COcoa **S**upplement and **M**ultivitamin **O**utcomes **S**tudy (**COSMOS**)

Study Protocol

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The **CO**coa **S**upplement and **M**ultivitamin **O**utcomes **S**tudy (**COSMOS**) Trial Protocol

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Table of Contents

Section	Topic	Page
1.0.	Overview	4
2.0.	Study Objectives	5
2.1.	Primary Hypotheses	5
2.2.	Secondary Hypotheses	5
2.3.	Tertiary Aim	5
2.4.	Ancillary Aims	5
3.0.	Background	6
3.1.	Cocoa Extract	6
3.2.	Multivitamins	6
4.0.	Study Design	8
4.1.	Schema	8
4.2.	Study Population	9
4.2.1.	Inclusion Criteria	9
4.2.2.	Exclusion Criteria	9
4.3.	Consent	9
4.4.	Intervention	10
4.5.	Randomization and Blinding	11
5.0.	Outcomes	11
5.1.	Primary Outcomes	11
5.2.	Secondary Outcomes	12
5.3.	Tertiary Outcomes	12
5.4.	Ancillary Outcomes	12
6.0.	Sample Size and Power	12
7.0.	Study Procedures	13
7.1.	Recruitment Sources	13
7.1.1.	Initial Mailings	14
7.1.2.	Second Mailing	14
7.2.	Run-in	15
7.3.	Randomization, Dispensing and Blinding	16
7.4.	Follow-up	16
7.5.	Safety and Adverse Events	17

7.6.	Unblinding	17
7.7.	Early Discontinuation	17
7.8.	Study Closeout	18
7.9.	Data Collection and Management	18
7.10.	Outcomes Collection	18
7.11.	Documentation and Definition of COSMOS Study Outcomes	19
7.11.1.	Cardiovascular Disease (CVD) Events	19
7.11.2.	Cancer	20
7.11.3.	Deaths	20
7.12.	Passive Follow-up	20
8.0.	Biospecimen and Blood Pressure Substudy	21
9.0	Clinic-Based Substudy	21
9.1	Ancillary Studies	21
9.2	COSMOS Cancer Tissue Study	24
9.3	Coronavirus disease (COVID-19)	24
10.0.	Statistical Analyses	25
11.0.	Data Management	25
12.0.	Study Monitoring	26
13.0.	Quality Assurance	26
14.0.	Study Administration and Organization	27
15.0.	Study Timeline	27
16.0.	Ancillary Studies	28
17.0.	Appendix	28
18.0.	References	30

Abbreviations

BP: Blood pressure

BWH: Brigham and Women's Hospital (Boston, MA), also called "Partners"

CABG: Coronary Artery Bypass Graft, also called "Coronary Revascularization"

CCC: Clinical Coordinating Center (WHI; Seattle, WA)

CHD: Coronary Heart Disease

COSMOS: COcoa Supplement and Multivitamin Outcomes Study

CVD: Cardiovascular Disease

FHCRC: Fred Hutchinson Cancer Research Center (Seattle, WA)

MI: Myocardial Infarction

NHLBI: National Heart, Lung, and Blood Institute

VITAL: VITamin D and omegA-3 TriaL

WHI: Women's Health Initiative

Overview

The **CO**coa **S**upplement and **M**ultivitamin **O**utcomes **S**tudy (**COSMOS**) is a randomized, double-blind, placebo-controlled, 2x2 factorial trial of a high-quality cocoa extract supplement (containing 600 mg/d cocoa flavanols , including 80 mg (-)-epicatechins) (please see section 4.4 Intervention), and a multivitamin supplement to reduce the risk of cardiovascular disease (CVD) and cancer in approximately 13,000 women aged ≥65 years and approximately 9,000 men aged ≥60 years with an average of 4 years of treatment and follow-up. These promising interventions have both already shown favorable results in observational studies and randomized trials, and are well-tolerated, safe, and relatively inexpensive. For cocoa flavanols, several small randomized trials have demonstrated benefits for intermediate outcomes, including blood pressure, lipids, insulin sensitivity, and flow-mediated vasodilation; for multivitamins, a prior large-scale randomized trial in men showed a significant reduction in cancer, but trial data in women are lacking. For both interventions, a confirmatory large-scale randomized trial such as the COSMOS in women and men could have major clinical and public health implications.

The trial utilizes an innovative and highly time- and cost- efficient approach leveraging the existing infrastructure of the Women's Health Initiative (WHI) and the VITamin D and OmegA-3 TriaL (VITAL). After an initial screening contact by the WHI, the Brigham and Women's Hospital (BWH) will recruit and consent into COSMOS up to 13,000 women among active WHI Extension Study participants who have demonstrated a commitment to scientific research and are free of baseline CVD, and free of cancer diagnosed within the last 2 years. BWH will recruit an additional 9,000 men among non-randomized respondents of the VITAL who expressed interest in research studies and already reported preliminary eligibility information. If necessary to reach the enrollment goals, additional men and women who contacted BWH for information about COSMOS may be included, along with additional women among non-randomized respondents of the VITAL, plus use of targeted mailing lists as needed. Thus, a total of up to 22,000 women and men will be randomized into the COSMOS trial. The study plan involves recruitment over one or more years, including a placebo run-in, and an average of 4 years of intervention and outcome ascertainment that will provide sufficient power to detect small-to-moderate reductions in primary CVD and cancer outcomes. The trial will be conducted primarily by mail, with study pills mailed by BWH to participants and CVD and cancer outcomes confirmed by medical record review. Existing WHI resources provide follow-up for ascertainment of self-reported health events and adjudication of cancer endpoints in all WHI participants, and for CVD outcomes in a subcohort. COSMOS will expand this to include adjudication of all CVD outcomes for those randomized into the COSMOS. Baseline blood and spot urine specimens will be collected from a subcohort of approximately 3,500 women and 3,500 men participating in COSMOS; blood pressure and anthropometric measurements also will be obtained from these participants when possible. Follow-up measurements will be collected from a subset of the biospecimen project participants to assess changes in important nutritional and vascular/metabolic biomarkers related to our cocoa flavanol and multivitamin interventions. The design of the trial also allows for the development of multiple ancillary studies, including one which will assess the effects of the study agents on cognitive function at baseline and follow-up using a web-based application and one which will assess the effects on risk of cataract and age-related macular degeneration (AMD). Additional measurements will be obtained from in-

clinic visits at baseline and after 2-years follow-up for approximately 600 COSMOS participants residing in the greater Boston area at the Harvard Catalyst Clinical and Translational Science Center.

(COSMOS Trial PIs: Dr. JoAnn Manson and Dr. Howard Sesso at Brigham and Women's Hospital, Harvard Medical School, and Dr. Garnet Anderson at the WHI CCC, Fred Hutchinson Cancer Research Center.)

2.0. Study Objectives

COSMOS is a randomized, double-blind, placebo-controlled, 2x2 factorial trial of a cocoa extract and a multivitamin supplement in up to 22,000 subjects (13,000 women and 9,000 men), including women recruited from active, older WHI participants and men and women who expressed interest in research being conducted by BWH. The primary objectives of this trial are to evaluate these agents for the maintenance of health. The specific hypotheses guiding the design of the trial are as follows:

2.1. Primary Hypotheses:

1) Cocoa extract (2 capsules/day containing a total of 600 mg/d flavanols, including 80 mg (-)-epicatechin, and 50 mg theobromine) (please see section 4.4 Intervention) will reduce the risk of major cardiovascular events, defined for these purposes as a composite endpoint of MI, stroke, cardiovascular mortality, coronary revascularization, unstable angina or acute coronary syndrome (ACS) requiring hospitalization, carotid artery surgery, and peripheral artery surgery or angioplasty;
2) A daily multivitamin will reduce the risk of invasive cancer (excluding non-melanoma skin cancer).

2.2. Secondary Hypotheses:

1) Cocoa extract will reduce the risk of a composite endpoint of MI, stroke, cardiovascular mortality, and coronary revascularization;
2) Cocoa extract will reduce the risk of invasive cancer (excluding non-melanoma skin cancer);
3) A daily multivitamin will reduce the risk of major cardiovascular events;
4) Cocoa extract and/or a daily multivitamin will reduce the combined endpoint of major cardiovascular events plus all-cause mortality;
5) Cocoa extract and/or a daily multivitamin will reduce the risk of individual cardiovascular events, including MI, stroke, cardiovascular mortality, coronary revascularization, unstable angina or ACS requiring hospitalization, carotid artery surgery, and peripheral artery surgery or angioplasty, and total mortality; plus site-specific cancers, including breast, colorectal, and lung cancer;
6) A daily multivitamin will reduce the risk of cancer among women and men with a history of cancer at baseline;
7) In a subset of equal numbers of female and male COSMOS respondents who provide baseline bloods and/or urine samples, cocoa extract and/or a daily multivitamin will significantly change blood and/or urinary levels of flavonoids or their metabolites, other pertinent nutritional biomarkers, and vascular/metabolic biomarkers from baseline to 1, 2, or 3 years of follow-up.

2.3. Tertiary Aim:

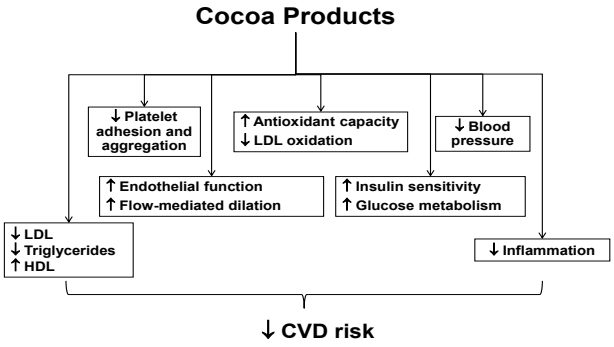
To assess whether the cocoa extract and/or a daily multivitamin exhibit synergistic effects on risk of major cardiovascular events or cancer, and if the effects vary by nutritional status or medication use.

2.4 Ancillary Aims:

To assess whether the random assignment to daily cocoa extract, compared to placebo, has favorable effects on cognitive function over 3 years of follow-up in men and women.
To assess whether daily cocoa extract and/or multivitamin have favorable effects on the risk of cataract and age-related macular degeneration (AMD).
To assess whether daily cocoa extract and/or multivitamin have favorable effects on the risk of injurious fall(s) resulting in healthcare utilization, recurrent falls, physical performance, and fractures.
To assess whether daily cocoa extract and/or multivitamin have favorable effects on inflammaging and epigenetic aging.

3.0. Background

Figure 1. Multiple mechanisms through which cocoa products may lower the risk of CVD



COSMOS is a large-scale randomized trial testing a patented, well-studied cocoa extract supplement (Mars Symbioscience) and a standard multivitamin (Pfizer Inc) in the maintenance of heart health and cancer risk among older women and men. Evidence for the potential benefits of these agents for chronic disease risk comes from multiple sources. For the cocoa extract intervention, randomized trials have demonstrated consistent benefits on intermediate outcomes, including blood pressure (BP), lipids, insulin sensitivity, and flow-mediated vasodilation, but there are no large-scale trials. For multivitamins, a large-scale randomized trial in men showed a reduction in cancer occurrence which appeared to be concentrated in men with prior cancer diagnoses.[1] There are no corresponding randomized trial data for women, and the nature of the data in men indicates that replication of this finding is needed. For both interventions, a confirmatory large-scale randomized trial could have enormous clinical and public health impact.

The WHI and VITAL study infrastructures provide key resources for COSMOS, eliminating the need for de novo recruitment (a major expense of most trials), supporting data collection and cohort follow-up, and facilitating adjudication of trial outcomes already collected for all cancer endpoints and CVD endpoints in a subcohort of WHI. This approach to recruitment and follow-up is highly innovative and extremely time- and cost-efficient. COSMOS also represents an important collaboration among NIH, academia, and industry partners donating the cocoa and multivitamin supplements and packaging and is directly responsive to the goal of the April 2013 NHLBI Workshop to facilitate cost-effective clinical trials that leverage existing cohort infrastructure such as WHI, and a 2012 Institute of Medicine Workshop on “Large Simple Trials.”

3.1. Cocoa extract

Cocoa and chocolate are ancient fermented products that the Aztecs made from the bean of the cacao tree, Theobroma cacao. Cocoa and chocolate contain catechins and epicatechins, readily water soluble flavonoids classified as flavan-3-ols,[2] a subclass of flavonoids with vascular health benefits. In the last decade, experimental and clinical studies testing the consumption of chocolate (as dark chocolate), cocoa, and high-dose cocoa flavanols note beneficial effects on antioxidant capacity,[3,4] LDL oxidation,[5] platelet activation,[6,7] lipid profiles,[8,9] endothelium-dependent vasodilation,[10,11] inflammation,[12,13] blood pressure (BP),[14] and glucose metabolism, [15] which may translate into important cardiovascular and cancer benefits (**Figure 1**).

3.2. Multivitamins

Multivitamins are the most common dietary supplement, taken by at least one-third of US adults.[16,17] Observational studies of multivitamin use and cancer have had inconsistent findings.[18-24] In the recently completed Physicians' Health Study (PHS) II, a randomized, double-blind, placebo-controlled clinical trial testing a Centrum Silver multivitamin in 14,641 men aged ≥50 years, we found a modest but significant 8% reduction in total cancer,[1] and no effect on CVD.[25] Notably, in men aged ≥70 years there was a stronger 18% reduction in total cancer (P, interaction by age=0.06) and a nonsignificant 9% reduction in CVD (P, interaction by age=0.04).[1,25] Further, among 1,312 men with a baseline history of cancer in PHS II, we found that daily multivitamin use was associated with a significant 27% reduction in total cancer. In addition, there was a significant 39% reduction in fatal myocardial infarction (MI) overall. Because the PHS II is the *only* large-scale clinical trial testing a daily multivitamin, these findings require replication to determine whether a multivitamin reduces the risk of cancer, particularly among older women and those with a history of cancer.

The popularity of vitamins, with $23 billion spent annually, the increase in vitamin and mineral fortification of foods and beverages, and recent safety concerns over some supplements[26,27] all heighten the importance of understanding the effects of vitamin supplements. Over half of US adults take vitamins,[28-30] with multivitamins by far the most common dietary supplement taken. More than one-third of US adults take multivitamins[12,13,31] such as Centrum Silver. The purpose of multivitamins is to reduce nutritional deficiency. Although supplementation with high-dose individual vitamins has not reduced cancer risk, multivitamins represent a different paradigm of RDA-level supplementation across dozens of vitamins and minerals. Despite the lack of clear benefits, US adults still take multivitamin supplements to reduce chronic diseases[30] or promote general health and well-being.[32] In NHANES, less than 25% of supplements taken were recommended by a physician or health provider.[33]

Cognitive Function

Dietary interventions have shown that consuming cocoa flavanols can reduce platelet aggregation and improve insulin sensitivity, blood pressure and peripheral and central blood flow.[34] Given that deleterious changes in these markers of health are associated with cognitive decline and dementia, cocoa flavanols may confer neuroprotective effects that benefit cognitive function and potentially reduce the risk of neurodegenerative disease. In one human study, daily consumption of cocoa flavanols was associated with improved cognitive function (attention, executive function and language) in older men and women with mild cognitive impairment, a transitional state between normal cognitive aging and dementia.[35]

Eye Health

The rationale for the COSMOS Eye ancillary study is based on recent findings from the Physicians' Health Study II trial of a beneficial effect of a daily multivitamin in cataract reduction and the plausible benefits of cocoa extract in preventing age-related macular degeneration (Protocol #2016P001612: Cataract and AMD in a Trial of a Multivitamin and Cocoa Extract).

Falls and Physical Performance

Several lines of evidence suggest that flavanols commonly found in cocoa confer beneficial effects on muscle function and physical performance. Cocoa is a rich source of water soluble flavanols, specifically epicatechins. Results from preclinical and clinical studies have shown promising effects of epicatechins on decreasing oxidative stress, improving markers of mitochondrial biogenesis, and increasing grip strength and walking speed. Studies in older adults indicate that flavanols have promising effects on musculoskeletal physiology and/or physical performance. This ancillary study will test whether

supplemental cocoa flavanols prevent falls, slow age-related declines in physical performance, and/or reduce fall-related fractures among older persons in the U.S.

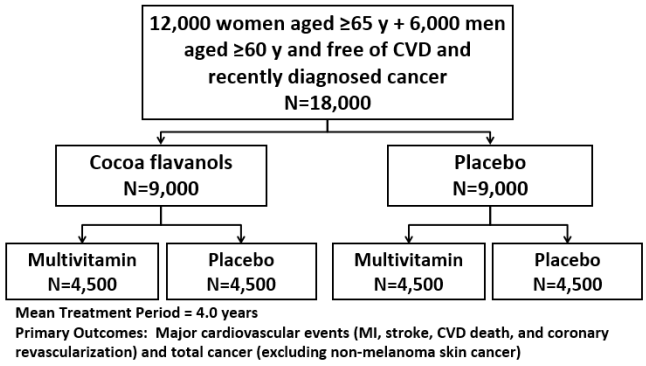
Inflammaging and Epigenetic Aging

Human aging is characterized by chronic, low-grade inflammation, which has recently been termed inflammaging. Inflammaging is exemplified by mild chronic elevation in blood levels of C-reactive protein (CRP) and several cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which increase with aging. Inflammaging is a highly significant risk for both morbidity and mortality in the elderly. Accelerated epigenetic aging that comprises DNA methylation changes has recently emerged as a powerful, novel predictor for lifespan and health span, disease susceptibility, morbidity, and mortality risk. DNA methylation is a major epigenetic process that regulates gene expression and can be modified by different bioactive compounds in foods. This ancillary study will provide insight into the underlying molecular mechanisms for cocoa flavanol and multivitamin supplements, and holds great promise for targeting by nutritional interventions.

4.0. Study Design

4.1. Schema

Figure 2. Overview of COSMOS trial of cocoa and multivitamin supplements



The COSMOS trial will be a randomized, double-blind, placebo-controlled trial to evaluate the benefits and risks of a patented cocoa extract supplement (600 mg/d of cocoa flavanols, (please see section 4.4 Intervention)) and a daily multivitamin (Centrum Silver) in the maintenance of heart health and cancer risk among 13,000 women aged ≥65 years and 9,000 men aged ≥60 years among WHI participants and those contacted but not randomized into VITAL, for a total of 22,000 randomized participants (**Figure 2**). In order to reach the recruitment goals, men and women who contact BWH for information about participating in the COSMOS trial may also be included as well as respondents to targeted mass mailings. This large, simple trial represents collaboration between the Division of Preventive Medicine at BWH, Fred Hutchinson Cancer Research Center (WHI CCC), WHI Regional Centers, and the WHI Project Office at NHLBI. Each participant will be randomly assigned in a 2x2 trial to a cocoa flavanol and/or multivitamin supplement with an average of 4 years of treatment and follow-up. Follow-up questionnaires at 6 and 12 months and semi-annually thereafter will assess compliance, dietary changes, new endpoints, and risk factors. Baseline blood and spot urine specimens will be collected from a subcohort of approximately 3,500 women and 3,500 men participating in COSMOS; blood pressure and anthropometric measurements also will be obtained from a sample of these participants. Follow-up measurements will be collected to assess changes in important nutritional and vascular/metabolic biomarkers related to our cocoa flavanol and multivitamin interventions. Additional measurements, including cognitive function, physical function, blood pressure, and other assessments, will be obtained from in-clinic visits at baseline and after 2-years follow-up for approximately 600 COSMOS participants residing in the greater Boston area at the Harvard Catalyst Clinical and Translational Science Center. The design of the trial also allows for the development of multiple ancillary studies including one which will assess the effects of the study agents on cognitive function at baseline and follow-up using a web-based

application. An ancillary study on eye health will assess the effects of the study agents on risk of cataract and AMD.

The trial builds on existing data collection and outcomes adjudication within the WHI CCC, 4 WHI Regional Centers (RCs), the WHI Study Database (WHIX) to facilitate participant tracking and security, and the VITAL trial, coordinated at Brigham and Women's Hospital (BWH) as a recruitment source. Active and placebo supplements and packaging will be donated by manufacturers. A detailed medication inventory on all WHI participants will also allow us to assess potential modifying effects of regular use of common medications, including statins, aspirin, and other medications.

4.2. Study Population

4.2.1. Inclusion criteria

1. Women will be ≥65 years of age and participating in the current WHI Extension Study. If fewer than 13,000 women from WHI agree to participate, additional women aged ≥65 years who were contacted for but not randomized into VITAL, women who contact BWH to volunteer for the COSMOS trial, and those responding to invitations by mail will be eligible. If the women from VITAL proceeded to the stage of the VITAL run-in, they must not have missed >10 days of pill taking per month.
2. Men will be ≥60 years of age who were contacted for but not randomized into the VITAL Trial, men who called for information about COSMOS, and those responding to recruitment invitations by mail. If the men from VITAL proceeded to the stage of the VITAL run-in, they must not have missed >10 days of pill taking per month.
3. ResearchMatch.org will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository (see IRB #090207).
4. Willing to participate, as evidenced by providing informed consent to the BWH and completing all required baseline forms.

4.2.2. Exclusion Criteria

1. History of myocardial infarction or stroke.
2. Diagnosed with invasive cancer other than non-melanoma skin cancer in the last 2 years prior to randomization unless the participant is willing and able to take the study pills and complete questionnaires for the duration of the trial.
3. Current active chemotherapy or radiation treatment for recurrent cancer.
4. Any serious illness that would preclude participation and/or completion of the trial, including the diagnosis of kidney failure and current dialysis treatment.
5. Taking cocoa or multivitamin supplements and not willing to forego use during the trial.
6. Taking total supplemental vitamin D >1000 IU/day and not willing to forego use during the trial.
7. Taking total supplemental calcium >1200 mg/day and not willing to forego use during the trial.
8. Extreme sensitivity to caffeine.
9. Consume < 75% of the expected number of both types of supplements during the run-in phase.
10. Unable to communicate in English due to language barrier or mental incapacity.

4.3. Consent

The participant's full understanding of the COSMOS trial is important ethically and for adherence to the protocol. Consent forms will be mailed to all individuals who have expressed interest in participating in the main COSMOS trial in response to the initial screening survey. All COSMOS subjects are consented by BWH and sign the Partners Consent form. Consent forms for WHI participants will be returned by mail to the WHI CCC for data entry. Consent forms for all others will be returned to BWH. A BWH toll-free telephone number will be provided with the corresponding consent form and recipients will be encouraged to call with any COSMOS study related questions. A toll-free line for the CCC will also be provided with the corresponding consent and recipients will be encouraged to call with any WHI study-related questions.

The BWH COSMOS consent will permit sharing of all data between the WHI CCC and BWH on the WHI participants who consent to COSMOS, including personal identifiers, for the purposes of intervention delivery. Similarly, the consent will permit BWH to share COSMOS data with the WHI CCC to facilitate active follow-up and passive follow-up through linkage to other sources such as the National Death Index and Medicare. The consent will not allow access to participant PHI by ancillary studies, but will include consent to contact for additional studies.

COSMOS participants who agree to provide blood samples, urine samples, blood pressure, and anthropometric measurements will sign a separate consent form specifically related to the biomarker project.

COSMOS participants in clinic-based visits at the Harvard Catalyst Clinical and Translational Science Center will also be required to sign a separate consent form that is included under a separate study protocol that specifically relates to the clinic-based COSMOS study project.

Separate study protocols and/or consent forms will be developed as appropriate for all ancillary studies to COSMOS, including a project for web-based cognitive function assessments for approximately 4,000 participants at baseline and follow-up.

The BWH COSMOS consent forms conform to all Human Subjects Protections as outlined by the US Department of Health and Human Services (DHHS) Office for Human Research Protections (OHRP) and guided by the Institutional Review Offices (IROs) of the participating institutions. The consent will be reviewed by each participating institution's (BWH, FHCRC) Institutional Review Boards (IRB).

4.4. Intervention

Cocoa Extract supplements: Active cocoa extract (600 mg/d of cocoa flavanols) and matching inert placebo with no cocoa flavanols will be provided by Mars Symbioscience. Participants will be asked to take two capsules per day, totaling 600 mg/d. After the COSMOS trial began, an advanced method to analyze cocoa flavanols was accredited by AOAC International as a First Action Official Method of Analysis https://doi.org/10.1093/jaoacint/qsaa132). This updated method relies on a reference material (RM8403) recently standardized and made commercially available by the U.S. National Institute of Standards and Technology. While the actual cocoa flavanol content of the COSMOS intervention remained unchanged throughout the trial, the application of this new analytical method led to expected changes in how the total cocoa flavanol content is now reported. Applying AOAC 2020.05/RM8403 to the COSMOS intervention, the total cocoa flavanol content of the COSMOS intervention is now 500 mg/day. Reporting of (-)-epicatechin content remained unaffected. Going forward, we will therefore apply AOAC 2020.05/RM8403 and report that the COSMOS intervention tested 500 mg/day of cocoa flavanols, including 80 mg of (-)-epicatechin.

Multivitamin supplements: Active multivitamin (marketed as Centrum Silver™) and matching inert placebo will be provided by Pfizer Inc. Participants will take one tablet per day.

The manufacturers will perform quality control testing to ensure their products contain the correct amounts and are free of contaminants.

Study supplements will be packaged in calendar packs provided by Contract Pharmacal Corp (CPC) to promote adherence to daily dosing and facilitate self-reports of adherence to pill-taking. Participants will be instructed to take their study supplements at the same time each day.

4.5. Randomization and blinding

Eligible participants will be randomized into one of 4 treatment arms in equal proportions:

Active Multivitamin and Active Cocoa Extract;

Active Multivitamin and Placebo Cocoa Extract;

Placebo Multivitamin and Active Cocoa Extract;

Placebo Multivitamin and Placebo Cocoa Extract.

The algorithm will use a randomized permuted block approach, stratified by (at minimum) sex, age group, and recruitment center (WHI or BWH). The date of randomized treatment assignment will be considered the formal enrollment date.

Whenever possible, household partners (e.g., spouse/partner) of a randomized COSMOS participant will be assigned to the same treatment group, as the corresponding randomized participant. To ensure balance within strata at the participant-level, the household partner will occupy the next available allocation with the same treatment, as the randomized participant. The strata of the household partner will be determined by the household partner's individual characteristics, and not the characteristics of the corresponding randomized participant.

BWH will receive all calendar packs for distribution to COSMOS participants and maintain blinding to treatment assignments.

5.0. Outcomes

Primary and secondary outcomes will be ascertained initially through self-report, proxy-report, or death certificate, and confirmed through medical record review using definitions and procedures established in the WHI. For COSMOS participants recruited from WHI, all cancer events and ~25% of CVD events in WHI will be adjudicated through currently funded ongoing WHI procedures. The remaining CVD events among WHI participants and all cancer and CVD events in the remaining participants will be documented and adjudicated using identical definitions and procedures by WHI physician adjudicators and COSMOS staff at BWH who are blinded to randomization assignment.

5.1. Primary Outcomes

Cocoa Extract: Centrally adjudicated major cardiovascular events, including myocardial infarction, stroke, cardiovascular mortality, coronary revascularization, unstable angina or ACS requiring hospitalization, carotid artery surgery, and peripheral artery surgery or angioplasty;

Multivitamin: Invasive cancers of any site other than non-melanoma skin cancer as confirmed by medical record review and coded according to SEER standards.

5.2. Secondary Outcomes

Cocoa Extract: Composite endpoint of MI, stroke, cardiovascular mortality, and coronary revascularization;

Invasive cancers of any site other than non-melanoma skin cancer as confirmed by medical record review and coded according to SEER standards.

Multivitamin: Centrally adjudicated major cardiovascular events, including myocardial infarction, stroke, cardiovascular mortality, coronary revascularization, unstable angina or ACS requiring hospitalization, carotid artery surgery, and peripheral artery surgery or angioplasty.

Cocoa extract and a daily multivitamin:

Major cardiovascular events (the combined endpoint of composite endpoint of MI, stroke, cardiovascular mortality, coronary revascularization, unstable angina or ACS requiring hospitalization, carotid artery surgery, and peripheral artery surgery or angioplasty) plus all-cause mortality;

Individual cardiovascular events, including MI, stroke, cardiovascular mortality, coronary revascularization, unstable angina or ACS requiring hospitalization, carotid artery surgery, peripheral artery surgery or angioplasty, and total mortality; plus site-specific cancers, including breast, colorectal, and lung cancer;

Invasive cancer in participants with a history of cancer at COSMOS baseline.

5.3. Tertiary Outcomes

Cocoa extract and/or a daily multivitamin: Synergistic effects on risk of major cardiovascular events or cancer, and if the effects vary by nutritional status or medication use.

5.4. Ancillary Outcomes

COSMOS Mind – Cocoa extract and/or a daily multivitamin: Telephone-based assessments of global cognitive function, executive function, and memory.

COSMOS Web – Cocoa extract: Internet-based neuropsychological testing battery, including the ModBent test (cognitive test of novel object recognition), the Spatial Learning Task, and the Flanker Task (cognitive test of attention, inhibition, and executive control).

COSMOS Eye – Cocoa extract and/or a daily multivitamin: cataracts, cataract surgery, and age-related macular degeneration (AMD).

COSMOS Falls and Physical Performance – Cocoa extract and/or a daily multivitamin: Injurious fall(s) resulting in healthcare utilization (visit to a healthcare provider or hospital); recurrent falls; physical performance as assessed by grip strength (by dynometer), walking speed (6 meters); and the Short Physical Performance Battery including tests of standing balance, walking speed, and chair stands; incident total, spine, forearm/wrist, and hip fractures; and bone density changes.

COSMOS Inflammaging and Epigenetic Aging – Cocoa extract and/or a daily multivitamin: epigenetic aging; inflammaging; and effects on inflammaging are mediated by epigenetic aging, and the impact on CVD risk.

6.0. Sample size and power

Power calculations for the primary endpoints of major cardiovascular events and total invasive cancer are based upon a 2x2 factorial trial in approximately 22,000 participants, including 13,000 women aged ≥65 years and 9,000 men aged ≥60 years. Based on the log-rank test (equivalent to score tests for hazard ratios from a Cox model without covariates), Freedman[36] has shown that the power, 1-β, of this test to detect a specified hazard ratio HR based on a two-sided test with significance level α is a simple function of the number of accrued events, m, in the two groups combined: $1-\beta = \Phi(\sqrt{m}\,(|HR-1|)/(|HR+1|) - z_{1-\alpha/2})$, where Φ is the standard normal cumulative distribution function. The factorial design of this trial is motivated primarily by efficiency. The scientific justification for testing each intervention is independent of the other and stands alone. Therefore each comparison (multivitamins versus placebo and cocoa extract versus placebo) will be based on a marginal test (but controlling for the other intervention) at a level of $\alpha = 0.05$.

Estimates of study power and the expected numbers of confirmed, accrued events in Table 1 are based on the following assumptions: (1) independent and equal allocation of participants to each treatment; (2) an age distribution based on that observed among WHI women ≥65 years and men aged ≥60 years responding to the

VITAL mailings; (3) annualized age-specific event rates based on observed rates during WHI and VITAL follow-up, adjusted for aging; (4) same event rates for men and women given that women are approximately 5 years older than men; (5) mean trial follow-up of 4 years, with nominal loss to follow-up as seen previously in WHI, VITAL, and mail-based trials by the BWH Division of Preventive Medicine; and (6) an average of 80% compliance. These assumptions remain the same as proposed for our original power calculations for 18,000 participants.

With approximately 22,000 randomized participants, there will be at least 80% power to detect a 11% relative hazard reduction in total CVD, and >95% power to detect such a reduction in the important composite of total CVD plus all-cause mortality with the expected number of events. Similarly, there would be at least 90% power to detect a 14% reduction in total cancer. Power for key secondary outcomes are highly dependent on the frequency of the events.

Table 1. Power for effects of a single agent on specified endpoints in a factorial trial of 22,000 women and men, with 4 years of treatment and follow-up (#s in parentheses are expected number of events in both groups combined)

Observed RR *	True RR †	Total CVD	Total CVD + Mortality	Total Cancer	CVD death	Stroke	MI	Breast cancer
Placebo event rate (per 100 p-y)		*3.0*	*5.0*	*2.2*	*1.0*	*0.9*	*0.8*	*0.8*
0.90	**0.875**	76% (2479)	93% (4106)	63% (1837)	- (824)	- (743)	- (661)	- (661)
0.85	**0.812**	98% (2416)	>99% (4005)	93% (1790)	64% (803)	59% (723)	54% (644)	54% (644)
0.80	**0.750**	>99% (2353)	>99% (3903)	>99% (1743)	88% (781)	84% (704)	80% (627)	80% (627)
0.75	**0.687**	>99% (2290)	>99% (3801)	>99% (1695)	98% (760)	96% (685)	94% (609)	94% (609)
0.70	**0.625**	>99% (2226)	>99% (3697)	>99% (1648)	>99% (738)	>99% (665)	99% (592)	99% (592)
0.65	**0.560**	>99% (2162)	>99% (3592)	>99% (1600)	>99% (717)	>99% (646)	>99% (575)	>99% (575)

*** Observed RR = intent-to-treat RR, including noncompliant participants and 80% compliance.**

† True RR = that with perfect compliance.

- Power < 50%

7.0. Study Procedures

7.1. Recruitment Sources

COSMOS participants will be recruited from three primary sources, the current WHI Extension Study participants, individuals who have responded to recruitment activities for VITAL but who were not randomized, and targeted mailings. The initial recruitment and screening of potential COSMOS participants will be conducted separately, by the CCC for WHI participants and by BWH for VITAL respondents . If necessary to meet recruitment goals, additional men and women who called for information about the COSMOS or respond to mass mailings will be recruited by BWH. ResearchMatch.org, a national electronic, web-based recruitment

tool created through the Clinical & Translational Science Awards Consortium in 2009 and maintained by Vanderbilt University as an IRB-approved data repository (see IRB #090207), will be utilized to help meet targeted recruitment goals.

7.1.1. Initial Mailings

WHI Participants: The CCC will establish pre-eligibility of WHI participants from the most recently available database of all WHI questionnaires and outcomes data (expected yield of 77,011). The CCC will mail each identified WHI participant a recruitment packet containing a letter and information explaining the rationale for the COSMOS trial and what participation would entail, a brief eligibility and interest questionnaire and a prepaid return envelope. The questionnaire will collect information on eligibility and demographics. Women who do not respond to the first mailing will be sent a repeat mailing within 8 weeks.

The CCC will scan the returned packets from WHI participants into the central COSMOS database maintained at the CCC and a computer program will evaluate the responses on the enrollment questionnaire along with the WHI questionnaire to classify respondents as ineligible, pending eligible, or eligible.

VITAL respondents:

Men in the COSMOS trial will be recruited from those contacted for the VITAL trial. BWH will identify men who were contacted for VITAL, but not randomized and are eligible for COSMOS based on all available data. BWH will also recruit men who called to request information about the trial and those who respond to targeted mass mailings.

Responses will be returned to BWH. BWH staff will scan the documents into the central COSMOS database and a computer program will evaluate the responses on the enrollment questionnaires to classify the respondents as ineligible, pending eligible, or eligible. For pending eligibles, BWH will either call the potential participant or mail a brief follow-up form to clarify incomplete or inconsistent responses.

If needed to meet enrollment goals, women who responded to the VITAL recruitment process, those who called for information about the trial, and those who respond to the targeted mass mailings will be recruited into COSMOS following the same protocol as for men, but limited to women ≥65 years of age.

Respondents who were willing but not eligible to participate will be sent a brief thank you note after all phases of screening have been completed. The CCC will mail for WHI participants and BWH will mail for non-WHI participants.

Additional men and women who are interested in joining the trial will be given the option of completing questionnaires online. The data will be collected and managed using REDCap (Research Electronic Data Capture), a secure, HIPAA-compliant web-based application hosted by Partners HealthCare Research Computing Enterprise Research Infrastructure & Services (ERIS). These potential participants will be directed to the study website where they will find a link to a REDCap survey that will allow them to submit their name, gender, and contact information. At that time, they will also have the option of completing the COSMOS 1 screening questionnaire through REDCap.

7.1.2. Second Mailing

Each site (CCC and BWH) will send a second mailing to those potential COSMOS participants who remain willing and eligible for the trial after the initial mailing. This packet will contain an expanded cover letter; BWH COSMOS trial consent form; an additional baseline questionnaire with questions on eligibility; demographic,

lifestyle, and clinical risk factors; dietary factors; and a return envelope. The Fred Hutchinson Cancer Research Center (WHI CCC) sends recruitment mailings and the Partners consent form to women they previously enrolled into the WHI.

Participants with any questions before they sign and return their BWH COSMOS informed consent will all be offered the opportunity to call a BWH toll free number and speak with COSMOS research staff or key Investigators as needed. Responses will be returned to the originating sites for evaluation of eligibility. Non-respondents will be sent a repeat mailing within a few months. Those with uncertain eligibility will be contacted by mail or phone to resolve any issues.

Respondents who are willing but not eligible will be sent a brief thank you note after all phases of screening have been completed. The CCC will mail for WHI participants and BWH will mail for non-WHI participants.

7.2. Run-in

Potential participants who remain eligible and interested after the second mailing will be asked to complete at least a 2-month placebo run-in (optimally 3-4 months) to eliminate poor compliers prior to randomization and increase study power.[37] The participants will not be told that the pills they are provided are placebo.

BWH will send up to a 6-month supply of multivitamin placebo tablets and cocoa extract placebo capsules to all respondents to the second mailing who remain eligible and who provided a signed consent form. The additional supply will allow daily pill taking to continue uninterrupted before randomization. Cocoa placebo capsules and multivitamin placebo tablets will be packaged in calendar packs, and dispensation will be tracked using lot numbers and tracking systems in place at BWH. Participants will be asked to take three pills (two cocoa placebo and one multivitamin placebo) per day.

During the placebo run-in, we will send a food frequency questionnaire (FFQ) to all participants that includes questions on dietary sources of cocoa flavanols, the use of nutritional supplements (including multivitamins), and other foods.

Also during the run-in, participants who are willing to provide blood samples will be sent information and materials inviting them to provide study data. Those who are willing and eligible for the Boston area clinic-based visits will be sent an invitational mailing with information concerning the opportunity to participate in this portion of the COSMOS trial. Participants who express interest in any ancillary projects such as the web-based, cognitive function sub-study, will receive separate mailings with detailed information concerning the projects and informed that their participation is optional to the main trial.

We will partner with Wake Forest (COSMOS Mind) and Columbia University (COSMOS Web) to enroll participants in these sub-studies. Once the participants enter the run-in, those who meet the requirements for these 2 sub-studies will be contacted by letter to opt-in in either substudy. If the participant returns the letter requesting participation in 1) COSMOS Mind, we will grant Wake Forest access to contact the participants and complete the phone interview. BWH will develop a data sharing agreement with Wake Forest to allow for these cognitive data to be analyzed, and Wake Forest has separate IRB approval for these COSMOS Mind activities. If the participant returns the letter requesting participation in 2) COSMOS Web, we will send the participants a letter or email with instructions on how to access and complete the web-based test. BWH will receive the web-based data, and will develop a data sharing agreement with Columbia University to allow for these cognitive data to be analyzed. Participants who complete a baseline test for COSMOS Mind (goal of 2,200) and COSMOS Web (goal of 4,000) will be contacted again at years 1, 2, and 3.

After at least 8 weeks from the start of the run-in, the CCC will mail WHI participants, and the BWH to non – WHI participants, a follow-up adherence and safety questionnaire asking about continued eligibility, willingness to participate in the trial, pill compliance, potential side effects, and other relevant lifestyle and clinical risk factors. Non-responders or those with uncertain eligibility will be contacted by mail or phone to resolve any issues. Responses will be scanned into the database and final eligibility will be determined. Those who are willing but not eligible will be sent a brief thank you note after all phases of screening have been completed from the CCC for WHI participants, and from BWH for non-WHI participants.

7.3. Randomization, Dispensing and Blinding

The WHI CCC will be responsible for randomizations and BWH will be responsible for dispensing study supplements. Once all eligibility data are collected and recorded in the central database, the WHI CCC will run a computerized function in the COSMOS database that will confirm all items of eligibility and if appropriate, allocate eligible participants into one of the 4 intervention groups. Once the randomization is done, BWH staff will receive notification of the participant ID to dispense. BWH will mail a supply of the appropriate supplements to the participant. All supplements will be labeled with unique lot numbers for tracking purposes and will not indicate whether the contents are active or placebo to assure blinding of all participants and staff involved in participant contact and outcomes ascertainment and adjudication.

WHI participants are assigned to one of 4 WHI Regional Centers or one of 5 affiliated subsites or the CCC for selected follow-up and outcomes documentation responsibilities. These 4 Regional Center sites (RCs) are: University at Buffalo (Jean Wactawski-Wende, PI), Wake Forest University (Sally Shumaker, PI), Ohio State University (Rebecca Jackson, PI), and Stanford University (Marcia Stefanick, PI). The 5 affiliated subsites include Brigham and Women's Hospital (JoAnn Manson, PI), University of Pittsburgh (Lewis Kuller, PI), University of Florida (Marian Limacher, PI), Iowa State University (Jennifer Robinson, PI) and the University of Arizona (Cynthia Thompson, PI). For COSMOS, all participants recruited from the list of VITAL responders and others recruited by BWH will be assigned to BWH for the purposes of these follow-up related activities.

7.4. Follow-up

Approximately 6 months and 12 months following randomization and semi-annually thereafter, COSMOS participants will be mailed a follow-up packet containing a cover letter, scannable follow-up questionnaires, and a return envelope. Questionnaires will assess compliance with randomized treatments, use of non-trial cocoa and/or multivitamins, dietary intake of chocolate, cocoa, and related foods, risk factors, and update medical history. Participants who do not respond to the follow-up mailing within 8 weeks will be sent a second mailing. Those who do not respond to the second mailing within 8 weeks will be contacted by phone. COSMOS participants who are in the WHI will receive separate annual mailings from the WHI CCC that include the WHI Form 33-Medical History update and a WHI medical records.

Up until the 12-month post-randomization follow-up phase, follow-up packets will be returned to the CCC from participants recruited by WHI and to BWH for participants recruited by BWH for scanning into the COSMOS database. Starting with the 12-month questionnaires, all COSMOS-specific questionnaires will be returned to the BWH for scanning into the database and follow-up. Packets will be reviewed to determine whether there are any notes from participants requiring attention. Then data collection forms will be scanned and imaged and those indicating there are safety concerns will be immediately flagged for follow-up by BWH staff. Forms that indicate a study outcome may have occurred will be evaluated and processed according to existing WHI procedures. For WHI participants, Regional Centers will receive reports of all potential outcomes that require documentation as well as the contact information for the health care-provider associated with that outcome.

For BWH participants, reports of study outcomes will be evaluated by BWH staff in accordance with existing WHI guidelines.

BWH will resupply each participant with their blinded calendar packs of assigned supplements at approximately 12 months post-randomization and annually thereafter. The timing of these mailings will be determined to reduce the likelihood of interruptions in use without considerable overlap.

At 6-month intervals between annual questionnaires, all participants will receive an interim questionnaire to address compliance, potential side effects related to the interventions, and collect records to confirm key endpoints. COSMOS participants will also be given the option of completing annual and interim follow-up questionnaires online through an e-mailed link. The online data will be collected and managed using REDCap (Research Electronic Data Capture), a secure, HIPAA-compliant web-based application hosted by Partners HealthCare Research Computing Enterprise Research Infrastructure & Services (ERIS). WHI participants already get a newsletter for the purposes of maintaining contact information at 6 month intervals; all COSMOS participants will receive annual newsletters to maintain contact as well.

7.5. Safety and Adverse Events

The interventions used are generally considered to be safe; indeed multivitamin use is ubiquitous so few if any serious adverse effects attributable to use of these supplements are expected. Participants will be fully informed of the known risks and benefits and participants will be provided with a toll-free number to call at any time if they have concerns about potential adverse effects of these interventions. In addition, routine data collection will assess participant experience with a range of potential conditions that may be affected. Study staff will follow standardized IRB and Human Subjects guidelines for determining whether the event is an adverse event (AE), serious adverse event (SAE), or unanticipated problem (UP) and follow national and institutional reporting requirements. These reports may come to either BWH or the CCC. If the report comes initially to the CCC, it will be referred to BWH within 24 hours for evaluation. BWH staff will determine whether the participant should continue with the COSMOS intervention or stop, either permanently or temporarily. Regardless of intervention status (continue, stop, stop temporarily), participants will be followed for study outcomes. For cocoa extract, potential side effects include gastrointestinal tract symptoms (constipation, diarrhea, dyspepsia, gastritis, and nausea). For multivitamins, fatigue, drowsiness, skin rash or discoloration, migraine, and minor bleeding (hematuria, epistaxis, easy bruising/other bleeding) will be considered potential side effects. We will also consider other potential side effects as appropriate.

Blood drawing may cause a small amount of pain. In addition, there may be a temporary bruise or “black and blue mark.” Rarely, people faint when their blood is drawn. Very rarely, the vein may become red and swollen, or infected.

BWHI staff will be responsible for all regulatory reporting.

7.6. Unblinding

If in the judgment of the BWH staff reviewing the status of a participant reporting a safety concern, the participant and/or her/his health care provider needs to be informed of the supplements they were using, an authorized unblinding officer in the BWH staff will log into the COSMOS database and execute a database function that will provide the treatment assignment. The unblinding officer will be required to record the reason for unblinding, and the staff member who requested it, if she or he is not the unblinding officer directly.

7.7. Early Discontinuation

Participants in COSMOS will be allowed to change the nature of their participation at any time. Those who wish to discontinue one of the supplements will be allowed to continue all other aspects of the protocol. Similarly those who are not willing to take any of the supplements but are willing to provide other follow-up information will continue to receive all follow-up mailings but will not be dispensed supplements. For WHI participants, withdrawal from COSMOS will not affect their participation in the WHI Extension Study.

7.8. Study Close-out

To assure complete ascertainment of outcomes, a final data collection packet containing a cover letter and a final COSMOS follow-up questionnaire will be sent to each participant at the end of their intervention period. COSMOS participants who are in WHI will also receive the WHI Form 33 Medical History Update and a medical release. Participants will not be unblinded without cause until the final Form 33 has been collected to assure unbiased outcomes data collection. Participants will be sent their unblinded treatment assignment and a brief lay-version of the primary study results when available.

7.9. Data Collection and Management

All trial self-reported information will be obtained directly from COSMOS participants or their designated proxies through the baseline, interim, and follow-up postcards and questionnaires. COSMOS participants recruited by the WHI will be followed by the WHI CCC prior to the 12-month post-randomization phase. Beginning with the 12-month follow-up phase, all participants will be followed by BWH staff.

The WHI CCC study documents mailed from and returned to the WHI CCC will use mark sense technology (bubble forms) consistent with the 20 year history of WHI participant data collection forms. The CCC will review, mark comments, and scan all the questionnaires using Scantron forms and imaging so that the image of the form will be accessible to WHI staff. The CCC will key enter the consent forms of WHI participants and store images of all forms electronically.

BWH questionnaires will be optically scanned and the data extracted into the computer. The relevant software, Cardiff TeleForm, has been successfully used at BWH for several years in the WHS, PHS, and VITAL trials. Inconsistent and unclear data will be reviewed by BWH staff who will edit misread variables. All data will undergo additional within-form and across-time checks to verify accuracy. The BWH database will be backed up nightly, ensuring at least two current copies maintained at BWH at all times.

The BWH computing system, which was fine-tuned in previous trials conducted by the BWH Division of Preventive Medicine, will track each participant's stage in the study and level of participation. Letters, questionnaires, and follow-up reminders will be automatically generated. Names, addresses, telephone numbers, participant status, and processing information will be kept up to date and data from questionnaires, letters, and phone calls will be entered directly into the database. When talking to study participants, study personnel will have ready access to identifying information, participation level, and content of previous study-related telephone calls.

Nightly transfers of study data between the WHI CCC and BWH will ensure the availability of the most current participant information for both study sites. COSMOS participant data is available only to authorized staff members at both BWH and the WHI CCC.

7.10. Outcomes Collection

For efficiency and to maintain consistency with previous work in WHI, all COSMOS outcomes processing will use the existing WHI procedures to ascertain and validate outcomes of interest for WHI and non-WHI participants. The CCC will send the Form 33--Medical History Update and a standardized medical release form as part of their follow-up packet to ascertain self-reported outcomes of interest to all female COSMOS participants from the WHI. CCC staff will scan these returned forms into the WHI database.

COSMOS participants who are part of the WHI Medical Records Cohort (MRC) will have medical records retrieval and adjudication performed per normal WHI procedures. COSMOS participants who are part of the WHI Self-Report Cohort (SRC) will be flagged in the database and will enter the records retrieval and adjudication process exactly as the MRC participants. The flagging will be used to segregate these cases from the self-report cohort for billing purposes.

All potential COSMOS outcomes among WHI participants will be flagged and identified in the appropriate Regional Center reports. Regional Centers will review questionnaires for completeness and for events requiring medical records. The Regional Centers will contact these COSMOS participants as needed to clarify or complete responses and confirm the participant signed the Release of Information form on the Medical History Update questionnaire. If not, or if an institution-specific medical release form is needed, Regional Center staff will obtain the necessary release form. Regional Centers will then obtain the required medical records and submit them to the CCC electronically following existing WHI procedures. Returned documents will be reviewed by CCC staff. For CVD outcomes that fall within the scope of the WHI adjudication plan, the CCC will send the associated medical records to WHI physician adjudicators for review and confirmation (or denial) of outcomes.

COSMOS participants recruited from BWH who report any of the COSMOS outcomes will be sent a medical release form by BWH staff and will enter a records retrieval and adjudication process similar to the WHI at BWH. CVD outcomes will be sent to BWH adjudicators who are familiar with WHI outcomes definitions and procedures. BWH adjudicators will use an online adjudication system to adjudicate these events according to existing WHI definitions.

Since unstable angina and ACS requiring hospitalization are not adjudicated for WHI participants in COSMOS, all of these self-reports will be adjudicated by BWH staff using the existing medical release document, record retrieval, and adjudication procedures used by BWH for all other outcomes.

All cancers for WHI participants will be reviewed and coded according to SEER standards by trained cancer coders at the CCC. BWH staff will provide equivalent coding to cancers for COSMOS participants who are not also part of WHI.

All outcomes adjudicators and cancer coders will be blinded to individual randomization assignment and all interim treatment arm contrasts for all study outcomes.

7.11. Documentation and Definition of COSMOS study outcomes

7.11.1. Cardiovascular Disease (CVD) events: For CVD events, discharge summaries, ECGs, laboratory values, and test reports will be sought.

Definite and probable nonfatal MI requiring overnight hospitalization is defined with an algorithm based on standardized criteria including cardiac pain, cardiac enzymes and troponin levels, and ECG findings. MI during surgery or aborted by thrombolytic therapy or procedures are also included.

Coronary heart disease (CHD) death includes deaths consistent with CHD as an underlying cause.

Coronary revascularization includes documented coronary artery bypass graft (CABG) surgery, percutaneous transluminal coronary angioplasty (PTCA), coronary stent, or artherectomy.[38]

Unstable angina or ACS requiring hospitalization includes review of medical records for reports of increased pain, use of medications to alleviate pain, and evidence of troponin leak, as well as other related factors.

Carotid artery surgery and peripheral artery surgery documentation includes review of surgical and radiology reports.

Stroke is classified according to the Trial of Org 10172 in Acute Stroke Therapy (TOAST)[39] and Oxfordshire subtype.[40] Supporting the confirmation of major CVD events by medical record review through the COSMOS would be of tremendous value to the WHI cohort.

7.11.2. Cancer: For incident cancers, discharge summaries, pathology reports, operative reports, and diagnostic or treatment procedure reports, including both inpatient and outpatient diagnoses will be requested.

Invasive cancer is considered confirmed if a pathology report substantiates a malignant primary invasive cancer at any location other than non-melanoma skin cancer.[38] All histologic types and anatomic subsites are included. Noncancerous colorectal polyps, atypical benign breast disease and other premalignant benign conditions are not included. Second primaries at a site and recurrences of cancer will be ascertained.

Tissue samples will be collected from COSMOS participants with confirmed diagnoses of incident cancer. (Please see section 9.2 regarding the proposed COSMOS Cancer Tissue Study.)

7.11.3. Deaths: For participants determined to be deceased, BWH or WHI Regional Center staff, as appropriate, will retrieve a death certificate. A condolence letter will be mailed to the family, requesting permission to obtain medical records and a copy of the death certificate. If the family does not provide the death certificate, a copy will be requested from the state vital records bureau where the participant died.

If there is indication on the death certificate of a COSMOS outcome of interest, a case will be opened, and medical records pursued to confirm (or deny) the outcome.

7.12. Passive follow-up.

The CCC periodically conducts a linkage to the National Death Index data base to ascertain vital status and cause of death information for all WHI participants who have been lost to follow-up or for known deaths for cases where we could not obtain records. The CCC will expand this search to include all COSMOS participants including a final search at the end of the COSMOS follow-up.

To complement the standardized methods for endpoint validation in COSMOS, we will use Centers for Medicare & Medicaid Services (CMS) database linkage. We will obtain annual updates of information on all inpatient and outpatient claims as well as durable medical equipment and we anticipate access to the prescription drug databased shortly. This database includes US adults aged ≥65 years, matching the entire WHI population and the majority of men and women recruited by BWH for whom we also expect high coverage. Linkage to other national databases, for example cancer registries or the Health Maintenance Organization Research Network, may also be done to improve the efficiency or the comprehensiveness of outcomes collection.

8.0. Biospecimen and Blood Pressure (BP) Substudy

To determine whether cocoa flavanols and/or a daily multivitamin use will significantly change levels of blood flavonoids, other pertinent nutritional biomarkers, vascular/metabolic biomarkers, and blood pressure from baseline, we will conduct a substudy in a sample of approximately 3,500 male and 3,500 female COSMOS participants who sign a separate consent for this additional procedure at baseline. COSMOS participants selected will either have baseline bloods and urine collected on their own through mailed blood kits that are returned by the participant, or have bloods, urine, BP, and brief anthropometric measurements collected and shipped through Examination Management Services, Inc (EMSI). As an alternative to EMSI, Quest diagnostic labs are available to assist with the specimen collections and shipments. A subgroup of these participants will also have follow-up samples and measurements collected again during follow-up using similar procedures. The total amount of blood collected will be 80 ml: 40 ml at baseline and 40 ml at follow-up. The total amount of urine collected will be 20ml: 10ml at baseline and 10ml at follow-up.

BWH will manage this protocol at baseline and post-randomization time points. BWH will be responsible for consenting participants and tracking, storing, and distributing COSMOS bloods and urine to appropriate laboratories. For assays related to COSMOS, BWH will coordinate with the labs for shipping, testing protocols and receipt of results data. One half of the specimen vials from WHI participants which are not used for the COSMOS study will be shipped to the WHI repository for storage at the conclusion of the COSMOS trial. These specimens will be inventoried and tracked in the WHI Study Tracking System (WHIST). Should the COSMOS blood collected for the parent trial fail to yield adequate lab results, BWH may request the CCC to ship a replacement vial for retesting.

9.0. Clinic-Based Sub-study

Additional measurements will be obtained from clinic-based visits at baseline and after 2-years follow-up for approximately 600 COSMOS participants residing in the greater Boston area at the Harvard Catalyst Clinical and Translational Science Center (CTSC). An equal number of women and men will be recruited. The clinic-based visits will include cognitive function assessments, physical assessments, blood pressure, and other measurements. A separate protocol and consent form will be developed and approved by the BWH IRB for the clinic-based COSMOS study visits.

9.1. Ancillary Studies

COSMOS Mind

COSMOS Mind cognitive data collection protocol will be modeled after the ongoing WHIMS-ECH and WHIMS-Y telephone-based protocols. Each assessment will be conducted by trained and certified examiners from the COSMOS-Mind Coordinating Center at Wake Forest and will be electronically audio-recorded for quality assurance. The interview consists of Story Recall 1 & II, Oral Trail Making test, Verbal Fluency, Number Span Test, Digit Ordering Test, Cognitive Change Index, Geriatric Depression Scale-Short Form and the Women's Health Initiative Insomnia Rating Scale. The interview will take approximately 45 minutes to complete. Participants' Responses will first be recorded on paper versions of the test and later entered into a dedicated and secure data management website. A secure web-based call tracking system will be used to monitor participant contact information. Data will be managed from a secure ftp site at Wake Forest. The interview takes approximately 45 minutes.

COSMOS Web

COSMOS Web was designed as a 'novel object recognition' task. During the immediate matching trials, participants view a single complex stimulus for 10 seconds; following a 1 second delay they are asked to select which one of two objects is identical to the studied stimulus. Following all matching trials, participants are shown serially individual complex objects and asked to indicate whether each object is identical to any of the target stimuli studied during the immediate matching trials. On these recognition trials, half the stimuli are targets and half are foils. The primary dependent variable for the COSMOS Web is the mean reaction time (*ms*) for correct rejections of foil stimuli on the delayed recognition trials. Multiple versions (alternate forms) were created for repeat administration. There are 2 other cognitive assessments, Spatial Learning task and Flanker Task, in the web-based assessment. The interview takes approximately 25 minutes to complete.

COSMOS Eye

COSMOS Eye endpoints will be identified from reports on baseline and annual follow-up COSMOS questionnaires and from interim reports received through mail, email, or telephone conversations. Participants will be asked to provide the name(s) and contact information for treating physicians and written consent to obtain medical records. Treating physicians will be asked to complete a questionnaire related to the diagnoses or to provide relevant medical records. Participants with medical record confirmed diagnoses of AMD will be sent follow-up questionnaires to assess progression of AMD.

COSMOS Falls and Physical Performance

Falls and decreased physical performance are major public health problems among older adults. Each year in the U.S., 30% of adults aged ≥65 years fall, resulting in increased risk of injury, hospitalization and death. Several lines of evidence suggest that flavanols, commonly found in cocoa, confer beneficial effects on muscle function and physical performance. The effects of supplemental cocoa flavanols on recurrent falls, injurious falls resulting in healthcare utilization, and musculoskeletal health, will be examined in a cost-efficient ancillary study. The benefits and/or risks of multivitamins on musculoskeletal health among the study population will also be assessed.

Throughout the COSMOS intervention phase testing cocoa flavanols and a multivitamin, information on falls, fractures, and the use of medications for bone health was collected at baseline and on the periodic follow-up health questionnaires. Information from the main trial questionnaires will be used to determine whether cocoa extract reduces risk of recurrent falls (defined as 2 or more falls per year), injurious falls resulting in healthcare utilization, and fractures. A follow-up Fall Questionnaire will be sent to assess circumstances surrounding injurious fall(s) with healthcare utilization (e.g., reason for fall, location of fall) and medical records will be obtained for adjudication of the fall and fall-related injuries.

Information obtained from the clinic sub-cohort (n = 603) at baseline and 2-years post randomization will be used to test whether cocoa flavanols improve physical performance as assessed by grip strength (by dynamometer), walking speed (6 meters), and the Short Physical Performance Battery (SPPB) that includes tests of walking speed, standing balance, and chair stands. The effects of the study agents on changes in bone density will also be explored.

Results from analyses of the biospecimens will be used to examine effect modification by changes in flavanol concentrations on the fall(s) and fractures outcomes. Parallel analyses of the multivitamin supplement also will be performed. COSMOS provides a unique opportunity to maximally leverage existing resources to test, at minimal cost, efficacy of cocoa flavanols and/or a multivitamin on improving clinically meaningful musculoskeletal outcomes among older women and men in the U.S.

Dr. Carolyn Crandall, Professor of Medicine at the David Geffen School of Medicine, UCLA, is an investigator on the COSMOS Falls and Physical Performance ancillary study to COSMOS. She will assist with design and conduct of the sub-study, adjudication of outcomes, interpretation of data, and presentation of the results. Mass General Brigham (MGB) staff will communicate with study participants to collect the sub-study questionnaires, signed authorization forms, and copies of medical records from healthcare providers. Once received, the records will be scanned onto a secure MGB network server managed by the Brigham and Women's Hospital Division of Preventive Medicine (DPM). Using an encrypted laptop, Dr. Crandall will have access to identifiable data on the secure, web-based online medical record review/outcome adjudication system through the MGB Virtual Private Network (VPN). No data will be transferred to Dr. Crandall or to the David Geffen School of Medicine at UCLA. A Research Data Sharing Plan will be put into place.

COSMOS Inflammaging and Epigenetic Aging

The effects of cocoa extract and multivitamins on longitudinal change in biological aging will be examined in an ancillary study conducted in collaboration with investigators from Augusta University. Accelerated epigenetic aging that comprises DNA methylation changes has recently emerged as a powerful, novel predictor for lifespan and health span, disease susceptibility, morbidity, and mortality risk. DNA methylation is a major epigenetic process that regulates gene expression and can be modified by different bioactive compounds in foods. In addition, human aging is characterized by chronic, low-grade inflammation, which has recently been termed inflammaging. Inflammaging is exemplified by mild chronic elevation in blood levels of C-reactive protein (CRP) and several cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which increase with aging. Inflammaging is a highly significant risk for both morbidity and mortality in the elderly, including cardiovascular disease.

Biospecimens collected from 600 COSMOS participants at baseline and after 1- and 2-years of follow-up will be analyzed for changes in markers of DNA methylation (including AgeAccelHorvath, AgeAccelHannum, LAgeAccelGrim, AgeAcfcelPhono, and DNAm TLadjAge) and inflammation. The relationship between cocoa extract and changes in epigenetic aging and inflammaging in relation to CVD will be evaluated. Parallel assessments of multivitamins on these markers of biological aging and CVD outcomes will also be performed. This ancillary study will provide valuable insights into the underlying molecular mechanisms for two popular supplements (cocoa and multivitamins) and holds great promise for targeting by nutritional interventions.

COSMOS Phase 1 Biomarker Assessments

BWH will send approximately 1,100 blood samples from 400 COSMOS participants to the University of California at Davis (UCD) for the measurement of key biomarkers linked with both the cocoa extract and multivitamin interventions. The assay results will be integrated into the analyses for the COSMOS Mind, Web, and coronavirus disease 2019 (COVID-19) projects. No additional samples will be collected from participants. No protected health information (PHI) will be sent to UCD.

COSMOS Phase 2 Biomarker Assessments

BWH will send approximately 11,600 spot urine samples from 6,795 COSMOS participants to the University of Reading (UR) for the measurement of key biomarkers linked with both the cocoa extract and multivitamin interventions. The assay results will be integrated into the analyses for the COSMOS interventions. No additional samples will be collected from participants. No protected health information (PHI) will be sent to UR.

9.2. COSMOS Cancer Tissue Study

We plan to establish a biobank of tumor tissue and slides for the future examination of the mechanistic effect, if any, of multivitamin and cocoa flavanol use on risk and survival from common cancers, such as breast, bladder, prostate, and lung, among the COSMOS population. The COSMOS trial provides a unique and cost-efficient resource to elucidate the molecular mechanisms by which the study supplements exert their benefit(s). We plan to examine the biologic effect of multivitamins and cocoa flavanols by leveraging cutting-edge molecular and genomic technologies (including but not limited to immunohistochemistry, RNA-sequencing, whole genome sequencing, epigenomic assays) to better understand cancer etiology.

To determine the feasibility of establishing a biobank of cancer tissue, we plan to conduct a pilot study to assess willingness of COSMOS participants to provide consent for their tissue samples to be collected and the success of obtaining tissue blocks and/or slides from hospitals and providers' offices. We will request samples only from those participants who have confirmed diagnoses of incident cancers. As per study protocol, we will first request consent to review medical records from participants who self-report a new or recurrent cancer diagnosis on the periodic COSMOS health questionnaires. For those who provide consent, we will obtain the relevant medical records from treating physicians or healthcare facilities. Once the records are received, reviewed by COSMOS investigators, and the diagnoses are confirmed, we will send participants a letter to request their tissue samples. Our goal is to conduct the pilot study in a sample of up to 80 participants with confirmed cases of incident cancers.

9.3 **Coronavirus disease 2019 (COVID-19)**

In response to the coronavirus disease 2019 (COVID-19) pandemic, we will send COSMOS participants email invitations for online REDCap surveys that have been prepared to collect critically needed, time-sensitive data on the epidemiology of COVID-19 in older adults, who have been disproportionately affected by the ongoing pandemic.

COSMOS participants will be combined with participants in two other active, large-scale, long-term prospective studies conducted at our Division of Preventive Medicine, the VITamin D and Omega-3 TriaL (VITAL) (protocol #2009P-001217), and the Women's Health Study (WHS) (protocol #2004P-001661), for a total of 52,000 participants who will be sent the email invitation with the secure link to the COVID-19 survey. Participants in these three studies have demonstrated interest, dedication, and continued participation in medical research and disease prevention through the completion of questionnaires and other study activities.

Participants who provided email addresses and have indicated willingness to be contacted by email to complete surveys online will be sent the invitation with the secure link to the COVID-19 survey. We will send reminder emails to non-respondents. The survey is consistent with our REDCap surveys sent and completed in the past and will collect critically important and time-sensitive information about symptoms, testing, diagnoses, treatments, severity of illness, and risk factors for the coronavirus. If willing, anyone who reports having the coronavirus infection and/or COVID-19 hospitalization will be contacted to provide consent to review their medical records following standard procedures already in place for the study.

COVID-19 surveys will be sent at three different time points spaced approximately one month apart to update participant status with regard to coronavirus testing, symptoms, diagnoses, and other relevant risk factors during this evolving pandemic. The data from these COVID-19 surveys will make seminal contributions to our understanding of the causes and consequences of coronavirus infection and COVID-19 during this pandemic.

COVID-19 is an ongoing global pandemic currently affecting the US, yet little is known about risk factors for illness, including more severe illness and indicators of recovery. COSMOS will collaborate with the **Re**searching **COV**ID to **E**nhance **R**ecovery (RECOVER) study, a National Institutes of Health (NIH) COVID-19 Initiative Project to address this issue. COSMOS will send an initial email to inform participants about this new research opportunity. Participants that are interested to learn more about the RECOVER study have the option to click on a secure REDCap link to view the invitation letter and brief screening survey. The COSMOS group will identify eligible and willing participants, and their screening information will be sent to the RECOVER group through a secure MGB server.

10.0. Statistical Analysis

In this 2x2 factorial trial, the primary aim is to compare the main effects of intention-to-treat with cocoa extract on incident CVD events, and multivitamins on rates of diagnoses of new cancers. The primary analysis be based on the intention-to-treat principle and use the Cox proportional hazards model for time-to event data[41] and estimate the HR for each intervention using indicators for the specific treatment, controlling for the second intervention, and stratifying the baseline hazard by (at minimum) sex, age group, and recruitment center (WHI or BWH). P-values will be based on a score tests for each intervention hazard ratio. Because the cohort consists of older women and men, competing risks due to deaths from other causes will be also be incorporated. Specifically, the primary analyses will estimate the cause-specific hazard and HR comparing intervention groups for each outcome by censoring individuals with deaths due to competing causes. We will estimate the cumulative incidence function for each comparison (multivitamin versus placebo, cocoa flavanols versus placebo) and plot the subdistribution of each endpoint over time.[42,43]

The alternative Fine and Gray approach[44] models the effect of treatment on the subdistribution hazard or directly on the cumulative incidence function. Although we will consider this model, Cox proportional hazards will be our primary analysis. Additional analyses of composite endpoints that include total mortality will provide a practical approach to the evaluation of the impact of competing risks.

In secondary analyses, we will examine effect modification by the other randomized intervention, gender, age, other key risk factors, time, and concomitant medications such as statins and aspirin. Interactions will be interpreted cautiously as hypothesis generating. We will also stratify by past randomization status in WHI trials. We will also consider baseline dietary intake and a subcohort of women and men with biomarker data analyzed as a case-cohort study using methods for proportional hazards regression.[45] Because of frequency matching by age, we will use appropriate stratum-specific weighting of observations.[46]

11.0. Data Management

The COSMOS data repository will be a single centralized database maintained by BWH

All pre-randomization participant data collected from all BWH participants and WHI participants who signed a COSMOS consent form are stored on the BWH data systems. Prior to the 12-month post-randomization phase, follow-up for missing and incomplete information is done by each institution separately but the data are stored centrally at BWH. Secure file transfer protocols are used to share data between the two institutions. Eligibility assignment during pre-randomization is performed by the WHI CCC for each participant and the data is transferred back to BWH securely.

Separate web applications at each institution allow their respective staff to perform the necessary tasks. The CCC will implement systems to support recruitment and screening mailings, data entry, and scanning of forms for WHI participants who enroll into COSMOS; modify the outcomes processing system to accommodate

changes in outcomes collection required for WHI participants; and create datasets and reports to support study monitoring and analyses. BWH will be responsible for recruitment and screening of BWH participants; scanning of BWH forms; ancillary study and biospecimen project mailings; post-randomization form mailings beginning with the 12-month follow-up phase; and study pill dispensing. The CCC will support BWH's participant unblinding efforts and create final datasets for analyses and data sharing purposes.

Starting in September 2016, some study data will be collected electronically using REDCap (Research Electronic Data Capture), a free, secure, HIPAA-compliant web-based application hosted by the Partners HealthCare Research Computing, Enterprise Research Infrastructure & Services (ERIS) group. Vanderbilt University, with collaboration from a consortium of academic and non-profit institutional partners, has developed this software and workflow methodology for electronic collection and management of research data. Our research team will develop REDCap surveys that mirror the standard paper-based questionnaires used throughout the trial. REDCap provides features that can be used for a variety of research projects and provides an intuitive interface to enter data with real-time validation (automated data type and range checks). The system offers easy data manipulation with audit trails, reports for monitoring and querying participant records, and an automated export mechanism to common statistical packages (e.g., SAS).

Participants' names and contact information will be accessible only to staff members who need the information for their jobs. Endpoint and health-related questionnaire data will be stored in separate files from the processing data and will be accessible only to approved investigators and programmers. In these files, participants are identified only by study ID.

11.1 Sending Data/Materials to Research Collaborators outside Mass General Brigham

A deidentified data set will be sent to Dr. Linda Neal, a retired professional data analyst, for dementia research. The data sent will contain deidentified variables from the COSMOS Mind ancillary study tests outlined in Section 9.1. The data will be securely transferred using the Partners Secure File Transfer system.

Limited data sets will be sent to Dr. Yu-Han Chiu at Penn State College of Medicine in Hershey, Pennsylvania and Dr. Soshiro Ogata at the National Cerebral and Cardiovascular Center in Suita, Japan for data analysis purposes. The data sets will contain descriptive, adherence, and outcome variables. The data will be securely transferred using the Partners Secure File Transfer system.

12.0. Study Monitoring

An independent Data and Safety Monitoring Board (DSMB) for COSMOS will be convened with appropriate expertise in clinical trials, epidemiology, biostatistics, and relevant clinical areas such as CVD and cancer. Representatives from the VITAL DSMB and the parallel WHI oversight committee (currently the DSMB) will be invited to participate.

The role of the COSMOS DSMB will be to examine the unblinded data on adherence, endpoints and adverse effects and recommend continuation, alteration in study design, or early termination as appropriate to assure the ethical conduct of the study.

Interim trial results will be assessed with the Haybittle-Peto rule,[47,48] adjusting for multiple looks. In this method, 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. This rule appropriately requires very strong evidence for early stopping, is more conservative than the Pocock[49] and O'Brien-Fleming[50] rules

and the alpha-spending function,[51] and can be conducted at convenient times without inducing statistical complexity.[52]

While these rules are intended for the primary endpoints, the goal is to assess the overall balance of benefits and risks of the two agents. Thus, consideration will also be given to the secondary endpoints that are needed in the interpretation of overall results. The monitoring rules will serve as guidelines in evaluating the strength of the existing data regarding benefits and harms. All decisions for continuation or stopping of treatment arms must be made after examining the totality of evidence, including other trial data, on these agents. Each treatment arm comparison will be considered independently of the other, i.e., one intervention may be stopped while the other may continue without any disruption in the planned follow-up.

13.0. Quality Assurance

Redundancies will be built into the data processing systems to ensure accurate recording of data and proper follow-up. All research forms will be scanned in and the data read by a character-recognition software program (Cardiff TeleForm). Inconsistent, and unclear data will be reviewed by a verifier who will edit misread variables. Information from forms that cannot be scanned will be copied onto new forms. The new data forms will be scanned and the original, damaged forms will be filed for future reference, if necessary. All data will undergo additional within-form and across-time checks to verify accuracy. Following data entry, all questionnaire responses that require additional follow-up for missing data, participant comments on the form or for endpoint validation will be manually reviewed to ensure correct processing and accurate follow-up.

Great care will be taken to ensure that participants receive their randomly assigned drugs. Upon receipt of drug shipments from the manufacturer, each of the four drug groups will be stored in segregated areas. A random sample of drugs bottles or calendar packs will be pulled from each drug group and blindly tested to insure that the packaged contents match the study label. At the time of packaging for shipment to individual participants, listings will be divided according to drug group assignment and only one group will be packaged at a time. The packaging area will be cleaned and cleared prior to packaging and shipment of the next drug group. If drug packages are returned by the postal service as undeliverable or declined by a participant, the contents of the package will be blindly tested as an additional level of quality control.

14.0. Study Administration and Organization

The BWH, WHI CCC, WHI Regional Centers, and WHI and VITAL investigators have worked closely to develop leadership and collaborative infrastructure. The Steering Committee, Ancillary Studies Committee, Publications and Presentations Committees, and other committees will include both WHI and BWH representatives. We expect to include all interested investigators in future projects involving the trial, and in the leadership and conduct of ancillary studies.

15.0. Study Timeline

The study plan assumes that recruitment, placebo run-in and randomization procedures will be completed for all 22,000 participants within 1-3 year of study initiation. This will allow an average of 4 years of intervention and follow-up; and 6 months for trial close-out and analyses (Table 2):

Table 2. Anticipated timeline for completion of the COSMOS trial

Activity	Y1	Y2	Y3	Y4	Y5

Finalize protocol and arrangements for study pills	X				
IRB approvals	X				
Subject recruitment	X	X	X		
Placebo run-in	X	X	X		
Treatment and follow-up	X	X	X	X	X
Biomarker and blood pressure collection	X	X	X	X	X
Clinic-based assessments	X	X	X	X	X
Study closeout					X
Analyses and publication of final manuscripts				X	X

16.0. Ancillary Studies

Ancillary studies to the COSMOS will proceed through the WHI Ancillary Studies Committee approval process. The WHI DCC (Project Coordinator) will provide administrative support as needed for ancillary study committee activities related to this trial.

17.0. Appendix

- COSMOS Consent
- Cover Letter for WHI participants for COSMOS 1
- Cover Letter for non-WHI participants for COSMOS 1
- COSMOS 1 (Screening form)
- COSMOS 2 (Baseline form)
- COSMOS 3 (Post run-in Adherence form)
- COSMOS Food Frequency Questionnaire
- COSMOS 6-Month Questionnaire
- COSMOS 12-Month Questionnaire
- COSMOS 18-Month Questionnaire
- COSMOS 2-Year Questionnaire
- COSMOS Food Frequency Questionnaire 2
- COSMOS 30-Month Questionnaire
- COSMOS 3-Year Questionnaire
- COSMOS 42-Month Questionnaire
- COSMOS 4-Year Questionnaire
- COVID-19 Survey
- June COVID-19 Survey
- August COVID-19 Survey
- COSMOS January 2021 Questionnaire
- COSMOS Obs 1 Questionnaire
- COSMOS Obs 2 Questionnaire
- COSMOS Obs 3 Questionnaire

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