DESCRIPTION. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

This proposal seeks 5 years of funding to continue the vitamin E (400 IU on alternate days), vitamin C (500 mg daily), and multivitamin (Centrum Silver daily) components of the Physicians' Health Study (PHS) II. This would extend average treatment and follow-up from 3.7 to 8.2 years. The PHS II is an ongoing randomized, double-blind, placebo-controlled trial using a factorial design to evaluate the role of vitamin E, vitamin C, a multivitamin, and β-carotene in chronic disease prevention among 14,642 U.S. male physicians aged 50 years and older who have demonstrated excellent morbidity and mortality follow-up and high compliance. The PHS II is uniquely positioned to clarify ambiguities regarding the role of vitamin E in the primary prevention of prostate cancer and cardiovascular disease (CVD) among those at usual risk; the role of a multivitamins and vitamin C and in the prevention of cancer and CVD; and all three agents in the prevention of eye disease and declines in cognitive function.

After reviewing the progress of the trial and the unblinded data, the Data and Safety Monitoring Board unanimously recommended an extension beyond the trial's scheduled termination of funding in December 2002. The primary rationale for the Board's recommendation was the likelihood, at the end of current funding, of ambiguous results due to insufficient numbers of endpoints and an inadequate latent period, which they feared could result in considerable clinical confusion. Because recruitment and 3.7 years of treatment and follow-up have been supported by nearly $20,000,000 of industry funds, the proposal to extend PHS II represents an extremely cost-efficient way to provide either clear positive or definitive null results on which sound clinical and public health recommendations can be based for the use of these commonly consumed vitamin supplements. Given the gaps in knowledge this study is intended to address, as well as the certain intense interest in its findings by the medical, lay, and regulatory communities, the proposed extension of this primary prevention trial in men is timely, important, and will complement other ongoing clinical trials.

PERFORMANCE SITE(S) (*organization, city, state*)

Brigham and Women's Hospital, Boston, Massachusetts

KEY PERSONNEL. See instructions on Page 11. *Use continuation pages as needed* to provide the required information in the format shown below.

Name	Organization	Role on Project
J. Michael Gaziano, MD, MPH	Brigham and Women's Hospital	Principal Investigator
Julie E. Buring, ScD	Brigham and Women's Hospital	Co-Investigator
JoAnn E. Manson, MD, DrPH	Brigham and Women's Hospital	Co-Investigator
William G. Christen, ScD	Brigham and Women's Hospital	Co-Investigator
Howard D. Sesso, ScD	Brigham and Women's Hospital	Project Director
Robert J. Glynn, ScD	Brigham and Women's Hospital	Statistician
Meir J. Stampfer, MD, DrPH	Brigham and Women's Hospital	EndPoint Reviewer
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Carlos S. Kase, MD	Boston University Medical Center	Consultant
Charlene Belanger	Brigham and Women's Hospital	Project Coordinator
Miriam Schvartz, MD	Brigham and Women's Hospital	Coding Specialist

A. SPECIFIC AIMS

This application seeks 5 years of funding to continue the vitamin E, vitamin C and multivitamin components of the Physicians' Health Study (PHS) II, extending average treatment and follow-up from 3.7 to 8.2 years. The PHS II is an ongoing randomized, double-blind, placebo-controlled trial using a factorial design to evaluate vitamin E, vitamin C, a multivitamin (Centrum Silver), and β-carotene in the primary prevention of cancer, cardiovascular disease (CVD), eye disease, and decline in cognitive function among 14,642 U.S. male physicians aged ≥50 years. Although numerous observational studies suggest that these agents may play important roles in preventing chronic disease, no large-scale randomized trial to date has confirmed these hypotheses in a usual-risk population.

The first Physicians' Health Study (PHS I) was a randomized, double-blind, placebo-controlled, 2x2 factorial trial of aspirin and β-carotene in the primary prevention of cancer and CVD among initially healthy middle-aged men. Upon its completion in 1995, industry funds were obtained to initiate PHS II. We have recruited and randomized 7,641 physicians who participated in PHS I and 7,001 new physicians. All costs for study start-up, recruitment, follow-up, study agents, and packaging have been covered by approximately $20 million of industry support. This support is scheduled to end on December 31, 2002, at which time there will have been an average of 3.7 years of treatment and follow-up in PHS II.

When the trial's independent Data and Safety Monitoring Board (DSMB) reviewed the unblinded data at its last meeting in May 2001, the clinical and statistical reviewers unanimously recommended continuing the trial beyond 2002 (see letter from the DSMB chair following Section I). The main reason they cited was the likelihood that the ambiguity of the findings at the scheduled end of the trial due to an insufficient number of endpoints and an inadequate latent period could cause considerable clinical confusion. An additional 4.5 years of randomized treatment and follow-up would greatly enhance the trial's power to detect the likely small to modest benefits of these agents.

We also plan to simplify PHS II in the proposed extension by eliminating β-carotene as a study agent. Given the average of 12 years of β-carotene treatment in PHS I plus an additional 3.7 years in PHS II, the added power gained by continuation of β-carotene treatment will be small.

Despite the lack of definitive data regarding the benefits of vitamin supplements in the prevention of chronic disease, millions of Americans take them for precisely this reason. Thus, positive or null results from this trial could have a substantial impact on personal and clinical decision making, as well as on public health policy. Given the widespread use of the study agents, this trial – the first among usual-risk men – will not likely be repeated. Because the costs of initiating PHS II have already been provided, the low incremental costs of continuing the study make PHS II an extremely cost-efficient way to assess whether or not commonly used vitamin supplements offer the protection against chronic diseases that has been seen in observational studies.

The primary and secondary aims of PHS II will continue to be:

A.1 Primary Aims

A.1.a. To test whether 400 IU of vitamin E taken every other day reduces risk of prostate cancer.

A.1.b. To test whether 500 mg of vitamin C taken daily or a multivitamin taken daily reduces risk of total cancer.

A.1.c. To test whether 400 IU of vitamin E taken every other day, 500 mg of vitamin C taken daily, or a multivitamin taken daily reduces risk of important vascular events.

A.2 Secondary Aims

A.2.a. To test whether 400 IU of vitamin E taken every other day, 500 mg of vitamin C taken daily, or a multivitamin taken every day reduces the risk of age-related macular degeneration or cataract.

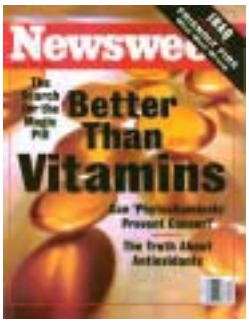
A.2.b. To test whether 400 IU of vitamin E taken every other day, 500 mg of vitamin C taken daily, or a multivitamin taken daily reduces the risk of early cognitive decline in men aged 65 years and older.

A.2.c. To test whether 400 IU of vitamin E taken every other day or a multivitamin taken every day reduces the risk of total cancer, colon cancer, and colon polyps.

A.2.d. To test whether 400 IU of vitamin E taken every other day, 500 mg of vitamin C taken daily, or a multivitamin taken every day reduces the risk of myocardial infarction and stroke.

B. BACKGROUND AND SIGNIFICANCE

Vitamin E, vitamin C, and a multivitamin each represent uniquely promising interventions hypothesized to play roles in the primary prevention of chronic conditions that include total, prostate, and colon cancer; cardiovascular disease; age-related eye diseases such as cataract and macular degeneration; and decline in cognitive function. Results from observational studies generally support these hypotheses. The media attention generated by these positive reports, like this Newsweek cover story calling antioxidants "better than vitamins," have helped fuel the widespread use of dietary supplements. Many men currently take vitamin supplements for their putative ability to prevent chronic disease – an estimated 17% of US men take vitamin E supplements,[1] and up to 40% take individual vitamin C supplements[2,3] or multivitamin supplements.[4] Annual sales of vitamins in the US exceed $5 billion.[5]



The widespread use of these agents has several implications. First, if individuals feel they are deriving benefits from supplements, they may be less likely to engage in other behaviors proven to prevent chronic disease. Second, supplement use poses a financial burden on individuals and health care payers given their daily use over a long duration. Finally, it is becoming increasingly difficult to conduct randomized trials of these agents in the US, as people are unwilling to be randomized to a placebo. We experienced this difficulty during recruitment for PHS II – among new physicians recruited for the trial, the major reason for ineligibility (over 90% of men who provided reasons for ineligibility) was unwillingness to give up use of a multivitamin, vitamin E, or vitamin C. Thus, it is critical to determine whether or not these commonly used supplements provide clear benefits, as well as to assess any potential risk of long-term use.

B.1 Vitamin Supplements and Cancer

Cancer is currently the second leading cause of death in the US.[6] Among US men, prostate cancer is the most common cancer, followed by cancers of the lung and bronchus, and colon and rectum.[7] Even modest reductions in cancer incidence would substantially affect public health.

Emerging evidence suggests that antioxidants such as vitamins E and C offer protection against cancer. Free radical damage (oxidation) to cellular components likely plays an important role in carcinogenesis.[8-10] Antioxidants defend against oxidative stress by scavenging free radicals and interrupting free radical-induced chain reactions. Fat-soluble vitamin E and water-soluble vitamin C apparently interact to link disparate antioxidant systems and may act synergistically to prevent oxidative damage.[11] These micronutrients have other activities that may contribute to the prevention of chronic disease. Vitamin E may enhance the immune response[12,13] and block the formation of carcinogenic nitrosamines.[14-16] Vitamin C can stimulate epithelial cells to produce collagen, which may stabilize these cells in a highly differentiated state, resulting in the development of less severe tumors.[17,18] Both of these vitamins have been shown to effectively prevent induced tumors in animals, including hormonally mediated tumors,[9,19-21] and may also modulate tumor growth. Vitamin E has been shown to inhibit growth of human lung cancer, melanoma, oral carcinoma, and breast cancer cell lines,[22] and to slow the growth of human prostate tumors in rats.[23,24] Data from observational epidemiologic studies indicate that individuals who consume high amounts of vitamins E and C tend to

have reduced risks of cancer. The combination of antioxidant vitamins and minerals, folic acid and other B vitamins, and other components found in a multivitamin may prevent cancer by several mechanisms, and observational studies raise the possibility that multivitamin constituents reduce cancer risk.[25-38]

B.1.a *Vitamin E*

B.1.a.1 Prostate cancer: Basic and animal research support the possibility that vitamin E may reduce the growth of prostate tumors. Vitamin E (α-tocopherol) is the major lipid-soluble, chain-breaking antioxidant protecting cell membranes from free-radical damage.[8,39-41] *In vitro* and *in vivo* experiments show that the free-radical quenching activity of vitamin E can decrease cancer growth.[15] Vitamin E may also enhance the immune system.[13] Vitamin E has been shown to slow the growth of human prostate tumors in vitro, as well as in rats receiving various doses of chemotherapeutic agents.[23,24,42]

Limited observational data, while not entirely consistent, support the possibility that vitamin E reduces the risk of prostate cancer incidence or mortality. Three case-control studies (conducted in Serbia, Greece, and Uruguay) observed inverse associations between dietary vitamin E and prostate cancer risk,[43-45] with statistically significant reductions of 40% or greater. In the Health Professionals Follow-up Study, conducted among 47,780 initially healthy, middle-aged men, although no overall clear association was found for self-reported vitamin E supplement intake and total, advanced, and fatal prostate cancer, a suggestive reduction in relative risk of metastatic and fatal prostate cancer (RR, 0.44; 95% CI, 0.18-1.07) was observed among ever smokers.[46] One study supports an inverse association which is strongest among those with higher levels of γ-tocopherol.[47] Other cohort[48] and case-control[49-53] studies have not observed significant associations between dietary vitamin E intake or supplemental vitamin E intake and risk of prostate cancer.

The most compelling data suggesting that vitamin E may reduce the risk of prostate cancer come from the Finnish Alpha Tocopherol/Beta Carotene (ATBC) Cancer Prevention Trial. The ATBC was a randomized, double-blind, placebo-controlled trial of α-tocopherol (50 mg daily) and β-carotene (20 mg daily) among 29,133 male smokers. Among those assigned to α-tocopherol supplementation, there was a 32% reduction in prostate cancer incidence ($p < 0.01$) during a median follow-up period of 6.1 years.[54] The effect of vitamin E was apparently strongest on more advanced tumors. A 41% reduction in prostate cancer mortality was also observed. However, since prostate cancer mortality was not a prespecified endpoint, it remains possible that this finding is due to chance.

Definitive proof for the hypothesis that vitamin E reduces the incidence of prostate cancer and risk of death from it will come only from additional large-scale randomized trials. Intriguing results notwithstanding (such as a nonsignificant trend toward reduced risk of prostate cancer among those randomized to vitamin E in the Heart Outcomes Prevention Evaluation trial [personal communication, JL Probstfield, 2001]) other completed trials of vitamin E, including several among individuals at high-risk for cardiovascular disease, were not powered to address the possible benefit of vitamin E on prostate cancer.

One recently initiated study, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), was designed specifically to assess the role of vitamin E and selenium in the prevention of incident prostate cancer among men initially free of prostate cancer based on baseline PSA values and digital rectal exams. PHS II will serve as an important complement to SELECT. PHS II will also contribute unique information regarding vitamin E and prostate cancer. Because the PHS II population is not screened for prostate cancer prior to randomization, the results from this trial should provide information regarding the effect of vitamin E across the spectrum of prostate cancer. If vitamin E slows the progression of early, undetected prostate cancer, as suggested by data from the ATBC trial and supported by animal studies, there will be greater power to detect this effect in the PHS II than in SELECT. In addition, since baseline blood samples have been provided by 76% of PHS II participants,

we have the ability to assess the effect of vitamin E stratified by baseline PSA levels. Thus, we feel that both PHS II and SELECT will provide important and complementary data regarding vitamin E supplementation at doses considerably above recommended daily values and prostate cancer risk. With the proposed extension, PHS II can provide 8.2 years of randomized vitamin E treatment at low cost (approximately $95/randomized participant/year in direct costs).

B.1.a.2 Total and colorectal cancer: Prospective cohort studies have employed two main strategies for investigating the association between vitamin E and the incidence of cancer: estimation of dietary intake or determination of plasma or serum levels. Although one study found an inverse association between intake of vitamin E supplements and cancer risk,[55] most studies evaluating dietary intake of vitamin E alone generally have found no association.[56-58] Several cohort studies have examined the association between serum or plasma vitamin E and subsequent risk of cancer. The Basel Study reported a significant inverse association with baseline α-tocopherol levels and risk of subsequent cancer after 7 years of follow-up,[59] but the effect was attenuated after 12 years of follow-up.[60] A meta-analysis of the results of several nested case-control studies[38,58,61-69] showed that mean baseline serum or plasma vitamin E levels were significantly lower among cases than controls for cancer from all sites.[37]

Mixed results have been obtained from trials of vitamin E supplementation and total cancer. In the ATBC trial, there was no reduction in risk of total cancer among those randomized to α-tocopherol (50 mg daily) and/or ß-carotene (20 mg daily), and vitamin E alone had no effect on the primary endpoints of lung or total cancer.[70] In the Chinese Cancer Prevention Trial, conducted among 29,584 poorly nourished residents of Linxian, China, those assigned to a combined daily treatment of vitamin E (30 mg), ß-carotene (15 mg), and selenium (50 µg) experienced statistically significant reductions of 9% in total mortality, 13% in cancer mortality, and 21% in gastric cancer mortality after nearly 6 years of treatment and follow-up.[71] However, these results may not be generalizable to well-nourished populations. Moreover, because three agents were tested in combination, the specific benefit of vitamin E, ß-carotene, or selenium cannot be determined.

Preliminary data suggest that vitamin E use may be associated with reduced risk of colorectal cancer. In the Health Professionals' Follow-up Study, men with supplemental vitamin E intake of at least 300 IU/day had an approximate 30% lower risk of colon cancer than never users, after multivariate adjustment.[72] In the ATBC trial, those assigned to vitamin E had a 17% reduction in colon cancer incidence.[54] This finding requires confirmation because colon cancer was not a prespecified endpoint in the ATBC.

B.1.b *Vitamin C*

Basic and animal research offer plausible mechanisms by which vitamin C may protect against cancer. Vitamin C stimulates epithelial cells to produce collagen, which may stabilize these cells in a highly differentiated state, resulting in the development of less severe tumors.[17,18] Vitamin C has been shown to effectively prevent induced tumors in animals.[20,21] It is a potent free radical scavenger,[73] and thus prevents the formation of mutagenic nitrosamines that can damage genetic material.[74] Finally, vitamin C may enhance immune system function.[75]

B.1.b.1 Total cancer: A wealth of evidence from observational studies of fruits and vegetables supports a role for vitamin C in the prevention of cancer. In a review of data from more than 90 epidemiologic studies of intake of vitamin C (or fruits that supply vitamin C) and cancer, Block found that almost all showed a protective relationship, with a median 2-fold increased relative risk for low compared with high intake.[35] The effects were statistically significant in three-fourths of the studies. Both dietary intake[58,76,77] and blood-based studies have shown inverse relationships.

Substantial epidemiologic evidence supports an inverse association between intake of vitamin C

and risk of a variety of specific cancers.[35] Published reports show significant protective effects of vitamin C on breast, oral, gastric, esophageal, pancreatic, lung, cervical, and rectal cancer, while none have reported elevated risk with increasing intake.[35,36,78] The epidemiological data have been consistent in indicating inverse associations for non-hormone dependent cancer sites. Risk reductions as large as 70% have been observed comparing individuals in the highest versus lowest levels of vitamin C intake.[58,77] In the first National Health and Nutrition Examination Survey (NHANES I), vitamin C intake among more than 11,000 men and women aged 25-74 was examined.[79] The standardized cancer mortality rate was considerably lower than expected among participants with the highest vitamin C intake. Vitamin C supplement use rather than dietary intake explained most of the observed association.

Plasma vitamin C can be measured reliably only in fresh blood specimens or in those stored at very low temperatures (-70°C) after being chemically stabilized.[80] Only one large-scale prospective investigation, the Basel Study, has assessed prediagnostic vitamin C levels and cancer risk. In fresh blood samples from 2,974 men taking part in the 1971-1973 follow-up examination, a statistically significant difference in mean plasma vitamin C levels was observed between cases and controls that achieved statistical significance for cancer mortality.[60] There were also significant inverse associations between vitamin C levels and stomach and total gastrointestinal cancer among subjects ≥60 years.

A major gap in the evidence regarding a possible role of vitamin C in the prevention of cancer is lack of data from large-scale clinical primary prevention trials. The existing trial evidence, limited to small-scale studies among high-risk populations, is a mix of promise[81-83] and disappointment.[84,85] Secondary prevention trials focusing on vitamin C for the recurrence of colon cancer or polyps have also yielded mixed results, ranging from a nonsignificant reduction (RR, 0.86; 95% CI, 0.51-1.45) among individuals with colon polyps assigned to a combination of vitamin E and C, compared with placebo,[86] to no evidence that either combined vitamins E and C or ß-carotene alone reduced the incidence of subsequent colorectal adenomas among patients with previous adenoma,[87] or no evidence that calcium and vitamin supplementation (vitamins A, C, and E) reduced cell kinetics of the colonic epithelium among patients with colorectal cancer.[88]

PHS II is the only large-scale trial testing the independent effects of vitamin C at doses well above the daily recommended intake in cancer prevention.

Table 1. Content of Centrum Silver multivitamin tablets*

Constituent	Dose
Vitamin A (20% as β-carotene)	5000 IU
Vitamin C	60 mg
Vitamin D	400 IU
Vitamin E	45 IU
Vitamin K	10 µg
Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20 mg
Vitamin B_6	3 mg
Folic Acid	400 µg
Vitamin B_{12}	25 µg
Biotin	30 µg
Pantothenic Acid	10 mg
Calcium	200 mg
Phosphorus	48 mg
Iodine	150 µg
Magnesium	100 mg
Zinc	15 mg
Selenium	20 µg
Lutein	250 µg

*Other minerals include copper, nickel, manganese, chromium, molybdenum, chloride, boron, silicon, potassium, and vanadium

B.1.c *Multivitamins and cancer*

PHS II is the first randomized trial to assess the impact of taking a daily multivitamin on the development of cancer. The findings from this ongoing trial in a low-risk population of men will have substantial public health implications, given the high proportion of Americans who take a daily multivitamin. The ingredients of Centrum Silver (American Home Products), the multivitamin used in PHS II, are listed in **Table 1**.

B.1.c.1 Total cancer: While the wealth of epidemiologic data on the inverse association between a diet rich in fruits and vegetables and a reduced risk of cancer have led many to isolate single vitamins or minerals as possible explanations for the observed effects,[89,90] it is also possible that the combined effects of multiple micronutrients at doses near the recommended daily values may explain this association. Certain micronutrients require the presence of adequate cofactors for optimal function. As described earlier, in the Chinese Cancer Prevention Trial, randomization to a daily antioxidant cocktail at doses approximating recommended dietary intakes (ß-carotene, 15 mg;

vitamin E, 30 mg; and selenium, 50 µg) was associated with statistically significant reductions in total mortality, cancer mortality, and gastric cancer mortality after nearly 6 years of treatment and follow-up.[71]

Folate, vitamin B_6, and vitamin B_{12} are essential precursors in cellular metabolism (methylation reactions and DNA synthesis) through their regulation of the transfer and utilization of one-carbon moieties.[91,92] Numerous clinical observations and epidemiological studies (many by our group) support an inverse association between low intake of folic acid, assessed by dietary intake or by measurement of blood or red cell folate levels, and increased risk of colorectal adenoma or cancer.[89,90,93-96] A causal relationship has also been seen in a rodent model of colorectal neoplasia.[97] A recent pooled analysis of data from nine prospective cohort studies in North America and Europe estimated an 11% (95% CI, 2%-19%) reduction in risk of colorectal cancer for every 400 µg/day increase in total folic acid intake.[98] The observational data strongly suggest that higher folic acid intake may reduce the risk of colorectal cancer. Data from randomized clinical trials such as PHS II, which includes folic acid in the multivitamin arm, are needed to confirm these observational results. PHS II provides the opportunity to explore the relationship of multivitamins with colon cancer as well as colon polyps.

Other minerals and vitamins contained in the multivitamin used in PHS II – including selenium, calcium, and vitamin D – also demonstrate promise in the prevention of cancer among usual-risk individuals. Clinical trials suggest that selenium may have a role in the prevention of several site-specific cancers,[99,100] and mounting epidemiologic evidence support roles for calcium and vitamin D, especially in the prevention of colon cancer.[101-103]

The widespread use of multivitamins and the billions of dollars spent on them annually make the question of benefit as well as risk timely and important. The PHS II will provide the only large-scale trial data on this question. No other trials are testing a multivitamin for the prevention of chronic disease and it is not likely that this trial will be repeated.

B.2 Cardiovascular Disease

Basic research has demonstrated plausible mechanisms by which antioxidants may prevent or retard atherogenesis,[104-106] and numerous observational epidemiologic studies, including many conducted among those at usual risk of cardiovascular disease (CVD), support the hypothesis that individuals with high intakes of various antioxidants have a decreased risk of CVD.[59,79,107-118] In sharp contrast to these data, however, are largely negative results from completed randomized trials among those at elevated risk of CVD. At present, only two long-term randomized trials of vitamin E, vitamin C, or multivitamins among individuals at usual CVD risk have been completed, and the interpretation of results from these trials is problematic.

Given that CVD is currently the leading cause of death in the US and most developed countries,[119] even a small reduction in CVD risk among those at usual risk due to use of one or more of these agents could have a substantial impact on public health.

B.2.a *Vitamin E and important vascular events*

The hypothesis that vitamin E (and other antioxidant vitamins) may reduce the risk of CVD has been supported by findings from basic research and observational epidemiologic studies. In laboratory research, vitamin E and other antioxidants prevent tissue damage by trapping organic free radicals and/or deactivating excited oxygen molecules.[12,19,120] Such activity may slow or prevent atherosclerotic plaque formation by inhibiting oxidation of low-density lipoprotein cholesterol (LDL) and thus protecting the vascular wall from oxidized LDL and other cytotoxic oxidative products.[121] Vitamin E may also modify platelet activity,[122-124] reduce thrombotic potential,[125] and modify vascular reactivity[126-128] via antioxidant-related modifications in prostaglandin metabolism and nitric oxide production.

The vast majority of observational data support a role of high levels of either supplemental or dietary vitamin E in the reduction of CVD. These epidemiologic studies have been conducted in usual-

risk populations of men and women, similar to the population of PHS II, whereas many clinical trials of vitamin E and CVD have been conducted in high-risk populations with existing CVD or multiple risk factors. In the Health Professionals' Follow-up Study, among 39,910 apparently healthy men followed for 4 years, use of vitamin E supplements was associated with a statistically significant 36% reduction in risk of coronary heart disease (CHD), and there was a suggestion of an inverse association between high dietary intake of vitamin E and CHD.[118] Similar results have been noted in the Nurses' Health Study, in which analyses for the risk of CHD (nonfatal myocardial infarction [MI] and fatal CHD) over an 8-year period suggested a 34% lower risk for women in the highest versus lowest quintile of vitamin E intake (*P* for trend, <0.001).[116] When vitamin E intake was examined separately by source (diet or supplements), the observed association was almost entirely due to supplement use.[118] In the Iowa Women's Health Study, conducted among 34,486 postmenopausal women with no history of CVD, a 62% lower risk of CHD mortality was observed after 7 years of follow-up among women in the highest quintile of dietary vitamin E intake (*P* for trend, 0.004),[129] while use of vitamin E supplements was not associated with a lower risk of CHD death. In the Rotterdam Study, conducted among 4,802 men and women aged 55 to 95 years who were free of CVD at baseline, no association between dietary intake of vitamin E and MI was observed over a 4-year follow-up.[130]

Determining whether the observed benefits are due to vitamin E itself or to other healthy diet or lifestyle factors requires data from large-scale randomized trials. Yet the vast majority of randomized trial data available to date comes from trials conducted among high-risk individuals. Moreover, these data are inconsistent (**Table 2**).

Table 2. Competed trials of vitamin E and CVD among high-risk individuals using clinical endpoints

Trial	Population	Vitamin E Treatment	Results
CHAOS[131]	2002 men and women with atherosclerosis	400 or 800 IU daily; 1.4 years	Nonfatal MI plus cardiovascular death: RR, 0.53 (95% CI, 0.34-0.83)
ATBC[54]	29,133 male smokers aged 50-69	50 mg daily; 6 years	Nonsignificant 5% reduction in incidence of ischemic heart disease and 16% reduction in ischemic stroke
GISSI[132]	11,324 MI survivors of an acute MI	450 IU daily; 3.5 years	Combined endpoint of death, nonfatal MI, and nonfatal stroke: RR, 0.95 (95% CI, 0.86-1.05)
HOPE[133]	9,541 men and women with existing CVD or at high risk	400 IU daily; 5 years	Combined endpoint of MI, stroke, or cardiovascular death: RR, 1.05 (95% CI, 0.95-1.16)
SPACE[134]	196 hemodialysis patients	800 IU daily; 1.5 years	Composite endpoint of MI, ischemic stroke, PVD, and unstable angina: RR 0.46 (95% CI, 0.27-0.78)

MI = myocardial infarction; PVD = peripheral vascular disease

A meta-analysis of completed large-scale trials among individuals at high risk of CVD indicates little effect of vitamin E on risk of MI, stroke, or cardiovascular death.[133] Interpreting these findings in the context of the existing observational data raises several questions. First, it is possible that vitamin E inhibits early atherosclerosis but does not prevent events in those with existing disease. The benefit among those at low or average CVD risk observed in epidemiologic studies may not extend to those at high risk with existing CVD or multiple risk factors. Second, among patients with pre-existing CVD or at high risk of it, the use of statins, ß-blockers, ACE inhibitors, and other prescribed medications may blunt the possible modest effect of vitamin E. Third, the average 5-year duration of treatment in these trials may not have been sufficient to allow a beneficial effect of vitamin E on atherosclerosis to emerge and that the exposure in observational studies represents much longer duration. Finally, it is possible that the observational results could be attributable to confounding.

To date, only two randomized trials of vitamin E in the primary prevention of CVD among usual-risk individuals have been completed. In the Chinese Cancer Prevention Study, participants receiving the combined daily treatment of ß-carotene (15 mg), vitamin E (30 mg), and selenium (50 µg) had a

relative risk of 0.90 (95% CI, 0.76-1.07) for cerebrovascular mortality and 0.91 (95% CI, 0.84-0.99) for total mortality compared with those on placebo.[71] The study design made it impossible to distinguish separate effects for any one supplement and the relevance of these findings to a well-nourished population is unclear. More recently, the Italian Primary Prevention Project examined the effects of low-dose aspirin (100 mg/d) and vitamin E (300 mg/d) among 4,495 men and women (mean age, 64.4 years) with one or more of the following CVD risk factors: hypertension, hypercholesterolemia, diabetes, obesity, family history of premature myocardial infarction, or advanced age. The trial was terminated prematurely after 3.6 years of treatment when "newly available evidence from other trials on the benefit of aspirin in primary prevention was strictly consistent with the results of the second planned interim analysis."[135] After less than 4 years of follow-up, vitamin E had no observable effect on any prespecified endpoint.

In addition to identifying possible benefits of vitamin E in the primary prevention of cardiovascular disease, PHS II will also provide important data regarding the safety of this agent. Although vitamin E appears to be relatively safe with few documented side effects,[136] there was an apparent increase in the risk of hemorrhagic stroke in the vitamin E treatment group in the ATBC trial, with 66 fatal hemorrhagic strokes occurring among subjects assigned to vitamin E compared with 44 among those assigned to placebo ($P<0.05$). This endpoint was not prespecified, and was confined largely to those with elevated blood pressure. In contrast, in the Chinese Cancer Prevention Trial, those assigned to supplemental vitamin E had a nonsignificant reduction in risk of fatal stroke (RR, 0.90; 95% CI, 0.76-1.07) which in this population would have included a large proportion of hemorrhagic strokes. Other large-scale trials of vitamin E, including HOPE and GISSI, have not reported any increased risk of total or hemorrhagic stroke.

Extending PHS II would provide ample power to determine whether individuals at low or usual CVD risk benefit from vitamin E supplementation. PHS II would complement the ongoing Women's Health Study (WHS), which is testing the effects of vitamin E and aspirin in the primary prevention of CVD and cancer among 39,876 female health professionals at usual risk of CVD. The WHS was recently extended for three additional years.

B.2.b *Vitamin C and important vascular events*

Vitamin C is a powerful water-soluble antioxidant. At physiological concentrations, it protects LDL from oxidation and reduces harmful oxidants in the stomach.[2] Its antioxidant role in vivo is, however, unclear. Plasma ascorbic acid concentrations may be low in chronic or acute oxidant states such as in diabetes, in smokers, or following acute pancreatitis or myocardial infarction.

As with the observational studies of vitamin E, those of vitamin C have been conducted in populations at low or usual risk for CVD. For example, among a large cohort of US adults examined in NHANES I, subjects consuming high levels of vitamin C had a significantly lower risk of death from all causes, particularly from CHD, over a 10-year follow-up period.[79] The multivariate-adjusted relative risk was 0.75 (95% CI, 0.53-0.97) in men with the highest versus lowest levels of vitamin C intake (50 mg/d dietary vitamin C plus regular supplements containing vitamin C versus <50 mg/d dietary vitamin C). Data from the Health Professionals Follow-up Study revealed no significant inverse association between either dietary or supplemental vitamin C and stroke, but a more modest inverse association could not be ruled out.[137] A recent study investigating fruit and vegetable consumption in both the Health Professionals Follow-up Study and the Nurses' Health Study found a strong inverse association between intake of green leafy vegetables and vitamin C-rich fruits and vegetables with the risk of CHD.[138]

No large clinical trials of vitamin C for the primary prevention of CVD have been conducted. One small trial of 578 patients admitted to a geriatric hospital found no differences in 6-month mortality among those supplemented with 200 mg of vitamin C daily.[139] No reduction in total and cerebrovascular mortality was observed in the Chinese Cancer Prevention Study among participants

randomized to vitamin C and molybdenum;[71] however, these results may not be generalizable to a well-nourished American population of men at usual risk of CVD.

Results from recent small-scale trials of vitamin C in the secondary prevention of CVD have yielded mixed results. In the Multivitamins and Probucol Study, a combination of vitamin C (1000 mg/d), vitamin E (1400 IU/d), and ß-carotene (100 mg/d) had no effect on the rate and severity of restenosis, and vitamin C may have blunted the effects of probucol.[140] In contrast, a reduction in the incidence of restenosis (24% for vitamin C versus 43% for placebo) was recorded in a short-term trial of 500 mg of vitamin C daily following percutaneous transluminal coronary angioplasty in 101 patients.[141]

As mentioned earlier, PHS II is unique in that it is the only trial testing vitamin C at a dose well above the recommended daily intake as a single agent for the primary prevention of CVD in a usual-risk population.

B.2.c *Multivitamins and important vascular events*

It is possible that the combined effects of substances such as vitamin E, vitamin C, and other components of fruits and vegetables may account for reductions in cardiovascular risk associated with diets rich in fruits and vegetables. The multivitamin arm of PHS II represents an intriguing area of research for which no large-scale trial data are available.

Observational data specifically on multivitamin use and CVD are limited. Data from the American Cancer Society Cancer Prevention Study II, a prospective cohort of 1,063,023 adult Americans in 1982-1989, compared cardiovascular mortality for multivitamin users versus nonusers. Among men with no baseline history of CVD who used multivitamins, the age-adjusted relative risk of dying from ischemic heart disease was 0.91 ($P \leq 0.001$), though this was attenuated upon multivariate adjustment.[142] A similar result was observed for women.

In addition to antioxidants, multivitamins such as the Centrum Silver tablets used in PHS II contain three B vitamins that have been hypothesized to play roles in CVD risk reduction – folic acid (400 µg; 100% daily value [DV]), vitamin B_6 (3 mg; 150% DV), and vitamin B_{12} (25 µg; 417% DV). All three are cofactors in the enzymatic pathways of homocysteine metabolism, and deficiencies of any of these three can result in elevated homocysteine levels. Data from cross-sectional and case-control studies show inverse associations between homocysteine levels and blood folate.[143-148] In NHANES III[145] and the Framingham Offspring Study,[149] approximately two thirds of the cases of elevated homocysteine were associated with low plasma folate and, to a lesser extent, vitamin B_{12}. Direct associations between blood levels or dietary intake of folic acid, vitamin B_6, and vitamin B_{12} and risk of CVD have also been demonstrated in several cohorts. As with homocysteine and CVD, however, the data are not entirely consistent. For example, in the Nurses' Health Study the relative risk of CHD among women in the highest quintile of folic acid intake (median, 696 µg/d) compared with those in the lowest quintile (median, 158 µg/d) was 0.69 (95% CI, 0.55-0.87) after adjusting for CHD risk factors.[150] In comparison, no association was observed between plasma folate and CHD incidence in the Atherosclerosis Risk in Communities Study after adjusting for CVD risk factors, while a strong inverse association was observed for plasma pyridoxal 5'-phosphate (vitamin B_6).[151]

Vitamin B_6 may also lower plasma homocysteine levels. In one recent study, 120 mg/d of vitamin B_6 for 5 weeks lowered plasma homocysteine by 17% among healthy subjects, nearly as much as did 300 µg/d of folic acid,[152] and a combination of folic acid and vitamin B_6 lowered homocysteine almost twice as much as either supplement alone. The primary role of vitamin B_6, though, is to reduce abnormal homocysteine responses after a methionine load, which are more common in patients with CVD.[153-155] Vitamin B_{12} also lowers homocysteine levels to some degree even in those without a vitamin B_{12} deficiency,[156] and may potentiate the homocysteine-lowering effects of folic acid.[157] In a meta-analysis, vitamin B_{12} (mean 0.5 mg daily) produced an additional 7% (95% CI, 3%-10%) reduction in blood homocysteine above that seen with folic acid alone.[158]

Promising results have been reported from small studies evaluating the impact of supplementation

with these B vitamins on measures of subclinical disease. Supplementation with folic acid and B vitamin combinations improved measures of endothelial function in patients with CHD[159,160] and familial hypercholesterolemia,[161] decreased the rate of developing an abnormal exercise test among healthy siblings of patients with premature atherosclerosis,[162] and reversed the progression of atherosclerosis.[163,164] These effects could be due to the homocysteine-lowering properties of these agents or to a direct effect of the vitamins themselves on endothelial function and/or the progression of atherosclerosis.

While several trials are currently evaluating the effect of B vitamin supplements in populations at high risk of CVD, PHS II represents the only large-scale study testing a multivitamin in the primary prevention of cardiovascular events in any population.

B.3 Eye Diseases: Cataract and Age-Related Macular Degeneration

Cataract and age-related macular degeneration (AMD) are the leading causes of visual impairment in older Americans.[165-167] More than 50% of those aged ≥75 years suffer from visually significant cataract,[168] and 25% of those ≥65 years have some manifestation of AMD.[167] Basic science and animal studies have shown that reactive species of oxygen, generated through photooxidation and normal metabolic processes, likely contribute to degenerative changes in the lens[169-171] and retina[172-174] of the eye. Supplementation studies in animal models support a protective role for antioxidant vitamins in cataract[175-179] and AMD.[180,181] In humans, observational epidemiologic studies also generally support a link between antioxidant intake and risk of these age-related eye diseases, although the data for individual nutrients are often conflicting.

B.3.a *Vitamin E, vitamin C, and multivitamins and age-related macular degeneration*

Blood-based cross-sectional and case-control studies tend to support a beneficial effect of vitamin E in reducing risks of AMD.[182-189] Results of two dietary-based cross-sectional and case-control studies, however, are less supportive.[190,191] For vitamin C, data from cross-sectional and case-control studies provide little evidence for a possible beneficial effect of higher dietary or plasma levels in lowering risks of AMD.[192-198]

Two prospective observational studies have explored the association between antioxidant vitamins and risk of AMD. In the Beaver Dam Eye Study, conducted among 1,709 participants, past intake of vitamin E was associated with a significant 60% reduction in the 5-year incidence of large drusen (early AMD).[199] Current and past intake of pro-vitamin A carotenoids were also associated with reduced risk, while vitamin C intake was not. In PHS I, we examined the association between self-selection for antioxidant vitamin supplement use and incidence of AMD among the 21,120 participants who did not have a diagnosis of AMD at baseline.[200] A total of 279 new cases of AMD with vision loss to 20/30 or worse were confirmed during an average of 12.5 years of follow-up. Compared to non-users of vitamin supplements, men who reported taking vitamin E supplements at baseline had a nonsignificant 13% reduced risk of AMD, and users of multivitamins had a nonsignificant 10% reduced risk of AMD, after adjustment for other AMD risk factors.[201] No reduction in risk was observed for users of vitamin C supplements.

Data from the ATBC study indicated no beneficial effect of α-tocopherol (50 mg daily) on the incidence of AMD during six years of treatment and follow-up (RR, 1.13; 95% CI, 0.81-1.59).[202] However, the dose of α-tocopherol (50 mg/day) used in ATBC may have been too low to generate clinical benefits in the eye, and the higher dose used in PHS II (400 IU taken every other day) may be more appropriate in reducing the risk of eye disease.

B.3.b *Vitamin E, vitamin C, and multivitamins and cataract*

Most cross-sectional and case-control studies have reported lower risks of at least one cataract type among individuals with high dietary intake or plasma levels of antioxidant vitamins, in particular

vitamins C and E.[189,195-198,203-215] Data from prospective studies, however, have been inconsistent. In the Nurses' Health Study, among 50,828 women followed for an average of 8 years, a 45% lower rate of cataract surgery was observed among long-term users of vitamin C supplements.[216] This relationship did not, however, persist after longer follow-up.[201] In this population, the risk of cataract surgery was not related to dietary intake of vitamin E[216] or to long-term (≥10 years) supplemental use of vitamin E.[201] In contrast, in the Longitudinal Study of Cataract,[217] the risk of nuclear cataract during 5 years of follow-up was reduced by about 50% among users of vitamin E supplements; no association was observed with vitamin C intake from diet and/or supplements. In the Beaver Dam Eye Study, there was a nonsignificant 30% reduced risk of nuclear cataract after 5 years of follow-up among those with high intake of vitamin E from diet and supplements, but no association between vitamin C intake and risk of nuclear opacities.[218] Data from three blood-based prospective studies also support a beneficial effect for vitamin E.[219-221] In these studies, high plasma level of α-tocopherol was associated with a 40% to 60% reduced risk of cataract.

In PHS I, observational analyses based on the first 5 years of follow-up demonstrated no benefit of supplemental use of vitamin C and/or E alone (excluding multivitamins) on cataract.[222] However, men who reported at baseline that they took only multivitamins had a statistically significant 27% decreased risk of cataract compared to men who used no supplements. Data regarding multivitamin use from other prospective studies have been inconsistent. In the Nurses' Health Study, there was no association of multivitamin intake and risk of cataract extraction in analyses conducted after 8[216] and 12[201] years of follow-up. However, in the Longitudinal Study of Cataract, multivitamin supplement use was associated with a 30% reduced risk of nuclear opacities during 5 years of follow-up.[217] Similarly, in the Beaver Dam Eye Study, the use of multivitamins (or any supplement containing vitamins C or E) for more than 10 years was associated with 30% to 40% reduced rates of nuclear and cortical opacities.[223]

Randomized trial data for antioxidant vitamins and cataract are limited. In the Linxian Cataract Study, end-of-trial eye examinations conducted among a subset of participants with esophageal dysplasia indicated a reduced prevalence of nuclear cataract in the subgroup of participants aged 65 to 74 years who were randomly assigned to vitamin/mineral supplements (including vitamin C (180 mg/day), vitamin E (60 IU/day), and ß-carotene (15 mg/day) compared to those assigned to placebo (OR, 0.57; 95% CI, 0.36-0.90). No significant association was observed among persons aged 45 to 64 years.[224] In the ATBC study, overall results based on 425 cataract surgeries documented during more than 6 years of treatment and follow-up indicated a statistically nonsignificant RR of 0.91 (95% CI, 0.74-1.11) of cataract surgery for those assigned to α-tocopherol.[225]

With the proposed extension, the average 8-year duration of treatment and follow-up will provide useful data to either support or refute these promising earlier findings.

B.4 Declines in cognitive function

B.4.a *Vitamin E and vitamin C and declines in cognitive function*

Brain tissue readily undergoes oxidative damage, and oxygen free radicals are believed to be involved in aging of the brain.[226,227] As important antioxidants and free-radical scavengers, vitamins E and C could protect against oxidative damage in the brain, and thus cognitive decline. A variety of animal studies indicate that vitamin C and vitamin E individually protect brain tissue from oxidation-related damage and decline, and that their combination may be even more effective.[228,229]

Epidemiologic studies of vitamin E or vitamin C and cognitive function are limited. Data on vitamin E come from a clinical trial of 169 Alzheimer's patients given high-dose (2000 IU) vitamin E supplements or placebo. After two years, the treated group demonstrated significant delays in time to death, institutionalization, severe dementia, or loss of the ability to perform activities of daily living,[230] although scores on the Mini-Mental State Examination (MMSE), a common measure of cognitive function, were similar in the vitamin E and placebo groups. These data, while intriguing, do not address

the important issue of how vitamin supplementation may affect earlier stages in the development of dementia. In NHANES III, individuals with the highest levels of serum α-tocopherol had a significantly decreased risk of a low score on two tests of verbal memory (RR, 0.48; 95% CI, 0.34-0.98) compared to those with the lowest levels;[231] no effect was seen for serum ascorbate or β-carotene. While those with the highest levels were largely supplement users, it is impossible to determine whether these serum measurements represent short- or longer-term vitamin status.

In studies of supplement use, Masaki et al[232] observed a significant 20% decrease in the risk of a low cognitive screening score associated with use of either vitamin E or vitamin C supplements 3 years before the interview; long-term use of these vitamins together appeared to be associated with a significant 43% decrease. In contrast, Mendelsohn et al[233] found no relation between use of antioxidant vitamins and performance on 15 cognitive tests. The majority of supplement users in this study, however, were taking multivitamins that generally have low doses of most antioxidants, and there was no information on duration. While observational data suggest that long duration of high doses of antioxidant vitamins may be related to better scores on cognitive tests, these data are generally are not prospective (i.e., cognitive function measured only once), nor do they provide clear evidence regarding which antioxidant vitamins may be most effective.

B.4.b *Multivitamin and declines in cognitive function*

As described in Section B.2.c, accumulating data ascribe to homocysteine a role in the pathology of vascular disease, and intervention studies show that modest amounts of folic acid, vitamin B_6, and vitamin B_{12} reduce homocysteine levels. Recent data indicating shared risk factors for vascular disease and Alzheimer's disease (AD),[234,235] have sparked interest in the relation of homocysteine and folate levels to dementia.[236] By extension, early cognitive decline may also be associated with homocysteine and folate levels.

The limited epidemiologic evidence suggests the possibility of an association between homocysteine/folate levels and cognitive function. Among 30 participants in the Nun Study, low serum folate was correlated with atrophy of the cerebral cortex, especially among those with AD lesions.[237] Higher folic acid intake has been related to a decreased risk of cognitive impairment (defined by score on the MMSE) among 260 elderly subjects.[238] Among 1,171 non-demented subjects aged ≥65 years, better scores on the MMSE and a test of short-term memory were observed in those with high serum folate levels.[239] In the Rotterdam Study, the only prospective evaluation of this association to date, no clear relationship was observed between homocysteine and cognitive decline (OR, 0.91; 95% CI, 0.52-1.58 comparing highest to lowest tertile of total homocysteine).[130] Given that the average follow-up was only 2.7 years and the confidence interval was wide, a modest relation between homocysteine level and cognitive decline cannot be ruled out.

Epidemiologic studies of cognitive decline in non-demented subjects are likely to prove critical in public health terms. The small to modest benefits that may be attributable to use of antioxidant supplements or multivitamins may have substantial impact in delaying the long process of dementia onset. However, since such studies must focus on non-demented subjects, and changes in cognitive function will likely be modest as well as protracted, it is critical to conduct large-scale, long-term trials. We are currently assessing cognitive status using standard tests of cognitive function administered three times over five years among 6,012 men aged 65 years and older in PHS II. The ability to follow these men for an additional 3 years could be invaluable in determining whether antioxidant or multivitamin supplementation protect against cognitive decline and how long a duration of supplement use is necessary.

B.5 Potential for innovative substudies

Given the wealth of health and lifestyle data collected in PHS II and the fact that 76% of PHS II

participants have provided blood samples and 91% have completed detailed food frequency questionnaires, we will have the opportunity to explore whether the effect of any study agent is modified by lifestyle, biochemical and/or genetic factors. We can assess the impact of each agent among individuals with low baseline levels of a given micronutrient or stratify the population by a disease marker. For vitamin E and prostate cancer, we will have the opportunity to explore the effect modification by baseline PSA levels as well as dietary and plasma γ-tocopherol levels.[47] Further, we can explore the mechanistic underpinnings of any findings.

B.6 Summary

Observational studies of the three study agents proposed for continuation in PHS II – vitamin E, vitamin C, and a multivitamin (as Centrum Silver) – indicate that they have promise for the primary prevention of prostate and total cancer, cardiovascular disease, age-related eye disease, and cognitive decline. Data from large-scale randomized trials in populations at usual risk of these chronic diseases, however, are limited or nonexistent. In spite of the lack of definitive data on the benefits and risks of these agents, they are widely used by US adults. Surveys show that more than 1 in 3 adults aged ≥45 years take vitamin E,[240] and 40% or more take individual vitamin C supplements[2,3] or multivitamin supplements.[4] Growing use of these supplements also makes it increasingly difficult to study these agents in randomized trials, as potential subjects are often unwilling to forgo use of these agents.

PHS II will contribute to current knowledge by obtaining either clear evidence of effects for the study agents or truly informative null results. In either case, data from PHS II would influence personal and clinical decision-making as well as public health policy. A definitive null result from the trial would help redirect limited resources to other promising areas of research in the prevention of cancer, cardiovascular disease, age-related eye disease, and cognitive decline.

PHS II will also deliver unique information regarding the prevention of chronic disease. With regard to vitamin E and prostate cancer, only PHS II and SELECT are powered for this endpoint, and the results from both studies will be complementary. With regard to vitamin E and cardiovascular disease, PHS II will complement the Women's Health Study. The ongoing SUpplementation en VItamines et Mineraux AntioXydants (SU.VI.MAX) Study is using a lower dose of vitamin E (30 mg/day) as part of a cocktail that also contains vitamin C, ß-carotene, selenium, and zinc among almost 13,000 French men and women at usual risk for cancer and CVD.[241] Other ongoing trials of vitamin E and CVD prevention, including the Women's Antioxidant Cardiovascular Study (WACS),[242] the Heart Protection Study (HPS),[243] and the HOPE trial,[133] are all being conducted among high-risk populations. For vitamin C and cancer, PHS II is the only study employing high-dose vitamin C among individuals at usual risk – SU.VI.MAX is using a low dose of vitamin C (120 mg/d), and WACS and HPS are not powered for cancer. For vitamin C and cardiovascular disease, PHS II again is the only study employing high-dose vitamin C among individuals at usual risk, with SU.VI.MAX employing a low dose and WACS and HPS focusing on high-risk populations. Finally, PHS II is the only large-scale randomized trial testing a multivitamin in the primary prevention of chronic disease.

Since the costs of recruiting and randomizing 14,642 men into PHS II have already been covered, as well as the costs of conducting an average of 3.7 years of treatment and follow-up, the proposed continuation of the vitamin E, vitamin C, and multivitamin arms of PHS II represents an extremely cost-effective trial considering its size and potential to answer critical questions.

C. PROGRESS REPORT / PRELIMINARY STUDIES

C.1 Introduction and Overview

Physicians' Health Study II was initiated as a randomized, double-blind, placebo-controlled, 2x2x2x2 factorial trial testing the benefits and risks of vitamin E (400 IU of synthetic α-tocopherol or its placebo on alternate days), vitamin C (500 mg synthetic ascorbic acid or its placebo daily), a

multivitamin (Centrum Silver or its placebo daily), and β-carotene (50 mg Lurotin or placebo on alternate days) in the primary prevention of prostate and total cancer, cardiovascular disease (CVD), eye disease, and decline in cognitive function among 14,642 male physicians aged 50 years and older.[244] PHS II built on the experiences gained in PHS I, which was funded jointly by the National Cancer Institute and the National Heart, Lung, and Blood Institute in 1980 as a trial of aspirin and β-carotene in the primary prevention of cancer and CVD.[245,246] Upon the scheduled completion of PHS I in 1995, BASF and American Home Products provided funding to initiate and conduct PHS II through December 2002. This application proposes to extend the vitamin E, vitamin C, and multivitamin components of PHS II for an additional 5 years – 4.5 years of randomized treatment and follow-up and 0.5 years of close-out.

As of August 29, 2001, the average duration of treatment and follow-up was 2.4 years (median follow-up, 2.8 years; range, 0.01-4.0 years). The participants have high rates of compliance and follow-up. Baseline blood samples have been provided by 11,120 (76%) of the randomized men, and detailed dietary questionnaires were completed by 13,324 (91%) subjects. The details of the trial's design and conduct to date are given below.

C.2 Enrollment, Run-in, and Randomization

Recruitment and enrollment for PHS II were conducted in two phases. In Phase 1, beginning in July 1997, all living participants in PHS I who had not previously indicated they were unwilling to participate in future studies (n=18,763) were sent a detailed letter explaining the background and rationale for PHS II. This initial mailing also contained an informed consent form and a questionnaire (**Appendix A**) requesting information on the occurrence of relevant endpoints since the previous PHS I questionnaire, as well as use of various vitamin supplements over the past 12 months. Participants who indicated they were currently taking individual supplements containing more than 100% of the RDA of vitamin E, vitamin C, ß-carotene, or vitamin A, or a multivitamin were asked if they would forego the use of such supplements for the course of the PHS II trial. Physicians unwilling to avoid using these outside supplements were ineligible for PHS II, as were men who reported a history of cirrhosis or active liver disease and those who were on anticoagulants. As a result of these inclusion and exclusion criteria, 7,641 (41%) participants from PHS I were randomized into PHS II.

Phase 2 of recruitment for PHS II began in the summer of 1999 with the mailing of invitational letters and baseline questionnaires to 254,597 U.S. male physicians aged 50 years and older (excluding physicians who participated in PHS I) identified from a list provided by the American Medical Association. Between July 1999 and July 2001, 42,150 men returned questionnaires. Of these, 16,743 indicated they were willing to participate in PHS II, of whom 11,128 were eligible, e.g., 50 years of age or older; not currently taking anticoagulants; no previous history of liver disease or other serious illnesses that might preclude participation; and willing to avoid the use of non-study vitamin and multivitamin supplements.

As was done in PHS I, the 11,128 potentially willing and eligible physicians recruited into PHS II during Phase 2 of enrollment began a 12 week pre-randomization run-in phase to assess their willingness and ability to comply with the daily pill-taking regimen. During Phase 1, PHS I participants who subsequently enrolled into PHS II did not undergo a second run-in phase since they had, on average, demonstrated high compliance after 12 years. During the run-in, Phase 2 subjects received calendar packs containing placebos of vitamin E, vitamin C, a multivitamin, and β-carotene. Of the 11,128 physicians who entered the run-in phase, 7,001 (63%) who complied with the pill-taking regimen at least two-thirds of the time and remained willing and eligible to participate were then randomized into PHS II. The pilot studies for PHS I demonstrated that most non-compliers become evident during the first several months of participation.[247] Thus, the run-in helped to assure that nearly all participants randomized in PHS II will be excellent compliers, greatly increasing the efficiency of the trial to definitively test the hypotheses under study.[248]

Starting in August 1997, a total of 14,642 men (7,641 physicians from PHS I in Phase 1 plus 7,001 from Phase 2) were randomized in a 2x2x2x2 factorial design stratified by age and in blocks of 16, to vitamin E, vitamin C, a multivitamin, and ß-carotene or their placebos. PHS I participants who subsequently enrolled in PHS II retained their original β-carotene treatment assignment. Since the randomization process yielded equal distribution of lifestyle and clinical risk factors across the three treatment groups (**Table 3**; data as of last DSMB meeting on March 22, 2001), this provides reassuring evidence that unmeasured or unknown potential confounders are also equally distributed.

Table 3. Baseline characteristics of PHS II participants by treatment as of 3/22/01.*

	Vitamin E		Vitamin C		Multivitamin	
Characteristic	**Vit E**	**Plac**	**Vit C**	**Plac**	**MV**	**Plac**
N	7296	7301	7306	7291	7298	7299
Age (years)	64.3	64.3	64.3	64.3	64.3	64.3
Body mass index (kg/m^2)	26.0	26.0	26.0	26.0	26.0	26.0
Systolic BP (mmHg)	128.1	128.1	128.0	128.1	128.0	128.0
Total cholesterol (mg/dL)	196.5	196.1	196.2	196.4	196.2	196.5
Diabetes mellitus (%)	6.2	5.9	5.9	6.2	6.2	5.8
Myocardial infarction (%)	3.4	3.4	3.4	3.4	3.4	3.4
Cancer (non-skin) (%)	8.8	8.7	8.8	8.7	8.7	8.8
Benign prostatic hyperplasia (%)	26.7	27.1	27.1	26.8	27.0	26.9
Cataract (%)	18.2	18.6	18.3	18.5	18.4	18.4

** All p-values > 0.05. No significant differences noted for ß-carotene versus placebo.*

C.3 Gender and Minority Inclusion

Because prostate cancer was one of the primary endpoints in PHS II, the PHS II study population was confined to men. Complementary information on other endpoints in women at usual risk is being obtained from our ongoing Women's Health Study (CA 47988, HL 43851, NS 34108, AG 15933), which is testing the primary prevention effects of vitamin E and aspirin among 39,876 female health professionals, as well as the Women's Antioxidant Cardiovascular Study (HL 46959, AG 15933), which is testing the secondary prevention effects of vitamin E, vitamin C, folic acid/vitamin B_6/vitamin B_{12}, and ß-carotene among 8,171 women with pre-existing CVD or with three or more risk factors. With regard to minority inclusion, the PHS I participants randomized into PHS II were predominantly white (92.5% of participants), which represented the ethnic distribution of physicians aged over 40 years in 1980. Of the 7,001 new physicians recruited and randomized into PHS II, 12% are members of minority ethnic/racial groups: 7.2% Asian/Pacific Islander, 2.5% Hispanic, 1.1% Black, and 1.2% other. Thus overall in PHS II, 9.4% of men are members of minority ethnic/racial groups: 5.1% Asian/Pacific Islander, 2.4% Hispanic, 1.1% Black, 0.03% American Indian/Alaskan Native, and 0.7% other.

C.4 Blood Collection

Baseline blood collection for PHS II occurred in two phases. First, in Phase 1 all physicians derived from PHS I were sent blood collection kits prior to randomization in PHS II. Second, in Phase 2 new physicians recruited for PHS II were sent blood collection kits during the run-in phase if they indicated on their baseline questionnaire a willingness to provide an optional venous blood sample. Using procedures developed for PHS I, blood collection kits included instructions to have blood drawn into 3 EDTA and 3 citrate tubes that were provided along with supplies needed to draw a blood sample, a gel-filled freezer pack, and a completed overnight courier air bill. Participants recorded the time of venipuncture and the time of their last meal. The specimens were sent to our blood laboratory in the freezer packs within 24 hours of the blood draw. Upon receipt, the samples were centrifuged to separate plasma, red blood cells, and buffy coat, and aliquoted into twelve 2 mL Nunc vials (6 EDTA plasma, 3 citrate plasma, 1 EDTA red blood cell, and 1 EDTA and 1 citrate buffy coat). A computer printed labels for the vials and assigned 12 locations across 3 separate nitrogen freezers (maintained at -170 ºC) for security. The entire process was completed within several hours of receipt of the specimens to ensure that samples were frozen within 30 to 36 hours after venipuncture. Each freezer has a back-up power system that is connected to an electronic alarm that has protected earlier blood samples from inadvertent thawing or warming for nearly 20 years. Of the 14,642 men randomized in

the trial, 11,120 (76%) provided a baseline blood sample.

The primary purpose of collecting prerandomization blood specimens is to provide the opportunity to assess whether treatment effects for any of the agents are modified by baseline plasma levels of the respective study agents. In addition, effect modification by other micronutrients or disease markers can also be explored. This resource is also extremely valuable for exploring other genetic and biochemical hypotheses in this well characterized trial cohort. A nested case-control approach will be used to assess the relationship of blood-based markers and risk of disease endpoints, enabling us to explore a number of promising biochemical and genetic markers (e.g. plasma antioxidants and carotenoids, fatty acids, plasma hormone levels, and prostate specific antigen levels) at very low incremental cost.

C.5 Dietary Assessment

A self-administered food frequency questionnaire (FFQ) (**Appendix A**) was mailed to all PHS II participants in two phases. First, physicians from PHS I who continued on to PHS II were sent FFQs in April 2000. Second, new physicians recruited for PHS II were sent FFQs during the run-in phase of the trial prior to randomization. Overall, of the 14,642 randomized men, 13,324 (91%) have completed and returned the FFQ. This questionnaire, developed by Walter Willett, MD, DrPH, and colleagues, is an efficient, reliable, and accurate instrument for categorizing individuals according to their intake of 32 nutrients, including vitamin E, vitamin C, and folic acid.[249-251] Data from the FFQ will be used to categorize the participants according to their baseline intake of various nutrients and then to evaluate whether the effect of each randomized treatment varies according to baseline dietary intake.

C.6 Treatment and Follow-up

Once a year, participants receive their supply of study pills in calendar packs containing active or placebo tablets and capsules. This shipment is accompanied by an annual follow-up questionnaire. Follow-up annual questionnaires include questions about compliance with the study treatments, use of non-study medications, occurrence of major illnesses or adverse effects, and other risk factor information (**Appendix A**). Because the randomization process occurred in two stages, median follow-up is longer for the original PHS I subjects (3.7 years) than for newly randomized participants (1.2 years). Overall, as of August 29, 2001, the average length of treatment and follow-up was 2.4 years, with a range of 0.01 to 4.0 years.

Participants not returning follow-up questionnaires within 5 to 6 weeks of the initial mailing are sent a second follow-up questionnaire. Continued nonrespondents receive a third and fourth written request followed by a telephone call to obtain follow-up information. For the small minority of men not providing questionnaire information by mail or by phone, vital status is ascertained.

As of August 29, 2001, four follow-up phases (12-month, 24-month, 36-month, and 48-month) are ongoing among the 14,642 randomized men. Since the physicians newly recruited into PHS II have less follow-up time, follow-up rates largely reflect those of physicians recruited from PHS I: 12-month questionnaires have been returned by 98.6% of participants; 24-month questionnaires by 98.8%; and 36-month questionnaires by 98.9% of those who have reached this stage of the trial. The 48-month questionnaires are currently being mailed to physicians recruited from PHS I, and we are currently mailing and processing 24-month questionnaires to physicians newly recruited into PHS II.

At the 6-month midpoint between annual questionnaires, participants are sent a letter and postage-paid postcard. This card, sent primarily to keep in touch with participants, asks them to return it only if they have been newly diagnosed with cancer or cardiovascular disease, are having compliance problems, or need to report a change of address. Approximately 15% of the study cohort responds to this optional interim follow-up.

C.7 Compliance Rates

The primary measure of compliance in PHS II is self-reported adherence to pill taking reported on

the follow-up questionnaires. Compliance is defined as taking at least two thirds of the study pills. On each annual questionnaire, participants are asked to report whether they are currently taking their assigned regimen and the percentage of study pills taken during the previous year. Another aspect of compliance is whether or not participants avoid outside use of the study agents, defined as use >30 days/year. In the study guidelines that are sent with each annual mailing, participants are instructed to restrict their use of individual supplements of vitamin E, vitamin C, multivitamins, ß-carotene, and vitamin A preparations. Participants whose outside use of the agents exceeds these guidelines are contacted by phone or personal letter to remind them of the study guidelines. Summary data as of August 29, 2001 regarding pill compliance and avoidance of outside supplementation are detailed in **Table 4**. It is important to note that compliance with the study agents in PHS II tends to follow an L-shaped pattern, as demonstrated for Phase 1 physicians in **Table 4**. After an initial drop in compliance during the first year of the trial, it levels off for the remainder of follow-up with losses of about 1% per year in subsequent years.[252] As indicated above by 1-year rates, we anticipate slightly different compliance for Phases 1 and 2 physicians during PHS II treatment and follow-up.

Table 4. Pill compliance and avoidance of outside use in PHS II*

	Phase 1 physicians (from PHS I)		Phase 2 physicians (newly recruited)
Median follow-up	1.0 year	3.7 years	1.2 years
Vitamin E			
On treatment	85.4%	84.6%	92.7%
Avoidance of outside use	91.6%	91.2%	98.5%
Vitamin C			
On treatment	85.5%	84.7%	92.7%
Avoidance of outside use	94.9%	94.8%	98.9%
Multivitamin			
On treatment	85.2%	84.1%	92.4%
Avoidance of outside use	89.0%	88.1%	96.0%

* Rates for β-carotene are comparable to other agents.

Previously in PHS I, we reported findings supporting the validity of the self-reported compliance by measuring serum thromboxane B_2, which is decreased after aspirin use, and plasma β-carotene in study participants from three geographic locations in three different time periods. Thromboxane B_2 levels were markedly lower in those assigned to aspirin than in those given aspirin placebo ($P<0.0001$). Similarly, those assigned to β-carotene had significantly higher levels than those given placebo ($P<0.0001$). There was also a strong positive correlation between levels of these biochemical markers and self-reports of compliance ($r=0.65$ for thromboxane B_2 and $r=0.69$ for β-carotene, both $P<0.0001$). During PHS II, we will obtain blood samples during visits to a new sample of participants living in the Greater Boston area who are randomly selected from a list of those recently returning a questionnaire reporting compliance data, to verify self reported compliance for taking vitamin E, vitamin C, and a multivitamin.

The efforts made by the study staff to maintain personal contact with the trial participants, in spite of the large size of the study cohort, is a critical factor in maintaining compliance and follow-up rates. The small number of nonrespondents to annual follow-up questionnaires are called to obtain questionnaire data by phone and to directly discuss compliance issues. Problematic questionnaires, letters, or telephone calls received from participants are reviewed and followed up with a personal letter or phone call from the research staff. In addition, participants are encouraged to call the PHS II office toll-free at any time if they have questions regarding their participation. Each week an average of 163 letters and 114 phone contacts are made with participants. These efforts encourage good compliance, reinforce study guidelines, and help collect missing data not provided on the follow-up questionnaires. To keep participants informed of the progress of the trial, newsletters about the trial are mailed periodically to all participants (**Appendix B**). These newsletters emphasize issues such as the importance of compliance, the role of the DSMB, and the publication of findings from relevant studies.

C.8 Endpoint Ascertainment

Nonfatal endpoints are based on self-reports from follow-up questionnaires, letters, or telephone calls. For each reported cancer, CVD, or eye disease endpoint, we request permission from the participant to examine relevant medical records. Once the consent form is obtained, records are requested from the hospital or attending physician. For fatal endpoints, when a report of a deceased participant is received, a letter of condolence is mailed to the next of kin and permission is requested to obtain medical records. Copies of death certificates are obtained from the next of kin or from the state vital records bureaus in which the participant died. Additional records, such as autopsy reports, are requested as needed. At the end of the study, a search of the National Death Index will be made for any randomized participants for whom vital status is unknown.

An Endpoints Committee consisting of four physicians with expertise in cardiology, oncology, and neurology regularly reviews the medical records for final confirmation of a reported diagnosis. The Endpoints Committee is blinded to the participants' randomized treatment assignments. In brief, a diagnosis of cancer is confirmed using histologic or cytologic evidence. In the absence of these diagnostic tests, strong clinical evidence accompanied by radiologic evidence or laboratory markers (e.g. PSA levels) is used to confirm cancer occurrence. Nonfatal myocardial infarction is confirmed using World Health Organization criteria.[253] Nonfatal stroke is defined as a typical neurologic deficit, either sudden or rapid in onset, lasting more than 24 hours and attributed to a cerebrovascular event. Death due to a cardiovascular cause is confirmed by convincing evidence of a cardiovascular event from all available sources, including death certificates, hospital records, and observers' accounts (for deaths occurring outside the hospital).

Table 5 provides the numbers of self-reported cancers (including total, prostate, and colorectal) and cardiovascular primary endpoints as of August 29, 2001. Self-reported cases are classified as confirmed if they have been reviewed by the Endpoints Committee and fulfilled the necessary confirmation criteria. When consent is not provided or the records cannot be obtained, the reported endpoint is not considered confirmed. Self-reported endpoints are classified as unrefuted if they have been confirmed by the Endpoints Committee or if they have not yet been reviewed (i.e., self-reported endpoints that have been reviewed by the Endpoints Committee and disconfirmed are considered refuted). Both confirmed and unrefuted endpoints will be reported in final analyses. To date, medical records have been obtained and reviewed by the Endpoints Committee for approximately 73% of self-reported endpoints; the majority of the remaining 27% are in progress. The confirmation rates among the records reviewed and completed thus far include 89% for myocardial infarction, 78% for stroke, 90% for cancer, and 100% for mortality. Of those not confirmed, most subjects had a related diagnosis, such as angina, transient ischemic attack, or nonmelanoma skin cancer.

Table 5. Unrefuted and confirmed primary endpoints as of 8/29/01

Outcome	Confirmed	Unrefuted
Total cancer	310	443
Prostate cancer	158	212
Colorectal cancer	28	41
Cancer death	80	110
Important vascular events	169	274
Myocardial infarction	83	139
Stroke	56	113
Ischemic	52	--
Hemorrhagic	4	--
Cardiovascular death	68	113

We also ascertain colon polyps as an endpoint in PHS II, based upon self-reports that are confirmed by review of histopathologic reports. This parallels the approach used by colleagues at the Health Professionals Follow-up Study and the Nurses' Health Study.[254] Adenomas are classified as either small (<1 cm diameter) or large (≥1 cm diameter).[254] Only polyps from the initial work-up are considered.

The review of all eye endpoints is coordinated by PHS II Co-Investigator Dr. William Christen. The treating ophthalmologist or optometrist is requested to complete a questionnaire providing information

about the reported diagnosis. Cataract is defined as confirmed age-related lens opacity sufficient to reduce best-corrected visual acuity to 20/30 or worse. Macular degeneration is defined as a confirmed self-report associated with a best-corrected visual acuity of 20/30 or worse due to age-related macular degeneration.

Declines in cognitive function are determined by short telephone interviews among the 6,012 PHS II participants who were aged 65 years and older at baseline. During the interview we administer the Telephone Interview for Cognitive Status (TICS), a telephone version of the Mini-Mental State Examination, as well as additional tests that measure other aspects of cognition. The East Boston Memory Test is given at the start of the interview and again at the end to test immediate and delayed verbal recall of a short paragraph. We administer a test of category fluency in which the men are asked to name as many animals as they can during one minute, plus a delayed recall of the TICS 10-word list.

Baseline cognitive testing in PHS II participants was extremely successful, with 98% participation. In addition, scores on the tests administered demonstrated a broad distribution, ensuring that we will have sufficient ability to examine our hypotheses. This aspect of the trial is currently funded by NIA for follow-up through June 30, 2004 for a total of three cognitive assessments. This proposal, if funded, would allow for an additional 3.5 years of follow-up so that all subjects will be exposed to the study agents for at least 5 years at the final cognitive assessment.

C.9 Substudies and Previous Work

The availability of detailed baseline and follow-up data, the extensive repository of blood samples representing 76% of participants, and the dietary assessment on 91% of participants represent a rich and valuable resource for the investigation of other important and timely hypotheses. For some analyses, PHS I and PHS II cohorts are combined, yielding a well-characterized group of more than 30,000 older men. To take advantage of this database, numerous substudies of the PHS II have been initiated, funded by the NIH, foundations, and industry sponsors. These include use of specific antihypertensive medications and risk of cardiovascular disease; β-carotene and non-melanoma skin cancer; nutritional, biochemical, and genetic markers of cancer; dietary and biochemical markers of prostate cancer risk; modifiable risk factors for sudden death; physicians' awareness of cholesterol levels; the development of a cardiovascular policy model; plasma lycopene and CVD; infection, inflammation, and risk of CVD; molecular and genetic epidemiology of CVD; an epidemiologic study of dry eye syndrome; and lifestyle and biochemical markers and the risk of hypertension, diabetes mellitus, and decline in renal function. Substudies using data obtained in PHS I have resulted in more than 166 publications to date (**Appendix C**).

C.10 Summary

A total of 14,642 men have been randomized in a 2x2x2x2 factorial design to vitamin E, vitamin C, a multivitamin, and β-carotene, or their placebos. After an average duration of treatment and follow-up of 2.4 years, compliance and follow-up rates are high. Based on the unblinded data, the DSMB unanimously recommended continuation of the trial beyond the scheduled termination of industry funding in December 2002. Because the added power gained by continuation of β-carotene treatment will be incrementally small, we plan to terminate this component, thereby simplifying the regimen for participants. As a result, the proposed additional 5 years of the trial (4.5 years of treatment and follow-up and 0.5 years of trial close-out) will be a 2x2x2 factorial trial of vitamin E, vitamin C, and a multivitamin, and will provide a unique opportunity to answer a number of vital questions for men in the primary prevention of prostate and total cancer, CVD, eye disease, and declines in cognitive function.

D. RESEARCH DESIGN AND METHODS

D.1 Overview

The study methods for the proposed continuation of PHS II for an additional 5 years (4.5 years of randomized treatment and follow-up and 0.5 years for trial close-out) will match those of the ongoing trial, with the primary goal of maintaining high compliance and follow-up among the 14,642 randomized men (**Table 6** on the following page). BASF has supplied the active vitamin E and placebo, and vitamin C and placebo for PHS II, and will continue supplying these agents during the proposed continuation. American Home Products has supplied the multivitamin and placebo (Centrum Silver) for PHS II and will continue to do so for the proposed continuation.

Table 6. Timeline for the Physicians' Health Study II.

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
	Current Funding						*Proposed Continuation*				
PHS II Recruitment:											
Phase 1											
Phase 2											
PHS II treatment and FU											
Trial closeout											

D.2 Continuation of Follow-up

If funding is approved for the continuation of PHS II, each participant will be sent a personal letter explaining the overall reasons behind the DSMB's recommendation for continuation, informing them of the approved funding by the NIH, informing them of the termination of β-carotene as of December 31, 2002, and asking for their continued collaboration. In our experience with PHS I and the WHS, termination of a single agent and the resultant changes in the calendar packs had no material impact on compliance. Calendar packs of the randomized trial treatments will continue to be sent to each participant unless they indicate otherwise. For non-compliers, follow-up information will continue to be assessed by questionnaire, telephone, or the National Death Index.

For follow-up we will rely on the highly successful methods developed for PHS I that have continued to be effective in PHS II. Specifically, every year randomized participants will be sent a 12-month supply of calendar packs containing their study pills, as well as a follow-up questionnaire asking about compliance, adverse effects to study agents, endpoints of interest, and risk factors. At the 6-month midpoint between annual questionnaires, participants will be sent a letter and postcard. The aim of this mailing is to maintain frequent contact with participants without overburdening them.

To track study participants, computer files generated every week will identify participants due to receive an annual follow-up questionnaire or interim postcard. These listings differentiate between first, second, third, or fourth requests. Computer listings will also be generated on a weekly basis for men scheduled to receive telephone calls from senior research assistants regarding nonresponse to a scheduled follow-up.

All returned questionnaires, postcards, and written or telephone correspondence will be thoroughly reviewed by senior research assistants for problems with participation or newly reported endpoints. The PHS II has a toll-free telephone number that participants may call at any time and speak with a senior research assistant during office hours or leave a message after office hours. In addition, two study physicians are available by page 24 hours a day for medical emergencies related to study participation.

D.3 Assessment of Compliance

The primary assessment of compliance will continue to be self-reported data on the annual follow-up questionnaires, which ask about compliance with the randomized study treatments and outside use

of nonstudy medications. Historically, physicians taking part in PHS I have demonstrated consistently high compliance with their treatment regimens (see Section C.7).[252,255] In order to validate such self-reports, visits will be made in year 2 of this proposed continuation to a random sample of participants living in the Greater Boston area, all of whom had recently returned a questionnaire self-reporting their level of compliance. These participants will be asked to provide a blood specimen during the visit. Their questionnaire data will be correlated with biochemical markers of vitamin E, vitamin C, and key components of the multivitamin.

D.4 Assessment of Endpoints

Endpoints will continue to be ascertained and confirmed as described in Section C.8. When a nonfatal endpoint is reported, signed permission will be requested to obtain medical records, and these will be reviewed by the blinded Endpoints Committee. When deaths are reported, death certificates and other relevant records will be obtained and reviewed. Cognitive function will also continue to be assessed as previously described.

D.5 Study Close-out

At the conclusion of randomized intervention on June 30, 2007, final study data will be collected from all participants. To facilitate the return of the most critical endpoint information, this follow-up will take place in two stages. First, participants will be mailed a brief form asking about recent diagnoses of cancer, cardiovascular disease, and eye disease. Following the return of this form, participants will then be asked to complete a longer questionnaire updating risk factor and dietary information. Cognitive function testing will continue until unblinding. Participants will also be mailed a letter and certificate thanking them for their dedicated participation. This letter will include unblinded information about the participant's randomized assignments.

D.6 Data Management

Because of the large number of men returning questionnaires, the fact that PHS II participants are being followed solely by mail, and the multiple phases of follow-up ongoing simultaneously, our computing system is a critical feature of effective follow-up. The system we have developed tracks each participant's stage in the study, his trial experience and level of participation, and generates letters and questionnaires at the correct times. Name, address, telephone numbers, current status, and processing information are kept up to date, and data from questionnaires as well as information from communications such as letters and phone calls are entered into the study database. When talking with a participant, study personnel need ready access to identifying information, trial experience, and level of participation. However, it is also crucial that these data be available only to authorized staff members. The systems already developed and fine-tuned under the current grant will be used in the proposed continuation.

Data entry for PHS II has been performed using formatted screens with built-in error checking. Using a double-entry system, each questionnaire is entered by two different data entry clerks who are also responsible for checking the coding on the form. The two resulting files are compared using an interactive verification program that allows for immediate correction of any discrepancies. Before being added to the database, the data undergo more sophisticated within-form and across-time checks to verify their completeness and accuracy as well as to ensure they are being assigned to the correct participant. Any errors in coding or keying are corrected immediately. Questionable data that need more extensive review are sent to a separate file and then resubmitted to the validation program after resolution of the problems.

Endpoint data and the data from each follow-up questionnaire are stored in individual data files. A master processing file is maintained separately. This file contains all the personal information necessary for scheduling follow-up mailings and telephone calls. It includes current name, address,

and telephone numbers, treatment group assignment, current status in the trial (i.e. whether taking each of the study agents), codes regarding the response to each mailed questionnaire, and the date questionnaires were returned. The master processing file also contains information regarding endpoints, events, and previous interactions with the participants. This structure allows for flexibility when creating analysis datasets while safeguarding the participants' identities. Nightly backups of all data files are made. This ensures that there are two copies of all data files at all times. Each month a set of all data files is taken off site and permanently stored.

D.7 Data Analysis

The design of this proposed continuation of PHS II is a 2x2x2 factorial trial of three study agents. Primary analyses of treatment effects will be based on the intent-to-treat principle with participants classified according to their randomized treatment assignment. Previous analyses have shown that the extensive baseline characteristics that were collected, including demographic, lifestyle, and health history variables, are equally distributed among the treatment groups (see Section C.2, **Table 3**).

Initial analyses of primary endpoints will include simple contingency table displays in which the rate of each endpoint in terms of number of events per person-year among the participants allocated to active treatment will be compared with that among those allocated to placebo. Specifically, the incidence rate for an endpoint among those assigned to an active agent will be compared with the corresponding rate among the participants allocated to placebo, controlling by stratification for assignment to the other agents. Proportional hazards regression models will be used to estimate the hazard ratio over the entire randomized period, allowing for variable lengths of follow-up.[256] Relative hazards will be estimated for each independent arm of the factorial trial using indicators for treatment exposure. Additional proportional hazards analyses will include both other randomized treatments and baseline covariates. Similar analyses will be conducted for each of the secondary endpoints.

Primary analyses will consider incident events. Participants with prior cancer, other than non-melanoma skin cancer, will not be considered in primary analyses of this endpoint and those with prior major cardiovascular disease will not be considered in the primary analysis of this endpoint. Thus, the primary analyses of both total incident cancer and total prostate cancer will consider the 13,367 randomized participants who were free of cancer at the time of randomization into PHS II; and the primary analyses of cardiovascular disease endpoints will consider the 13,887 participants without prior myocardial infarction or stroke at the time of randomization. Secondary analyses will consider time to the first event after randomization, stratified on the number of prior events that a participant has, and including all randomized subjects.

Beyond these analyses, we will also examine effects of combinations of treatments. Specifically, we will examine the hazard ratios for a combination of two of these active agents, as well as combinations of three active agents, versus the placebos for these agents, testing the specific contrasts of interest using appropriate indicator variables. If effects are indeed additive, the estimated risk reductions in these combined groups should be larger than those for a single agent. We will also compare hazard ratios for a combination of two agents versus a single agent, again using indicator terms with the appropriate reference group. We will include multiplicative interaction terms in proportional hazards models to explore other possible interactions, including interactions between multivitamins and single supplements of vitamins E or C. It should be noted, however, that for the principal analyses, the main effects of each agent will be the most important, as the multivitamin contains low levels of the other agents (in the range of the recommended daily value) compared to the individual supplements which provide average daily vitamin E at 667% and daily vitamin C at 833% of the recommended daily values.

We will also use proportional hazards models to evaluate effect modification by other factors, including baseline dietary levels of the study agents for all endpoints, and smoking as a modifier of effects on eye endpoints. To determine whether treatment effects vary over time, we will examine

survival plots, interactions with time and patterns in scaled Schoenfeld residuals,[257] and also examine effects stratified by time.

The primary endpoint in the analysis of cognitive function is change over the course of the study in a composite measure of function. To characterize change over time utilizing the repeated measures of cognitive function, we will use growth curve models, with random effects error structure.[258] For hypothesis testing, models will estimate the average change per year of follow-up and test whether a given exposure is related to change in cognitive function or initial level.

The stored blood samples will provide the opportunity to explore effect modification by baseline plasma levels of study micronutrients. These analyses will use a nested case-control design to minimize the cost of blood studies. Specifically, cases of a primary endpoint and controls will be divided into quartiles of plasma level according to the distribution among controls. We will fit conditional logistic regression models that include interaction terms between baseline quartile and randomized assignment.[259] We will also test for trend in treatment effects across baseline quartiles using a linear term for quartile evaluated at the median plasma level within each quartile. Deviations from linearity, consistent with threshold and other effect shapes, will also be explored using cubic splines.[260]

D.8 Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) meets annually, or more frequently at the discretion of the chair, to examine the accumulating unblinded data for evidence of any extreme benefits or side effects that warrant alteration in the protocol or termination of one or more components of PHS II. Interim trial results are assessed with the Haybittle-Peto rule,[261,262] adjusting for multiple looks in interim analyses. In this procedure, interim results are compared to a z-score of 3 standard deviations (P=0.0027) throughout the trial. The final results may then be interpreted as having close to nominal significance levels. To more precisely characterize the significance level of the final look, we will also use an alpha-spending function to adjust the final P value.[263]

The DSMB used for PHS II is also responsible for the Women's Health Study and Women's Antioxidant Cardiovascular Study and its substudies, to ensure that a balanced view of the effects of these agents is investigated in men and women. Further, we have representation from NCI, NHLBI, NEI, and NIA. DSMB members include Drs. Lawrence S. Cohen, I. Craig Henderson, Andrea LaCroix, Ross Prentice, and Nanette Wenger (chair). *Ex officio* members include Drs. Jeffrey Cutler (NHLBI), Frederick Ferris (NEI), Peter Greenwald (NCI), Natalie Kurinij (NEI), Marjorie Perloff (NCI), Alan Zonderman (NIA), and Ms. Eleanor Schron (NHLBI). This committee will continue to consider any trial finding in light of the totality of evidence, and recommend whether to continue the trial uninterrupted, alter the protocol, or alter or terminate one or more of the arms. A letter of support from the DSMB is attached after Section I.

D.9 Power

The estimated statistical power to study the individual effects of vitamin E, vitamin C, and multivitamin on the incidence of the primary endpoints of total cancer, prostate cancer and important vascular events (nonfatal myocardial infarction (MI) + nonfatal stroke + cardiovascular disease (CVD) death) is shown in **Table 7** (on following page). Power for examining cancer endpoints is based on follow-up of 13,367 participants who were free of cancer at randomization and power for cardiovascular endpoints is based on follow-up of 13,887 participants who were free of important vascular events at randomization. We present power with both the currently funded follow-up and the proposed extended follow-up. The following assumptions are used in these calculations:

(i) With current trial funding, study treatment and follow-up is scheduled to end on December 31, 2002, giving an average length of randomized treatment and follow-up of 3.7 years. With the proposed extension, treatment and follow-up would be extended for 4.5 years, giving a total average follow-up of 8.2 years.

(ii) Event rates are based on those observed in this trial to date. When the database was closed for these analyses, a total of 310 incident cancers, 158 incident prostate cancers, and 169 important vascular events had been confirmed. Numbers of unrefuted endpoints (confirmed plus reported but not yet confirmed or disconfirmed) are: 443 total cancers, 212 prostate cancers, and 274 important vascular events. Because of the time needed for the confirmation process, as well as the high rates of endpoint confirmation in this trial, observed rates per person-year of observation based on unrefuted events are used in these computations. A lag time for questionnaire return is also included.

(iii) Both the observed rates and the observed relative risks have been adjusted for compliance during follow-up, based on self-reports. Compliance rates are similar in the active and placebo groups and across agents. We assume an average compliance over the entire treatment period of 85% for each agent based upon current compliance and projected future compliance. The estimated rates for these calculations incorporate use of non-study vitamins, since these rates are based on the actual experience of the placebo group.

(iv) We present power calculations for the marginal effect of each study agent under the assumption of additive effects. In a factorial trial, we must consider the possibility that two or more agents will be effective in reducing incidence of an endpoint. This would affect the power calculations by reducing the incidence in all exposed groups.

(v) Power is given using a 2-sample test of cumulative incidence rates with a significance level of 0.05. With the low incidence rates and the small relative effects anticipated in this trial, study power based on the logrank test[264] is virtually identical to estimates based on the above approach.

Table 7. Power (%) for the effect of randomized treatments on prostate cancer, total cancer, and important vascular events with current and proposed funding.

		3.7-year Follow-up (current)			8.2-year Follow-up (proposed)		
True RR	**Observed RR**	**Prostate Cancer**	**Total Cancer**	**Important Vascular Events**	**Prostate Cancer**	**Total Cancer**	**Important Vascular Events**
0.80	0.83	42.4	74.9	50.8	75.4	97.8	83.9
0.75	0.79	60.9	91.4	70.7	91.7	99.9	96.1
0.70	0.75	77.5	98.1	86.0	98.2	>99.9	99.5
0.65	0.70	89.3	99.8	94.8	99.8	>99.9	>99.9
0.60	0.66	96.0	>99.9	98.6	>99.9	>99.9	>99.9

For the primary endpoints of prostate cancer and important vascular events, power is limited with 3.7 years of follow-up, but greatly improved with 8.2 years of follow-up. With 3.7 years of follow-up, we have 80% power to detect true reductions of 31% for prostate cancer (observed RR=0.74) and 28% for important vascular events (observed RR=0.76). For the primary endpoint of total cancer, at 3.7 years of follow-up we have greater than 80% power to detect a true 22% reduction in risk, or an observed RR of 0.81. With the extended follow-up, we would be able to detect a true 22% reduction (observed RR=0.81) in risk of prostate cancer, a true 15% reduction (observed RR=0.87) in risk of total cancer, and a true 19% reduction (observed RR=0.84) in risk of important vascular events. We would still have good power to detect the hypothesized moderate effects with 8.2 years of follow-up if average compliance during the trial is 80%. Specifically, with this level of compliance we would have greater than 80% power to detect a true 23% reduction (observed RR=0.82) in risk of prostate cancer, a true 16% reduction (observed RR=0.87) in risk of total cancer, and a true 21% reduction (observed RR=0.83) in risk of important vascular events. Overall, these power calculations show that additional follow-up is required to provide definitive answers to the questions posed by PHS II.

We have also calculated power to detect differences in secondary endpoints with the currently

funded follow-up and the proposed extension **(Table 8)**. These analyses are also based on an alpha level of 0.05. However, in light of the multiple secondary endpoints, we interpret significance of these outcomes with caution.[265] These calculations use the rates of myocardial infarction and stroke observed in the trial to date, and the rates of confirmed cataract and age-related macular degeneration observed in PHS I. Results indicate that we currently only have good power to study confirmed cataract. Extended follow-up would allow us to reliably detect meaningful differences in other endpoints including age-related macular degeneration, myocardial infarction, and stroke.

To study change in cognitive function, assuming a standard deviation of 1.2 in change over time in our composite score, we have a power of 99% to detect a mean change of 1 point in our composite score, and of 89% for a mean change of 0.3 points. With the extended follow-up, we could detect this difference over a longer treatment period than would be available with the current funding, as well as whether the treatment effect persists over time.

Table 8. Power (%) for the effect of randomized treatments on myocardial infarction (MI), stroke, cataract, and age-related macular degeneration (AMD) with current and proposed funding.

		3.7-year Follow-up (current)				8.2-year Follow-up (proposed)			
True RR	Observed RR	MI	Stroke	Cataract	AMD	MI	Stroke	Cataract	AMD
0.80	0.83	29.1	23.0	83.7	32.7	55.6	44.5	99.3	61.5
0.75	0.79	43.0	33.9	96.1	48.0	75.6	63.4	>99.9	81.3
0.70	0.75	58.1	46.7	99.5	64.1	89.7	79.9	>99.9	93.3
0.65	0.70	72.5	60.1	>99.9	78.2	96.8	91.0	>99.9	98.3
0.60	0.66	84.1	72.7	>99.9	88.7	99.3	96.9	>99.9	99.7

D.10 Study Organization

The PHS II will continue to be conducted from the Division of Preventive Medicine at Brigham and Women's Hospital, located at 900 Commonwealth Avenue East, Boston, MA. The PHS II Steering Committee includes the professional staff, the project coordinator, and senior systems analyst. Functions of the Steering Committee include scientific direction of the study, assurance of the confidentiality of data, preservation of the rights of participants, quality control of data collection and statistical analyses, preparation of administrative reports to the NIH, a review of scientific reports from the study, and approval of publications and presentations of study results.

Dr. J. Michael Gaziano has served as the Principal Investigator of the trial since 1999. Co-Investigators include Drs. Julie E. Buring, JoAnn E. Manson, and William G. Christen. Dr. Robert J. Glynn will serve as Statistician for the trial. The Project Director will be Dr. Howard D. Sesso. Ms. Charlene Belanger will be Project Coordinator, as she has been since the start of PHS I. The PHS II Endpoints Committee is chaired by James O. Taylor, MD, and also includes Drs. Meir J. Stampfer, Samuel Z. Goldhaber, and Carlos S. Kase. The DSMB is described in detail in Section D.8.

E. HUMAN SUBJECTS

E.1 Characteristics of the Study Population

This continuing trial will consist of 14,642 U.S. male physicians aged 50 years and older randomized into the Physicians' Health Study II. Of these, 9.4% are members of minority ethnic/racial groups: Asian/Pacific Islander (5.1%), Hispanic (2.4%), Black (1.1%), American Indian/Alaskan Native (0.03%), or other (0.7%). Men randomized into the PHS II had to satisfy the following eligibility criteria: a) no reported personal history of liver disease or other serious illness that might preclude participation; b) not currently taking individual supplements of vitamin A, E, C, ß-carotene, or multivitamins; c) not currently taking anticoagulants or corticosteroids; d) willing to participate in the trial; and e) proven

good compliance during the run-in phase.

E.2 Sources of Research Material

Completed sources of research material include original computer tapes and roster listings of names and addresses obtained from the American Medical Association used to identify potential participants, and blood specimens from approximately 76% of the randomized cohort. Ongoing sources of research materials include follow-up questionnaires, death certificates, and medical records for men with self-reported endpoints of interest with signed medical record release forms. All information is held strictly confidential, and is used only in aggregate for medical statistical purposes.

E.3 Recruitment of Subjects and Consent Procedures

All participants were previously identified and contacted in order to assemble the study population for the PHS II. There will be no new recruitment efforts. When a study endpoint is reported, the participant will be asked to sign a consent form allowing us to obtain the relevant medical record. When a participant dies, and additional information is required to ascertain cause of death, we will request consent from the participant's next-of-kin to obtain copies of relevant medical records.

E.4 Assessment of Potential Risks

Potential risks to PHS participants include the possibility of side effects related to the use of 400 IU of vitamin E every other day, 500 mg of vitamin C daily, and a multivitamin (Centrum Silver) daily. Questions on the annual follow-up forms are designed to assess these potential side effects, and the unblinded data on side effects are reviewed by the DSMB yearly, or more frequently as warranted.

The potential risks of the long-term use of 400 IU of vitamin E on alternate days are unknown. No serious side effects of chronic vitamin E supplementation in humans have been demonstrated.[266,267] Isolated case reports have included fatigue, dermatitis, gastrointestinal disturbances and vasodilation. The only relative contraindication to vitamin E supplementation appears to be anticoagulation therapy. Vitamin E increases the anticoagulant effects of phylloquinone antagonists and may, therefore, make control of coagulation parameters more difficult.[268] Vitamin E reduces platelet adhesion and could therefore increase risks of bleeding. There are increasing safety data available from randomized trials concerning the long-term risks of moderate-dose vitamin E either alone or in combination with aspirin.[122,269,270] A concern regarding an increased risk of hemorrhagic stroke was raised in the Finnish ATBC trial, which reported a 50% increased risk of hemorrhagic stroke deaths among those randomized to 50 mg of α-tocopherol on alternate days.[54] This has not been observed in other large trials. In fact, in the Chinese Cancer Prevention Study, the overall stroke risk was lower in a population that is at high baseline risk of hemorrhagic stroke.

Vitamin C has been given at doses considerably higher than the proposed 500 mg per day without significant side effects. Transient diarrhea is the only side effect supported by the literature despite use of vitamin C in doses as high as 10 to 20 g per day. Because of the absence of adverse effects, a safe upper limit for vitamin C intake has not been defined.

Because the multivitamin tends to contain a large number of vitamins and minerals at doses closer to the RDA, we do not anticipate any risks associated with taking a Centrum Silver tablet daily. Further, taking a multivitamin daily already parallels the habits of millions of Americans, in whom there have been no serious risks reported regarding its use.

Other possible risks to PHS II participants involve the social-psychological risk for the individual resulting from inadvertent disclosure of confidential medical history information. Every possible precaution is taken to maintain confidentiality of medical information in our trials.

E.5 Procedures for Minimizing Potential Risks

To minimize the potential for adverse effects, we have chosen doses for each study agent where

side effects appear to be minimal but benefits appear to be plausible. Those with liver disease and who were on anticoagulants were excluded. Once in the trial, those who develop a coagulation disorder, intracranial bleeding, peptic ulcer, gastrointestinal bleeding, or hematuria are advised to consult their personal physicians. Participants taking corticosteroids are advised to inform their physician that they may be potentially taking vitamin E.

To further protect the interests of study participants, a Data and Safety Monitoring Board (DSMB) has been in place since the beginning of the trial. The DSMB is composed of external experts, including *ex officio* representatives from the National Cancer Institute, National Heart, Lung and Blood Institute, National Eye Institute, and the National Institute of Aging. Their role is to examine unblinded data on endpoints and adverse effects. Based on these data, the DSMB can recommend either continuation, alteration of the study design or early termination of the PHS II.

Confidentiality is maintained in the PHS II by storing completed questionnaires, death certificates, and medical records, identified by study number only, in secure offices accessible by authorized personnel only. In addition, each study employee signs a confidentiality agreement annually.

E.6 Risk/Benefit Ratio

The overall risks of participating in the PHS II are very small. If any of the agents studied prove to be beneficial, individuals assigned to the active agent will receive the benefit of the treatment. However, if the agents prove to have no effect, this would spare the expense of those who might purchase these agents as possible preventive agents. Whatever the outcome of the PHS II, it will add valuable knowledge to the role of vitamin E, vitamin C, and a multivitamin (Centrum Silver) in the primary prevention of major morbidity and mortality.

F. VERTEBRATE ANIMALS

None

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H. CONSORTIUM / CONTRACTUAL ARRANGEMENTS

None

I. CONSULTANTS

Carlos S. Kase, MD, will continue to review stroke endpoints for PHS II. His biosketch is included.