium and vitamin E could definitively evaluate the effect of these nutrients, in the formulations and doses tested, on prostate cancer incidence. Although the SELECT outcomes disagree with much of the observational data, they do, however, concur with other nutritional studies in not supporting preventive use of selenium or vitamin E supplements.

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