PARTNERS HUMAN RESEARCH COMMITTEE
PROTOCOL SUMMARY

Answer all questions accurately and completely in order to provide the PHRC with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.

PRINCIPAL/OVERALL INVESTIGATOR

Karen H. Costenbader

PROTOCOL TITLE

Vitamin D and Fish Oil for Autoimmune Disease, Inflammation and Joint Pain

FUNDING

National Institutes of Health R01 AR059086-01

VERSION DATE

November 9, 2009

SPECIFIC AIMS

Concisely state the objectives of the study and the hypothesis being tested.

1. To test whether vitamin D_3 and/or omega-3 fatty acid supplementation reduces the risk of incident autoimmune disease in five years in 20,000 elderly adults enrolled in an ongoing randomized controlled trial

2. To assess the effects of vitamin D_3 and/or omega-3 fatty acid supplementation upon levels of biomarkers of systemic inflammation over five years in a population of 2,000 older Americans in an ongoing randomized controlled trial

3. To investigate whether vitamin D_3 and/or omega-3 fatty acid supplementation decreases chronic knee pain among 2,000 older adults followed for five years in an ongoing randomized controlled trial

BACKGROUND AND SIGNIFICANCE

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

Vitamin D and omega-3 fatty acids are two inexpensive and nontoxic nutritional supplements, widely held to have anti-inflammatory, immune- and pain-modulating effects. The purported health benefits of the relatively inexpensive and available dietary supplements vitamin D and omega-3 fatty acids have received enormous attention in both the medical literature and the popular press[1-17]. We propose to assess the roles of vitamin D and omega-3 fatty acids for three important outcomes in the elderly: prevention of autoimmune disease, effects on biomarkers of systemic inflammation, and modeulation of chronic knee pain. We will leverage the infrastructure of a newly NIH-funded randomized, double-blind, placebo-controlled clinical trial, the ***VIT***amin D and Omeg***A***-3 Tria***L*** (***VITAL***), for the primary prevention of cancer and cardiovascular disease.

Autoimmune diseases, including rheumatoid arthritis, autoimmune thyroid disease, inflammatory bowel disease, polymyalgia rheumatica and psoriasis, are increasingly prevalent with age and are associated with significant morbidity and medical expenditures. These diseases are associated with autoantibody production and systemic inflammation. No preventive therapy currently exists. Systemic inflammation associated with these diseases, as well as with increasing age, body mass index, and cigarette smoking, independently predicts cardiovascular disease, diabetes mellitus and mortality[18-23]. Inflammation and immune activation, as well as muscle weakening are involved in the development of chronic knee pain, one of the most common causes of pain and disability in elderly Americans. Chronic knee pain, largely due to osteoarthritis, imposes a huge economic burden, increasing with the aging of the "baby-boomer" generation.

Understanding of the pluripotent immunomodulating and anti-inflammatory beneficial effects of vitamin D is rapidly advancing. In addition to its roles in regulating calcium homeostasis and bone turnover, vitamin D has effects on multiple cells of the immune system, inhibiting pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) and lowering C-reactive protein (CRP)[24]. Data from laboratory studies and cross-sectional and observational epidemiologic investigations strongly suggest a protective effect for vitamin D in autoimmune disease susceptibility, but there have been no large randomized trials of high-dose vitamin D supplements for the primary prevention of autoimmune diseases or effects on systemic inflammation in a general population. Data on vitamin D and joint pain point to multiple potential therapeutic mechanisms, but the effects of nontoxic doses of vitamin D on chronic knee pain in the elderly have not been tested.

Marine omega-3 fatty acids exert effects through the leukotriene and prostaglandin pathways, decreasing inflammatory mediators and cytokine production, and have multiple known anti-inflammatory properties[25]. High dose omega-3 fatty acids have shown promise in the treatment of those with ongoing autoimmune diseases, reduction of biomarkers of systemic inflammation in large observational studies, and treatment of various types of pain and arthritis. However, no randomized controlled trials of these supplements have ever tested their effects in the primary prevention of autoimmune diseases, reduction of circulating biomarkers of systemic inflammation, or amelioration of chronic knee pain in a large elderly population.

RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by Partners researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, "Enrollment at Partners will be limited to adults although the sponsor's protocol is open to both children and adults."

The VITAL trial (Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259) will involve 20,000 men and women from across the U.S., selected on age only (men aged ≥60 and women aged ≥65), with an oversampling of Blacks. In a 2x2 factorial design, participants will be randomized to moderate-to-high dose vitamin D_3 (cholecalciferol; 2000 IU [50 µg]/d) and marine omega-3 fatty acids (EPA [500 mg/d] + DHA [500 mg/d]) supplements (or placebos).

The proposed investigations will take advantage of an NIH-funded large RCT of vitamin D and omega-3 fatty acid supplementation for the prevention of cancer and cardiovascular disease. The ***VIT***amin D and Omeg***A***-3 Tria***L*** (***VITAL*** ,JoAnn Manson, MD, DrPH, PI) is a randomized, double-blind, placebo-controlled trial of the benefits and risks of vitamin D (vitamin D_3 [cholecalciferol], 2000 IU/d) and omega-3 fatty acids (800 mg/d; EPA to DHA ratio, 1:1) among

20,000 men and women, aged ≥60 and ≥65, respectively. VITAL cohort assembly has begun and baseline mailings will begin in January 2010. The three outcomes that we will test in this ancillary grant include: prevention of autoimmune diseases, and effects on systemic inflammation and chronic knee pain. At the end of a 3 month run-in period, participants will be randomly assigned to one of four treatment groups for 5 years: vitamin D_3 (2000 IU/d) and fish oil (EPA+DHA, 800 mg/d); vitamin D_3 and fish oil placebo; placebo vitamin D_3 and fish oil; and placebo vitamin D_3 and placebo fish oil. At 1-year intervals, participants will receive a new supply of pills, and assessments of incident autoimmune diseases, compliance and potential side effects. All autoimmune disease endpoints will be confirmed by medical record review by the physician endpoints committee. We anticipate validation of 600 incident cases of autoimmune disease, giving us sufficient statistical power to assess supplement effects on disease incidence. Blood samples at baseline and after 1-2 years follow-up will be collected in a subcohort of 2000 individuals and analyzed for changes in biomarkers of systemic inflammation: C-reactive protein, interleukin-6, and tumor necrosis factor-α. Approximately 2000 individuals with chronic, daily knee pain on baseline screening will be followed with annual questionnaires, providing ample statistical power to evaluate the effects of vitamin D and fish oil upon changes in chronic knee pain. This trial will be very cost-effective as it will be conducted by mail. Each participant will be randomly assigned in a 2x2 factorial design to vitamin D, fish oil, both agents, or both placebos (anticipated mean treatment duration of 5 years).

Study population. The study population will be that of the parent VITAL trial, described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov NUMBER01169259. It will include approximately 20,000 apparently healthy participants—10,000 men aged ≥60 and 10,000 women aged ≥65, ages at which rates of chronic diseases, including autoimmune diseases and knee OA pain, increase substantially.

Cohort assembly, eligibility, run-in and randomization are described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

Baseline autoimmune disease prevalence assessment. The enrollment questionnaire accompanied by a cover letter will be sent to all participants and will include a question regarding personal and family history of five specific autoimmune diseases (AITD, IBD, psoriasis, RA and PMR) as well as space to write in others. Individuals will also be asked about their family history of any autoimmune disease.

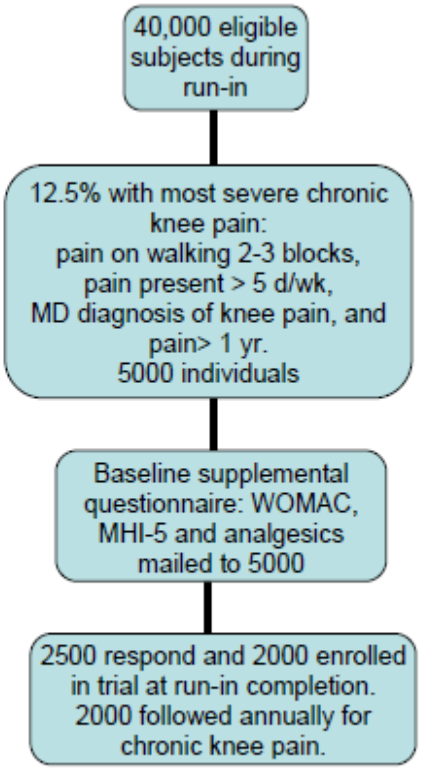

Fig. 2. Screening for chronic severe knee pain during run-in

Screening for chronic daily knee pain during run-in. As VITAL participants will be involved in the parent trial of cancer and cardiovascular outcomes and potentially other ancillary studies, to limit participant burden we will screen the entire population and select 2000 participants with chronic daily and severe knee pain who are willing and eligible to be in this subtrial. This number will provide ample statistical power for chronic knee pain outcomes.

For our purposes, the outcomes of interest will be <u>chronic frequent knee pain</u> (not radiographic knee OA). We will thus screen our trial population and classify those with: knee pain with walking 2-3 blocks, a doctor's diagnosis of knee OA, pain > 5 days per week and for > one year and classify those with all four of these as having chronic frequent knee pain. (**Fig. 2**). Individuals with total knee replacements at baseline will be excluded. Our goal is to include only those with the most severe, chronic knee pain at basline in this longitudinal RCT

of the two investigational agents. Based on estimates of the prevalence of knee pain in a population of adults over age 60[123, 404] and the added specificity of our screening questions, we anticipate that the prevalence of chronic severe knee pain by this method will be approximately 12.5%, or 5000 individuals of the 40,000 individuals in the run-in phase. Although the sensitivity and specificity of our screening questions for chronic frequent knee pain (highly likely to be OA) in this population is not known, it is likely higher that that of Lavalley and ultimately will ensure that we include individuals with chronic, frequent knee pain, regardless of diagnosis.

Baseline knee pain severity assessment. We will include supplementary longitudinal knee pain assessment questions **(Appendix B)** and a cover letter with run-in questionnaire to 5000 individuals during the run-in phase. After the run-in phase, 50% of the individuals who were compliant with > 66% of study medications and supplementary requests, and continue to be willing and eligible, will be enrolled in VITAL. Thus, we anticipate that 2500 of the 5000 will be eligible for this sub-trial, and of these 2000 individuals with chronic, severe knee pain will complete the baseline assessment and will be followed annually. In a subset of 100 men and 100 women participants, we will request access to medical records and radiograph reports and have them forwarded to us for review to validate the sensitivity, specificity, positive and negative predictive value of our screening strategy for the diagnosis of knee OA. A standard definition of knee OA for epidemiologic studies will employed: physician diagnosis of OA and the presence of one or more osteophytes on AP knee radiographs[405].

Baseline Blood Collection and Randomization are those of the parent VITAL trial, described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at Partners will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

Trial Procedures and Data Collection

Treatment with active vitamin D3 and/or fish oil or placebo is described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number01169259.

Follow-up procedures, dietary and supplemental intake assessments, are those of the parent VITAL trial, described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

Incident autoimmune disease ascertainment and validation. Incident autoimmune disease case identification during the trial will be a 2-stage procedure:

1. Questions inquiring about new onset autoimmune diseases will be sent with the annual questionnaires. Participants who self-report an incident autoimmune disease endpoints on any of these questionn

Table 3. Criteria for the Validation of Autoimmune diseases

Autoimmune thyroid disease: **Hashimoto's disease:** 1) no prior thyroid surgery or I^{131} therapy, TSH > 4.5 mu/L, andanti-TPO >20IU/mL; OR 2) no prior thyroid surgery or I131 therapy, anti-TPO >20IU/mL and currently receiving levothyroxine therapy[426]. **Graves' disease:** NOT receiving levothyroxine and either: 1) TSH <0.5mU/L for ≥ 3 months, with diffuse goiter and anti-TPO; OR 2) TSH <0.5mU/L and diffuse uptake on radionuclide thyroid scan[427]
Inflammatory bowel disease: Crohn's disease: ≥ 2 separated by ≥ 2 months: 1) abdominal pain, diarrhea, weight loss, malaise, and/or rectal bleeding; 2) endoscopic findings of linear ulceration, mucosal cobblestoning, skip areas, or perianal disease; 3) radiologic findings of fistula, stricture, mucosal cobblestoning, or ulceration; 4) laparotomy appearance of "creeping fat," bowel wall induration, mesenteric lymphadenopathy; 5) histologic transmural inflammation and/or epithelioid granulomas[428]. **Ulcerative colitis:** 2, separated by ≥ 6 months: 1) diffusely granular or friable colonic mucosa on endoscopy; AND 2) continuous mucosal involvement by endoscopy or barium studies[428].
Polymyalgia rheumatica: 1) bilateral aching and morning stiffness (≥ 30 min) for ≥ 1 month involving: neck or torso, shoulders or proximal regions of the arms, and hips or proximal thighs; 2) ESR > 40 mm/hr (Westergren); AND 3) prompt response to corticosteroid therapy[119]; AND/OR **Giant cell arteritis**: ≥ 3: 1) age > 50 years; 2) new headache; 3) temporal artery tenderness or decreased pulsation on exam; 4) elevated ESR; 5) temporal artery biopsy with vasculitis or granulomatous infiltration[145].
Psoriasis: Physician diagnosis of psoriasis in medical records[125, 429]
Rheumatoid Arthritis: ≥ 4 for ≥ 6 weeks: 1) morning stiffness > 60 minutes; 2) arthritis > 3 body areas; 3) hand arthritis; 4) symmetric arthritis; 5) rheumatoid nodules; 6) rheumatoid factor and/or anti-anti-cyclic citrullinated peptide antibodies;7) radiographic changes[430,431].

aires will be mailed a letter asking them to sign a medical release form authorizing VITAL staff to obtain hospital/physician records. The request will be accompanied by a cover letter expressing sympathy for the diagnosis and explaining guidelines and the scientific importance of record validation. Non-responders will be sent two additional requests for the medical release form. During the 10 year Women's Health Study trial, only 5% of cases refused to sign a medical release. After the release is obtained, a copy will be sent to the treating hospital or physician. If there is no response within 1 month, a second request will be mailed, followed by a phone call. In the Women's Health Study, the staff was able to obtain hospital/physician records 99% of the time.

2. Medical records will be reviewed independently by two trained physicians (including board certified rheumatologist, endocrinologist, and gastroenterologists) blinded to the randomized treatment assignment, will confirm or disconfirm the autoimmune disease according to classification criteria (**Table 3**). The specificity of autoimmune connective tissue disease detection using a staged series design is very high, reducing misclassification of healthy subjects[406]. Similar procedures have been used in the Nurses' Health Study for the validation of RA and SLE by self-report, with case confirmation rates of 69-70% of medical record reviewed[386, 387]. All participants who report autoimmune diseases that cannot be validated will be censored from analyses at the time of the ambiguous self-report. The primary autoimmune disease endpoint of VITAL will be total autoimmune disease incidence. We estimate that 1000 or more new cases of autoimmune disease should occur during the five years of this trial in this elderly population, although we will not be able to capture them all. We anticipate some over-reporting of incident autoimmune diseases, and may need to mail up to three separate medical record requests in some cases. We estimate a final confirmation of 60% of the true incident cases, or 600 cases. Individuals who report an autoimmune disease at baseline or in follow-up will not be excluded from analyses of other incident autoimmune diseases (e.g., an individual who reported pre-existing AITD will not be excluded from analyses of new autoimmune diseases).

Follow-up blood collection, biochemical assays for circulating 25(OH)D, EPA and DHA are those of the parent VITAL trial, described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

Biochemical assays for Inflammatory biomarkers: IL-6, TNFreceptor2 (TNFR2) and hsCRP assays. (Children's Hospital, Boston, MA, Nader Rifai, PhD) IL-6 will be measured by an ultra-sensitive quantitative sandwich enzyme immunoassay from R&D Systems. IL-6 is quantified in pg/ml and assay sensitivity is 0.94 pg/mL. IL-6 is a strong predictor of cardiovascular disease and type 2 diabetes[23, 412, 413], suggesting that a single sample represents long-term levels. TNFα cannot be reliably measured in stored plasma as it degrades rapidly. TNFR2 expression parallels TNFα levels and is a surrogate marker for inflammation[414, 415]. TNFR2 will be measured by a quantitative sandwich ELISA assay from R & D Systems. Day-to-day assay variabilities at concentrations of 54.8, 252 and 356 pg/mL are 8.8, 3.7 and 5.8%, respectively. TNFR2 stability was assessed in 17 fresh blood samples, at baseline, and after a delay of 24 hours and 36 hours, and the CV was 0.8 for the comparison of 0 to 36 hours (personal communication, Nader Rifai). High sensitivity CRP will be measured by high sensitivity latex-enhanced immunonephelometric assay on a BNII analyzer (Dade Behring, Newark, Delaware), with a coefficient of variation of < 5%. CRP levels by this technique, in mg/L, are predictive of cardiovascular disease and type 2 diabetes in cohort studies[23, 416].

Knee Pain Assessments Among those who screen positive for chronic knee pain (knee pain with walking 2-3 blocks, pain > 5 days per week for > 1 year, and a doctor's diagnosis of knee OA and no history of total knee replacement) during the run-in phase, baseline knee pain symptoms will be assessed prior to randomization and receipt of any study medications. Thereafter, knee pain symptoms will be assessed annually during the same season (month) in this chronic knee pain subcohort of 2000 participants. We will use the following validated, short

instruments to assess knee pain, analgesic use and depression:

a. Western Ontario McMaster Universities (WOMAC) Knee Osteoarthritis Index Developed in 1988, the WOMAC knee questionnaire is a multidimensional measure of pain, stiffness, and physical functional disability consisting of 24 items graded in a numerical rating scale ranging from 0 ("no symptoms") to 10 ("extreme symptoms")[417, 418]. It is the most widely used and accepted measure for monitoring[419] the course of the disease or to determine the effectiveness of anti-rheumatic medications in many knee OA trials. As several studies pointed to redundancy in the WOMAC function scale[68, 420], Whitehouse and colleagues have developed and tested a reduced WOMAC function scale that contains seven functional items[421]. This scale, when tested in 1578 total joint replacement patients, had a Cronbach's alpha of 0.85 with the WOMAC, indicating reliability and responsiveness to change was even greater than that of the extended scale. We will utilize the reduced WOMAC function scale, along with the pain, stiffness, and overall WOMAC scales.

b. Analgesic medications Participants will be asked to report which medications they are taking from a short list of typical medications taken for knee pain, how frequently and for how long they have taken them. Medication use will be categorized as follows: (0) none, (1) acetominophen/non-steroidal anti-inflammatory drugs (NSAIDs), (2) narcotics/adjunctive pain medications (such as anti-depressants and antiepileptics). A decrease in analgesic medication use (from higher to lower category) will be considered as a secondary outcome, and analgesic use will also be included as a covariate in other knee pain analyses.

c. Mental Health Inventory-5 (MHI-5) from the Short- form (SF-36) Health Survey The SF-36 is a widely-used and well-validated questionnaire that assesses eight domains of general health[422-425]. The SF-36 includes a subscale of five questions about mental health (the Mental Health Index-5,MHI-5)[426]. The MHI-5 is comprised of 5 questions and there are six possible responses to each question, scored between 1 and 6. Individuals' scores thus range from 5 to 30. This is then transformed into a variable from 0-100 using a standard linear transformation. This subscale will provide useful a covariate as mental health could potentially explain some of the outcome variance. The responses range from ''never'' to ''most of the time'' and will be scored following a standard protocol. The recommended cut-off for a mood disorder/depression is a score of 65 on the mental health questions (MHI-5). The SF-36v2, its most recent version, is copyrighted, and we have obtained permission to use it from the Medical Outcomes Trust and QualityMetric Incorporated.

Procedures for the Validation of Deaths, Compliance and Data Management will be those of the parent VITAL trial, described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

For studies involving treatment or diagnosis, provide information about standard of care at Partners (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

This trial will not affect standard of care for any disease state. The medications tested will not be for treatment or diagnosis.

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

Procedures to minimize risks including recruitment and informed consent, protection of medical records and personal health information, minimization of risks of the trial medications, DSMB structure and oversight, will be those of the parent VITAL trial, described in Partners' IRB

approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

For this ancilllary study, investigations of autoimmune disease incidence (Aim 1) and biomarkers of systemic inflammation (Aim 2) will involve subjects already consented for involvement in the parent VITAL trial and the ancillary studies utilizing mailings a separate blood draw consent form (already approved for parent trial) to obtain follow-up blood samples. For self-reports of autoimmune disease, a letter will be mailed describing the study and ask for consent for review of medical records. Medical record will be requested from physicians and hospitals and reviewed for published criteria autoimmune diseases. For the chronic knee pain investigations (Aim 3), a letter will be mailed to potential participants with the supplementary knee pain assessments, stating that they have reported chronic knee pain on the past questionnaire and requesting that they complete another annual questionnaire regarding their chronic knee pain symptoms. Return of these supplementary questionnaires will imply informed consent and subjects will also be given the option of stating "I am not interested", so that they are not followed further.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

Procedures for protection against risks will be those of the parent VITAL trial, described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259. This ancillary study poses only the minimal risk of inadvertent disclosure of private health information.

The potential risk of disclosure of confidential information is guarded against by maintaining completed questionnaires, including ancillary questionnaires and blood test results in locked files accessible by authorized personnel only. In these files, participants are identified only by study ID. Subject identifiers are stored in separate computer files with password protection, and results will be presented only in the aggregate. In addition, employees involved with the proposed study will be asked to sign a form agreeing not to disclose any information to which they might have access, regardless of their personal perception about its confidentiality. Each employee of the study with human subject contact participates in an institutional (Brigham and Women's Hospital) human subjects' educational program, which consists of reviewing regulatory and informational documents pertaining to human subjects' research; passing a test on ethical principles and regulations governing human subjects research; and signing a statement of commitment to the protection of human subjects. Finally, all such employees are required to participate in HIPAA training. Medical records will be reviewed by Brigham and Women's physicians who have completed Human Subjects Certification.

FORESEEABLE RISKS AND DISCOMFORTS

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

As above, there is the risk of inadvertent disclosure of private health information. The strict procedures we will follow to safeguard against this are outlined above.

The foreseeable risks and discomforts of the parent VITAL trial are described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

EXPECTED BENEFITS

Describe both the expected benefits to individual subjects participating in the research and the importance of the knowledge that may reasonably be expected to result from the study. Provide a brief, realistic summary of potential benefits to subjects, for example, "It is hoped that the treatment will result in a partial reduction in tumor size in at least 25% of the enrolled subjects." Indicate how the results of the study will benefit future patients with the disease/condition being studied and/or society, e.g., through increased knowledge of human physiology or behavior, improved safety, or technological advances.

For the majority of participants, there will be few direct benefits from participating in this primary prevention study other than the awareness of being involved in a large endeavor to answer relevant and timely questions regarding the possible benefit of vitamin D and fish oil. During the trial itself, we will receive inquiries from the participants regarding both specific and general health concerns. We will respond to each of those questions, primarily directing participants to published sources or recommending that they see their local health provider who is familiar with their medical history.

The potential benefits to society relate to the increasing use of both vitamin D and fish oil, with data not yet clearly indicating efficacy in the general population with respect to prevention of autoimmune disease, inflammation and knee pain. Such data will help guide individual decisions, clinical recommendations, and public health guidelines.

EQUITABLE SELECTION OF SUBJECTS

The risks and benefits of the research must be fairly distributed among the populations that stand to benefit from it. No group of persons, for example, men, women, pregnant women, children, and minorities, should be categorically excluded from the research without a good scientific or ethical reason to do so. Please provide the basis for concluding that the study population is representative of the population that stands to potentially benefit from this research.

The gender distribution of the 20,000 participants in VITAL will be 50% male and 50% female—specifically, 10,000 men aged ≥60 and 10,000 women aged ≥65. Estimates of the race/ethnicity of the participants come from the pilot study, in which the source list of individuals to whom we were mailing was enhanced with names identified as possible minorities. Based on the pilot results, we will be able to identify approximately 40,000 willing and eligible participants to enter the run-in, with at least 25% underrepresented minorities; 20,000 of these will be randomized into the trial, with the same proportion minority. Specifically, of the 20,000 randomized participants, we anticipate the ethnic distribution to be 1400 (7.5%) Hispanic and 18,600 (93%) non-Hispanic; with regard to race, we anticipate 5000 (25%) African-American, 500 (2.5%) Asian, 400 (2%) American Indian, 80 (0.4%) Pacific Islander, and 14,020 (70.1%) white individuals. Note that ethnic and racial categories can overlap—e.g., participants can be Hispanic-white or Hispanic-black.

When people who do not speak English are excluded from participation in the research, provide the scientific rationale for doing so. Individuals who do not speak English should not be denied participation in research simply because it is inconvenient to translate the consent form in different languages and to have an interpreter present.

Individuals who do not speak English will not be eligible for this trial as it will not be possible to translate informed consent forms and all study questionnaires and documents into other languages.

For guidance, refer to the following Partners policy:
Obtaining and Documenting Informed Consent of Subjects who do not Speak English
http://healthcare.partners.org/phsirb/nonengco.htm

RECRUITMENT PROCEDURES

Explain in detail the specific methodology that will be used to recruit subjects. Specifically address how, when, where and by whom subjects will be identified and approached about participation. Include any specific recruitment methods used to enhance recruitment of women and minorities.

Recruitment procedures will be those of the parent VITAL trial, described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

Provide details of remuneration, when applicable. Even when subjects may derive medical benefit from participation, it is often the case that extra hospital visits, meals at the hospital, parking fees or other inconveniences will result in additional out-of-pocket expenses related to study participation. Investigators may wish to consider providing reimbursement for such expenses when funding is available

VITAL subjects will not receive remuneration for their participation in the trial.

For guidance, refer to the following Partners policies:
Recruitment of Research Subjects
http://healthcare.partners.org/phsirb/recruit.htm

Guidelines for Advertisements for Recruiting Subjects
http://healthcare.partners.org/phsirb/advert.htm

Remuneration for Research Subjects
http://healthcare.partners.org/phsirb/remun.htm

CONSENT PROCEDURES

Explain in detail how, when, where, and by whom consent is obtained, and the timing of consent (i.e., how long subjects will be given to consider participation). For most studies involving more than minimal risk and all studies involving investigational drugs/devices, a licensed physician investigator must obtain informed consent. When subjects are to be enrolled from among the investigators' own patients, describe how the potential for coercion will be avoided.

Subjects will be mailed an informed consent document along with a letter explaining the parent VITAL trial and a screening questionnaire. Return of the signed informed consent document by mail will constitute informed consent.

NOTE: When subjects are unable to give consent due to age (minors) or impaired decision-making capacity, complete the forms for Research Involving Children as Subjects of Research and/or Research Involving Individuals with Impaired Decision-making Capacity, available on the New Submissions page on the PHRC website:
http://healthcare.partners.org/phsirb/newapp.htm#Newapp

For guidance, refer to the following Partners policy:
Informed Consent of Research Subjects
http://healthcare.partners.org/phsirb/infcons.htm

DATA AND SAFETY MONITORING

Describe the plan for monitoring the data to ensure the safety of subjects. The plan should include a brief description of (1) the safety and/or efficacy data that will be reviewed; (2) the planned frequency of review; and (3) who will be responsible for this review and for determining whether the research should be altered or stopped. Include a brief description of any stopping rules for the study, when appropriate. Depending upon the risk, size and complexity of the study, the investigator, an expert group, an independent Data and Safety Monitoring Board (DSMB) or others might be assigned primary responsibility for this monitoring activity.

NOTE: Regardless of data and safety monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care.

Describe the plan to be followed by the Principal Investigator/study staff for review of adverse events experienced by subjects under his/her care, and when applicable, for review of sponsor safety reports and DSMB reports. Describe the plan for reporting adverse events to the sponsor and the Partners' IRB and, when applicable, for submitting sponsor safety reports and DSMB reports to the Partners' IRBs. When the investigator is also the sponsor of the IND/IDE, include the plan for reporting of adverse events to the FDA and, when applicable, to investigators at other sites.

NOTE: In addition to the adverse event reporting requirements of the sponsor, the principal investigator must follow the Partners Human Research Committee guidelines for Adverse Event Reporting

The parent VITAL trial has established a DSMB for the parent trial and all ancillary studies. This is described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov number 01169259.

MONITORING AND QUALITY ASSURANCE

Describe the plan to be followed by the principal investigator/study staff to monitor and assure the validity and integrity of the data and adherence to the IRB-approved protocol. Specify who will be responsible for monitoring, and the planned frequency of monitoring. For example, specify who will review the accuracy and completeness of case report form entries, source documents, and informed consent.

NOTE: Regardless of monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for ensuring that the study is conducted at his/her investigative site in accordance with the IRB-approved protocol, and applicable regulations and requirements of the IRB.

Dr. Costenbader, PI of this ancillary VITAL study, will be responsible for collection and monitoring of data, including those concerning any adverse events, for the outcomes of autoimmune disease, inflammatory biomarkers, and knee pain. She will work closely with the parent trial PIs, steering committee and DSMB. A panel of BWH physicians, including Dr. Erik Alexander, Dr Abrar Qureshi, Dr. Sonia Freidman, Dr. Elizabeth Karlson, Dr. Jeffrey Katz and Maura Iversen, PT, DrPH will be involved in validating outcomes. They will follow hospital and research privacy procedures strictly and will be required to pass required Partners research privacy courses ad certifications.

The trial DSMB is also described in Partners' IRB approved protocol 2009-P-00127; clinicaltrials.gov NUMBER01169259.

For guidance, refer to the following Partners policies:

Data and Safety Monitoring Plans and Quality Assurance
http://healthcare.partners.org/phsirb/datasafe.htm

Adverse Event Reporting Guidelines
http://healthcare.partners.org/phsirb/adverse_events.htm

PRIVACY AND CONFIDENTIALITY

Describe methods used to protect the privacy of subjects and maintain confidentiality of data collected. This typically includes such practices as substituting codes for names and/or medical record numbers; removing face sheets or other identifiers from completed surveys/questionnaires; proper disposal of printed computer data; limited access to study data; use of password-protected computer databases; training for research staff on the importance of confidentiality of data, and storing research records in a secure location.

NOTE: Additional measures, such as obtaining a Certificate of Confidentiality, should be considered and are strongly encouraged when the research involves the collection of sensitive data, such as sexual, criminal or illegal behaviors.

The potential risk of disclosure of confidential information is guarded against by maintaining completed questionnaires, including ancillary questionnaires and blood test results in locked files accessible by authorized personnel only. In these files, participants are identified only by study ID. Subject identifiers are stored in separate computer files with password protection, and results will be presented only in the aggregate. In addition, employees involved with the proposed study will be asked to sign a form agreeing not to disclose any information to which they might have access, regardless of their personal perception about its confidentiality. Each employee of the study with human subject contact participates in an institutional (Brigham and Women's Hospital) human subjects educational program, which consists of reviewing regulatory and informational documents pertaining to human subjects research; passing a test on ethical principles and regulations governing human subjects research; and signing a statement of commitment to the protection of human subjects. Finally, all such employees are required to participate in HIPAA training. Medical records will be reviewed by Brigham and Women's physicians who have completed Human Subjects Certification.

SENDING SPECIMENS/DATA TO RESEARCH COLLABORATORS OUTSIDE PARTNERS

Specimens or data collected by Partners investigators will be sent to research collaborators outside Partners, indicate to whom specimens/data will be sent, what information will be sent, and whether the specimens/data will contain identifiers that could be used by the outside collaborators to link the specimens/data to individual subjects.

Specimens and data from this study will not be shared with collaborators outside of Partners.

Specifically address whether specimens/data will be stored at collaborating sites outside Partners for future use not described in the protocol. Include whether subjects can withdraw their specimens/data, and how they would do so. When appropriate, submit documentation of IRB approval from the recipient institution.

Not applicable.

RECEIVING SPECIMENS/DATA FROM RESEARCH COLLABORATORS OUTSIDE PARTNERS

When specimens or data collected by research collaborators outside Partners will be sent to Partners investigators, indicate from where the specimens/data will be obtained and whether the specimens/data will contain identifiers that could be used by Partners investigators to link the specimens/data to individual subjects. When appropriate, submit documentation of IRB approval and a copy of the IRB-approved consent form from the institution where the specimens/data were collected.

Not applicable.

Protocol Biostatistical Analyses

Separate analyses will be conducted for the 3 endpoints and for the 2 nutritional supplements, vitamin D and omega-3 fatty acids. Analyses of treatment effects will be based on the intent-to-treat principle. The first analysis will compare baseline characteristics by randomized treatment

assignment to ensure that balance was achieved by the randomization. Characteristics to be examined include known risk factors for autoimmune diseases, systemic inflammation, and chronic knee pain including: age; gender; race/ethnicity; family history of autoimmune disease; BMI; smoking; alcohol use; physical activity; co-morbid medical conditions such as hypertension, hyperlipidemia, diabetes, and cardiovascular disease; and baseline vitamin D and omega-3 fatty acid levels as assessed by dietary questionnaire in all participants and blood assays in the case-cohort subsample. The large sample size, as well as successful balance of known potential confounders, will provide assurance that unmeasured or unknown potential confounders will also be equally distributed across randomized treatment groups. For autoimmune disease, we will estimate the intervention effects on the primary endpoint of total incident autoimmune disease. For inflammatory biomarkers, we will assess changes in linear biomarkers (log transformed if necessary). For chronic knee pain, the primary endpoint will be assessed by the WOMAC pain scale; secondary analyses will test for decreases in WOMAC stiffness, function and global scales, as well as analgesic use separately. Data analyses will be performed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina).

Autoimmune Disease Incidence. In this 2x2 factorial design, the primary aim is to compare the main effects of intention-to-treat with vitamin D and with fish oil upon autoimmune disease incidence. Initially, we will compare Kaplan-Meier curves to graphically display the survival functions across different treatment groups and a log-rank test to test the difference. We will use Cox proportional hazards models to assess the main and interaction effects of the treatment groups. The unadjusted risk ratios and their 95% CIs will be used to measure the strength of risk across different treatment groups. The proportional hazard assumption will be tested based on the smoothed plots of the scaled Schoenfeld residuals [386]. To estimate the cumulative incidence function, a nonparametric estimate will be obtained using the Kaplan–Meier method. Given randomization and blinding of 20,000 individuals in this RCT, most important covariates will be equally distributed. But, in secondary analyses, we will assess the effects of controlling for age, sex and other possible strong predictors, such as family history of autoimmune disease; baseline levels of inflammatory biomarkers, vitamin D, EPA and DHA; dietary vitamin D, EPA and DHA intake; BMI and physical activity.

Inflammatory Biomarkers. In a randomly selected subcohort of 2000 participants, blood will be collected at both baseline and follow-up and changes in levels of plasma biomarkers of inflammation will be investigated. As inflammatory biomarkers are associated with age, BMI, medication use and cigarette smoking, we will again ensure that baseline characteristics are balanced in the four treatment groups in this subcohort and will additionally adjust for them in our analyses. Inflammatory Biomarkers (hsCRP, IL-6, TNFR2) will be analyzed as continuous outcomes. Descriptive statistics such as the minimum, maximum, range, median, and mean are used to summarize the variable as well as detect outliers and missing values. Exploratory graphical techniques such as Boxplots, Histograms, Quantile-Quantile plots, and Stem and Leaf plots will be used to further examine the distribution of each biomarker. Natural log transformation may be necessary based on our past analyses[105]. Unadjusted treatment effects will be evaluated using two-way Analysis of Variance (ANOVA) or Kruskal–Wallis nonparametric test. Multiple linear regression will be used to test the association between the biomarkers (or transformed scale) and treatment groups, while adjusting for age and gender. Model assumptions and goodness of fit will be evaluated using applicable techniques[387]. In a secondary analysis, additional adjustment for the other potential predictors including baseline biomarkers will be performed.

Chronic Knee Pain. In a separate subcohort of approximately 2000 individuals with baseline chronic daily knee pain and no history of total knee replacement surgery, we will evaluate changes in level of knee pain over the course of the RCT. The annual measure of WOMAC knee pain subscale will be the primary outcome. Angst and colleagues examined data from 122 prospectively-followed individuals with knee OA in a comprehensive rehabilitation program to define the minimally clinically important difference (MCID) in WOMAC scores (range 0-10), for

pain, stiffness and function[388]. Analgesic use will be secondary outcome, defined on a scale of 0-2: 0 (none), 1 (NSAIDS or acetaminophen only) and 2 (narcotics). As chronic knee pain is associated with age, BMI, medication use, comorbidities and depression (MHI-5), we will again ensure that baseline characteristics are balanced in the four treatment groups in this knee pain subcohort and will additionally adjust for these potential confounders in our analyses. Similarly, we will analyze repeated measures of WOMAC knee pain score, stiffness score, function score and globe score as continuous outcomes. The descriptive statistics and the distribution of each outcome variable will firstly be examined. Transformation will be considered if applicable. We will use two-way analysis of variance (ANOVA) or Kruskal–Wallis test for bivariable analysis and general linear mixed model for multivariable repeated measure analysis. For another secondary outcome, we will assess the effects of the supplements upon analgesic use, classified as: no medications, acetaminophen or non-steroidal use; and narcotic medication). Generalized linear mixed models for repeated binary outcomes will be used to examine the adjusted associations.

Statistical issues in resampling from a RCT. Our investigations of the effects of these agents on biomarkers of systemic inflammation and knee pain will not utilize the entire 20,000 individuals enrolled in the funded VITAL trial. Rather, each will follow a selected subcohort of approximately 2000 individuals. It is possible that these individuals will not be drawn completely equally or randomly from the 4 treatment groups. Possible unbalance of sample characteristics will be evaluated and be controlled for in multivariable analyses.

Secondary Analyses. Beyond the primary analyses, we will examine several pre-specified secondary outcomes for autoimmune diseases, inflammatory biomarkers, and chronic knee pain. We will examine effect modification by the other randomized intervention, by baseline risk factors, and by time. Given past studies showing interactions between intake of n-6 fatty acids and n-3 in reductions in biomarkers of systemic inflammation[285], we have a particular interest in exploring interactions between the interventions, as well as with the baseline plasma biomarkers of 25(OH)D for vitamin D and of EPA+DHA for fish oil, and dietary intake of both. We hypothesize that the intervention effects may be larger among those with below-median baseline levels and will examine treatment effects by quartiles of these biomarkers. When stratifying by 25(OH)D, season (assessed by date of return) and geographic location (assessed by zip code) will be considered. We also have a prior interest in the effects of the vitamin D intervention within groups defined by race/ethnicity and skin pigmentation, and by BMI. In addition, we will evaluate effect modification by age and gender, as well as by sunlight exposure, calcium and phosphorus intakes from the FFQ (as these nutrients affect vitamin D bioavailability[1]), and baseline risk factors for autoimmune disease, systemic inflammation and chronic knee pain. Interaction effects will be interpreted cautiously, as hypothesis-generating. Finally, we will examine whether treatment effects vary over time and duration of treatment by examining survival plots and interactions with time. There may be latent effects on outcomes, depending on the stage at which these agents act.

Analysis of potential adverse effects. We will also compare the incidence of potential side effects in the active vs. placebo groups for each agent, including the incidence of kidney stones with vitamin D assignment[389] and the incidence of GI symptoms, skin abnormalities, and bleeding with fish oil assignment.[329]

Power calculations. This section provides a detailed power analysis for each of the 3 aims. To calculate
power, the following assumptions were made: (1) a 2x2 factorial trial in 10,000 men aged $\geq$60 and 10,000 women aged $\geq$65; (2) independent and equal allocation of participants to each treatment in the trial (for Aim 1); (3) an age distribution based on that observed at baseline in our past RCTs for men aged ≥60 and women aged ≥65; (4) trial follow-up of 5 years, with little loss to follow-up as achieved in our past RCTs; and (5) compliance of 80% similar to that in published trials upon which our estimated rate ratio (RR) reductions are based. Power calculations were performed using the observed RR, given past observed effects and assuming

a compliance of 80%. The corresponding true relative risk (RR) with perfect compliance is also calculated.

a. Incident autoimmune diseases. As in **table 1**, we estimate the annual incidence of total autoimmune diseases in a U.S. population over age 60 to be approximately 1000 cases per 100,000 individuals per year: thus, in this trial of 20,000 individuals for 5 years, 1000 incident cases.(**Table 2**) Conservatively, we will validate 60% of these 1000 cases, or 600 cases, with 150 occurring in the placebo group and fewer in the intervention groups if they are effective. Given the clustering of autoimmune diseases within individuals, some incident cases will occur within the same individuals, and these will be analyzed as discrete endpoints. Power is given for a a logrank test[390] based on the proportional hazard rates with a significance level of 0.05.(**Table 4**) Assuming that only one agent is effective, with 5 years of treatment and follow-up, we will have 81% power to detect an observed RR of 0.70 (or 30% risk reduction) for the primary endpoint of autoimmune disease incidence. For the secondary endpoint of AITD, we will have 85% power to detect a 40% risk reduction. If both agents are effective in preventing autoimmune disease but act independently, power would be reduced slightly due to a somewhat smaller overall number of events. If the agents interact, however, power will be affected to the extent of the interaction. Should the agents act synergistically, power would increase. For example, if the effect of each agent alone is a reduction of 15%, but in combination the effect is stronger, with an additional 10% decrease, the RR comparing the combined group to the all placebo group would be 0.65, versus 0.72 with additive effects (on the multiplicative scale). Power for the total effects would increase to 91%. Power is shown for a given observed reduction in risk, assuming 80% compliance similar to that seen in trials such as WHS and PHS.

Table 4. Statistical power for effects of a single agent on autoimmune disease in a factorial trial of 10,000 men aged ≥60 and 10,000 women aged≥65 with 5 years of follow-up

Observed RR†	True RR	Autoimmune Disease	AITD
0.70	0.67	0.81	0.62
0.65	0.61	0.91	0.75
0.60	0.56	0.97	0.85

†Observed RR=intent-to-treat RR assuming compliance 80%. True RR= with perfect compliance. AITD= autoimmune thyroid disease

b. Inflammatory biomarkers. Our power calculations are based on past changes in systemic inflammatory biomarkers in large cohort analyses and we have first assumed that only one agent is effective, that compliance is 80% and that the cohort of 2000 followed over two years for changes in biomarkers is randomly drawn from each of the four treatment groups. (**Table 5)** Based on the distribution of hsCRP, IL-6 and TNFR2, natural log-transformation may be used for power analysis if applicable. According to NHANES 1999-2002 data, median hsCRP level is 2.8 mg/L for men aged ≥60 and women aged ≥65[391]. Using a two-sided type I error of 0.05, the sample size of 500 patients with compliance of 80% in each group will achieve 89% power to detect a 25% reduction of hsCRP using a two-sided t test (based on log-transformed scale). Pai and colleagues examined plasma levels of TNFR2 and IL-6 as biomarkers for cardiovascular disease risk among participants in the Nurses' Health Study and the Health Professional Follow-up Study (mean age 60.3 for women, 65.2 for men)[135]. The mean TNFR2 was 2370 pg/ml (SD 795 pg/ml) and the median IL-6 level was 1.6 pg/mL. A sample size of 500 per group with compliance of 80% will achieve more than 99% power to detect 20%-40% reduction of TNFR2 with a two-sided significance level of 0.05. Based on a log-transformed scale, we will have 88% power to detect a 30% reduction in IL-6.

Table 5. Statistical power for effects of a single agent on inflammatory biomarkers in a subcohort of 2000 individuals

Observed reduction %	hsCRP	IL-6	TNFR2
20	0.72	0.55	>0.99
25	0.89	0.74	>0.99
30	0.97	0.88	>0.99
40	0.99	0.99	>0.99

†Assuming compliance to be 80%.

c. Chronic knee pain. In the study by Angst and colleagues, the mean global score was 4.8 and the MCID for improvement in pain was 0.75, for stiffness 0.72, for function 0.67 and for global

Table 6. Statistical power for effects of a single agent on chronic knee pain in a factorial trial of 2000 (men aged ≥60 women aged ≥65), with 5 years of follow-up (80% compliance rate)

	Baseline Score	SD	MCID	Power
WOMAC knee pain	4.83	2.25	0.75	0.99
WOMAC knee stiffness	4.61	2.67	0.72	0.96
WOMAC knee function	4.81	2.18	0.67	0.99
WOMAC Global	4.80	2.09	0.67	0.99

SD= standard deviation per Angst, 2002 MCID= minimally clinically important difference

score 0.67 [388]. Assuming a type I error rate of 0.05 and a power of 0.80, this translated into sample sizes of 142, 216, 162, and 153 individuals respectively affording adequate statistical power to detect these changes in an RCT of an intervention using the WOMAC as an outcome. We will follow 2000 individuals with chronic daily knee pain longitudinally over up to 5 years. With estimated loss to follow-up and compliance of 80%, we will have generous statistical power to detect the MCID in improvement in WOMAC pain, stiffness, function and global scores. (**Table 6**) Moreover, repeated measurement of WOMAC scores will provide additional power to detect specified effect sizes. Again, unequal group sample sizes could reduce the power to some extent (although is unlikely to occur unless there is a strong chance imbalance). However, if we conservatively assume the intervention to placebo allocation ratio is 1:2, we will still achieve similar power to detect 25% reduction in both inflammatory biomarkers and the WOMAC MCID of knee pain. (The power to detect MCID for WOMAC knee pain would be > 99%, for WOMAC knee stiffness 95%, and for WOMAC knee function 98%.)