ClinicalTrials.gov Document Upload

Cover Page

Study Title:
Study of Vitamin D and Omega-3 Supplementation for Preventing Diabetes

---

Sponsor:
National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

---

Study Identifier(s):

- ClinicalTrials.gov Identifier (NCT Number): NCT01633177
- Protocol ID: 0308112690000

Document:
Statistical Analysis Plan

Date (Submission to NIH):
November 20, 2009

A. SPECIFIC AIMS

Emerging evidence suggests favorable effects of both vitamin D and marine omega-3 fatty acids on glucose homeostasis. Optimal vitamin D intake is essential for insulin secretion and action[8-17], and omega-3 fatty acids may reduce diabetes risk as a result of favorable effects on insulin sensitivity, endothelial function, chronic inflammation, or other metabolic abnormalities[18-25]. Although the metabolic effects of vitamin D and omega-3 fatty acids show considerable promise for the primary prevention of type 2 diabetes (T2D), there are no completed, ongoing, or planned randomized clinical trials of vitamin D or omega-3 supplements that include T2D as a primary outcome in a general population.

We thus propose to utilize an NIH-funded randomized trial (1 U01 CA138962) to test the hypothesis that vitamin D and omega-3 supplementation will reduce the risk of T2D. We will further assess whether and to what extent vitamin D or omega-3 supplementation will improve insulin sensitivity and pancreatic β-cell function in a subsample of the trial cohort. The **VIT**amin D and Omeg**A**-3 Tria**L** (***VITAL***) is a randomized, double-blind, placebo-controlled trial specifically designed to evaluate the benefits and risks of vitamin D_3 (2,000 IU/day) and marine omega-3 fatty acid (eicosapentaenoic acid [EPA] + docosahexaenoic acid [DHA], 1g/day) supplements in the primary prevention of cancer and cardiovascular disease (CVD). The VITAL trial will aim to enroll 20,000 men and women (aged ≥55 and ≥55 years, respectively). The planned treatment duration is 5 years after a 1.5-year recruitment period. Thus, the VITAL trial offers us a unique opportunity to examine diabetes prevention in a large trial of vitamin D and omega-3 supplementation. The current proposal seeks funds to implement an inexpensive and efficient strategy to validate self-reported incident T2D cases in the entire trial population and to collect pre- and post-intervention measures of glucose, insulin, and hemoglobin A1c (HbA1c) in a subset of participants. Two methods of diabetes case validation are proposed. First, we plan to collect detailed information about diagnostic glucose testing and anti-diabetic medication use from medical records and/or supplementary questionnaires completed by the participant's physician. Second, to complement our diabetes ascertainment process, we will also plan to retrieve additional data on diabetes diagnoses and hypoglycemic medications by linking the participants with the Centers for Medicare and Medicaid Services (CMS) database. In addition to evaluating whether the study interventions impact the onset of clinical diabetes, we propose to collect pre- and post-intervention measures of glucose, insulin, and HbA1c in a subset of the participants to reliably assess whether vitamin D or omega-3 supplementation alters insulin and glucose homeostasis. We plan to recruit 1,000 participants without prior clinical diabetes at four Clinical and Translational Science Center (CTSC) sites across the US (Boston, Chicago, San Francisco, and Houston) within the national CTSC network. A standard 2-hour oral glucose tolerance test (OGTT) and HbA1c measurements will be performed during the CTSC visits at baseline and at 2-year post-randomization (matched for season by month).

Primary Aims:

1. To test whether vitamin D_3 and/or EPA+DHA supplementation reduces the risk of T2D among all initially non-diabetic participants in the VITAL trial.
2. To test whether vitamin D_3 and/or EPA+DHA supplementation improves insulin sensitivity and β-cell function in a subset of 1,000 non-diabetic participants receiving OGTT at baseline and 2-year post-randomization.

Secondary Aims:

1. To test whether vitamin D_3 and/or EPA+DHA supplementation lowers HbA1c, fasting glucose and insulin, as well as other surrogate indices of insulin sensitivity and β-cell function as determined by the homeostasis model assessment (HOMA-IR and HOMA-%B, respectively) in our substudy.
2. To test whether vitamin D_3 and EPA+DHA supplementation exerts synergistic or additive effects on the risk of T2D among all initially non-diabetic participants in the VITAL trial.
3. To test whether vitamin D_3 and EPA+DHA supplementation exerts synergistic or additive effects on insulin sensitivity and β-cell function as assessed by OGTT in our substudy.

For the above main effect estimates, we will further explore whether the effect of vitamin D_3 or EPA+DHA supplementation varies by (1) age, (2) sex, (3) baseline intakes of these nutrients, (4) baseline levels of 25(OH)D (for vitamin D_3), (5) race/skin pigmentation (for vitamin D_3), (6) geographic region (for vitamin D_3), and (7) BMI (for vitamin D_3).

D. RESEARCH DESIGN AND METHODS

D.1. The Study Population of all Non-diabetic Participants in the VITAL Trial

D.1.1. Participant recruitment in the VITAL trial

The *VIT*amin D and Omeg*A*-3 Tria*L* (*VITAL*) is an NIH-supported (1 U01 CA138962), randomized, double-blind, placebo-controlled trial of the benefits and risks of vitamin D (vitamin D_3 [cholecalciferol], 2,000 IU/d) and omega-3 fatty acids (1 g/d; EPA to DHA ratio, 1:1) in the primary prevention of cancer and CVD. The VITAL trial will be conducted among 20,000 apparently healthy participants—10,000 men aged ≥60 and 10,000 women aged ≥65, ages at which rates of chronic disease increase substantially. Enrollment of racial/ethnic minority groups proportionate to the total minority population was a high priority of the VITAL. The VITAL was designed to recruit 20,000 men and women with at least 25% representing minority groups at the end of the recruitment period. Individuals will be eligible if they have no history of cancer (except non-melanoma skin cancer) or CVD (including myocardial infarction [MI], stroke, transient ischemic attack [TIA], angina pectoris, coronary artery bypass graft [CABG], or percutaneous coronary intervention [PCI]). Recruitment for the VITAL will begin in 2010 and end in 2012. 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 1,400 (7.5%) Hispanic and 18,600 (93%) non-Hispanic; with regard to race, we anticipate 5,000 (25%) African-American, 500 (2.5%) Asian, 400 (2%) American Indian, 80 (0.4%) Pacific Islander, and 14,020 (70.1%) white individuals. Participants will be randomized only if they: (1) demonstrate good compliance in pill taking, defined as taking at least two-thirds of the study pills during the run-in; (2) express willingness to continue in the trial; (3) report no new history of cancer (except non-melanoma skin cancer), MI, stroke, TIA, angina pectoris, CABG, PCI, kidney stones, hypercalcemia, sarcoidosis, or other serious illness during the run-in; and (4) demonstrate continued willingness to limit supplemental vitamin D intake to ≤800 IU/d and to forgo the use of fish oil supplements.

D.1.2. Treatment, compliance, and follow-up procedures

Active vitamin D_3 (2,000 IU [50 µg] per pill) and matching inert placebo will be provided by Pharmavite LLC. Active fish oil (1 g per soft-gel capsule, EPA to DHA ratio, 1:1) and matching inert placebo will be provided by Ocean Nutrition. With regard to follow-up, at 6 months and each anniversary date of randomization, participants will be mailed a follow-up questionnaire and a re-supply of bottles or calendar packs. The yearly follow-up questionnaire will include items on compliance with randomized treatments, use of non-trial supplements of vitamin D and fish oil, development of major illnesses, dietary intakes of vitamin D and fish, and updated risk factors related to cancer and CVD. Our primary measure of compliance will be the self-reported information provided on the yearly follow-up questionnaires, which will ask about adherence to the pill-taking regimen. Because VITAL participants will reside throughout the U.S., it will not be possible to obtain blood samples for a validity study on all, or even a random sample of, participants. Thus, as in our previous trials, each year we will visit unannounced 50 New England-area participants, to request an on-the-spot blood sample to be analyzed for 25(OH)D and EPA+DHA levels. The distribution of these values will be compared between the active and placebo groups, and compared with the questionnaire data on compliance, as a test for validity. Non-responders will be mailed a second and third request and will then be telephoned to collect study data. At the very least, vital status will be ascertained. In the WHS, we were able to collect morbidity data on 97% of participants and mortality data on nearly 100%; we anticipate similar rates in VITAL. Information on potential side effects of the study agents will be elicited for monitoring by the Data and Safety Monitoring Board. For fish oil, these side effects include GI upset (presence or absence of symptoms of peptic ulcer, nausea, constipation, diarrhea); bleeding (any GI bleed, GI bleeding requiring transfusion, hematuria, easy bruising, epistaxis); skin eruptions; and physician diagnosis of atrial fibrillation or other irregular rhythms. For vitamin D, these side effects include GI symptoms as listed above, and physician diagnosis of hypercalcemia or kidney stones.

As indicated in *Appendix A*, a baseline questionnaire will be completed by all VITAL participants to collect detailed information on demographics (age, gender, race/ethnicity, education, occupation, income); medical history (cancer, CVD, hypertension, diabetes, hypercholesterolemia, renal disease, kidney stones, hypercalcemia, kidney failure, sarcoidosis, other major illnesses); allergy to fish; current use of supplements containing vitamin D or fish oil; current use of other supplements or medications; dietary intake of vitamin D and consumption of fish; cancer and vascular risk factors (e.g., smoking, height, weight, BP, cholesterol, diabetes, alcohol use, physical activity, and family history of cancer and CVD); and potential effect modifiers of the vitamin D component such as skin pigmentation (using the Fitzpatrick scale[179], which ranges from ‘always’

to 'never burns') and sunlight exposure (time spent outdoors, outdoor physical activity, and sunscreen use [zip codes will be used to assess latitude and sun strength]). Respondents will also be asked to provide telephone number(s) and an e-mail address.

A validated, self-administered semi-quantitative food frequency questionnaire (FFQ) will also be mailed to participants during the run-in. **The performance of this FFQ, developed by Walter Willett, MD, DrPH, and colleagues, has been validated in many populations that were mainly European Americans**[180-184] **but included Hispanic Americans**[7] **and African Americans**[185]**. These validation studies comparing the FFQ with dietary records or biological measures in blood and adipose tissue have shown that this FFQ is an efficient, reliable, and accurate instrument for categorizing individuals according to their intake of 32 nutrients, including vitamin D and marine omega-3 fats**[180-184]. Participants are asked to estimate their average intake over the past year of various foods, beverages, and supplements that contain vitamin D, marine omega-3 fats, and other nutrients. Dietary vitamin D intake will be estimated from participants' reported intakes of certain foods, including dairy products, fortified breakfast cereals, fortified juices, dark-meat fish, and cod liver oil. Dietary marine omega-3 fat intake will be estimated from intake of dark-meat fish, canned tuna, other fish, and seafood. Classifying participants by baseline intake of various nutrients will allow us to evaluate whether the study agents' effects vary by such intake.

D.1.3. Ascertainment algorithm for identifying incident diabetes:

On the baseline and subsequent annual questionnaires supported by the parent VITAL trial, we will ask all the participants if and when they have ever been diagnosed with T2D. To confirm self-reported diagnosis, we seek funding to support retrieval of more detailed information regarding the onset of disease, symptoms, diagnostic tests, and hypoglycemic treatment for each incident diabetes case. For each participant who reports a diagnosis of diabetes on his/her annual follow-up questionnaire, written permission will be obtained prior to contacting his/her healthcare provider. We will contact the participant's physician for information from medical records pertaining to diagnostic glucose values and any antidiabetic treatment. The physician will also be mailed a supplementary questionnaire concerning disease onset, symptoms, additional diagnostic tests, and hypoglycemic treatment. **After all medical records and/or supplementary questionnaires are obtained, a study physician, blinded to the randomized treatment assignment, will review the records and make a final determination of case status.** Confirmation will be based upon American Diabetes Association guidelines[186] according to presence of: 1) One or more classic symptoms (thirst, polyuria, weight loss, hunger, pruritus, or coma) plus fasting plasma glucose (FPG) $\geq$ 126 mg/dl (7.0 mmol/L) or random PG $\geq$ 200 mg/dl (11.1 mmol/L); or 2) in the absence of symptoms, at least two elevated PG levels on different occasions (FPG $\geq$ 126 mg/dl and/or random PG $\geq$ 200 mg/dl and/or PG $\geq$ 200 at $\geq$ 2 hours on oral glucose tolerance testing); or 3) treatment with hypoglycemic medication (insulin or oral hypoglycemic agent). We will not perform glucose tolerance testing to screen VITAL participants for T2D at baseline because such an effort would be extremely costly and logistically challenging. However, we believe undiagnosed diabetes is likely to be evenly distributed in each comparison group as a result of randomization in a large population. Furthermore, any misclassification on this basis would only lead to only a slight underestimation of the intervention effect in a prospective setting; it should not produce spurious positive results [187].

D.1.4. Additional information from the Centers for Medicare and Medicaid Services (CMS):

We also plan to retrieve additional data on the occurrence of any diabetes-related hospitalizations or outpatient visits for diabetes, as well as use of oral hypoglycemic agents or insulin, by linking the participants with Medicare claims inpatient and outpatient databases from the Centers for Medicaid and Medicare Services (CMS). Based on results from our previous validation studies and our extensive experience, we believe that combined information from annual self-administered questionnaires and medical information provided by physicians through medical record of diagnostic glucose testing or antidiabetic medication, coupled with additional information from the CMS linkage inpatient and outpatient databases, will be a highly reliable and cost-effective approach to identify T2D in the cohort. **The utilization of the CMS linkage database for diabetes diagnostic information will be independently supported by the funding from the Lung VITAL ancillary study (the Principal Investigator, Dr. Diane Gold).**

D.1.4.1. Linkage to the CMS inpatient and outpatient databases.

Medicare is a US federal health insurance program that reimburses in-patient costs for most citizens and permanent residents aged ≥65 years. On an annual basis, we will obtain identifiable (Medicare) Data Files through ResDAC (www.resdac.unm.edu/Index.asp), which is a CMS contractor that provides free assistance to academic and non-profit researchers interested in using Medicare or Medicaid data. The data use agreement

(DUA) delineates the confidentiality requirements of the Privacy Act and data release policies and procedures. Participants can be identified via their social security number and date of birth or through their Medicare ID (HIC). The following data will be obtained:

i. *MedPar Inpatient and Skilled Nursing Facility*. This file contains inpatient stay records summarizing all services rendered to a beneficiary from the time of admission to facility through discharge. Each record may represent one claim or multiple claims, depending on the length of stay and the amount of inpatient services used throughout the stay. Data include diagnosis and procedure codes, DRG, dates of service, reimbursement amount, hospital /SNF provider, and beneficiary demographic information.
ii. *Outpatient SAF*. Contains final action claims data from hospital outpatient departments, rural health clinics, renal dialysis facilities, outpatient rehabilitation facilities, comprehensive outpatient rehabilitation facilities, community mental health centers, and ambulatory surgical centers. Information includes diagnosis and procedures codes, dates of service, reimbursement amount, outpatient provider number, revenue center codes, and beneficiary demographics.
iii. *Home Health SAF*. Contains final action claims data including the number of visits, type of visit (e.g., skilled-nursing care, home health aides, physical therapy), diagnosis, dates of visits, reimbursement amount, provider number, and beneficiary demographics.
iv. *Carrier SAF*. Contains final action claims data from non-institutional providers, including physicians, physician assistants, clinical social workers, and stand-alone ambulatory surgical centers. Information includes diagnosis and procedures, dates of service, reimbursement amount, provider number, and demographics.
v. *Hospice SAF*. Contains final action claims data including level of hospice are routine (e.g., routine home care, inpatient respite care), terminal diagnosis, dates of service, reimbursement amount, provider number, and demographics.
vi. *Durable Medical Equipment SAF*. Contains final action claims data including diagnosis, services provided, dates of service, reimbursement amount, provider number, and demographics. (DME files began in 1994).
vii. *Bene ID Conversion File*. This file is needed to link the SSN finder file we will submit with the Medicare administrative data from the Research Data Distribution Center (RDDC). The identifier on Medicare data files processed after November 2005 is called the RDDC Beneficiary Identifier (RDDC Bene ID).

In previous studies which diabetes (ICD-9: 250), chronic obstructive pulmonary disease (COPD, ICD-9: 490-496, except 493), pneumonia (ICD-9:480-487), congestive heart failure (CHF, ICD-9: 428), and myocardial infarction (MI), (ICD-9: 410) were examined, disease diagnosis indices have successfully been developed using physician claims data; these may be useful in secondary analyses evaluating whether vitamin D reduces T2D[188]. The sensitivity and predictive value of Medicare hospital claims data for major ICD-9-CM diagnoses was evaluated by Fisher and colleagues, who compared claims data with the original hospital record data. The sensitivity for diabetes diagnosis was excellent: 0.84 (0.81, 0.86); the predictive value was 0.85 (0.83, 0.88)[189]. Dr. Joel Schwartz and Dr. Antonella Zanobetti**,** consultants to the parent VITAL trial, are investigators at the Harvard School of Public Health and have many years of experience working with administrative Medicare data to evaluate a wide range of chronic disease outcomes, including diabetes. We will be able to consult with them as needed for support and assistance in the use of CMS linkage for the VITAL diabetes grant.

D.1.4.2. CMS data protection and confidentiality. When we obtain Identifiable (Medicare) Data Files through ResDAC (www.resdac.unm.edu/Index.asp), we will carefully adhere to the DUA, the confidentiality requirements of the Privacy Act, and data release policies and procedures. The VITAL data coordinating center will address the issues regarding ensuring data integrity, security and confidentiality through a System Security Plan to the NHLBI. All VITAL coordinating center staff will sign a confidentiality agreement as well as attending HIPAA and IT security training. The CMS data will be securely stored at Division of Preventive Medicine, with access to the building controlled by key card, with security personnel monitoring entrance. The original media on which the CMS data are supplied will be stored in a locked cabinet. Clinical coordinating center passwords will be required to conform to our password policy. Logon access to the database will be password protected. Individually identifiable or deducible data will not be transmitted by unsecured telecommunications. The data will not be physically moved or transmitted from the clinical coordinating center without written approval from CMS. At the conclusion of the study, or by the date of retention identified in the DUA, a CMS “Certification of Destruction” certifying the proper destruction of all data obtained will be sent to CMS. These data files will be obtained through independent funding and will be available to the VITAL parent trial and all VITAL ancillary studies.

D.2. Our Substudy with Data Collection at Baseline and after 2-Year Follow-up

D.2.1. Clinical and Translational Science Centers (CTSC) subcohort in VITAL

The main purpose of our 2-year substudy among 1,000 participants is to obtain detailed clinical assessments of glycemic status, insulin sensitivity and secretion in order to understand whether vitamin D or omega-3 fatty acid supplementation compared with placebo results in discernable changes in intermediate phenotypes of glucose and insulin homeostasis over a 2 year period. Such data will shed light on the physiologic mechanisms by which either or both supplements reduce T2D incidence, as well as identifying potential risks.

This will be accomplished by establishing a Clinical and Translational Science Center (CTSC) subcohort of 1,000 VITAL subjects who live in four racially and ethnically diverse large metropolitan areas – Boston, Chicago, San Francisco, and Houston – and agree to additionally participate in a series of ancillary studies, including this proposal on T2D. These CTSC sites among four major metropolitan areas also provide good geographic and racial/ethnic diversity (please refer to **Section O** for letters of support and collaboration from the Principal Investigators of the CTSCs in Boston, Chicago, San Francisco, and Houston). These CTSC sites are part of a funded, high-priority NIH program for translational research that streamlines and facilitates the translation of research findings into tangible health benefits through established infrastructure for space, experienced staff, dedicated equipment and administrative support for the clinical visits for VITAL and other clinical research studies. Most of the staff time and faculty collaborations will be funded independently. Staff at each of the CTSCs will be trained in the standardized protocol for the VITAL CTSC component and will undergo regular site visits to evaluate compliance with the protocol and to identify any quality assurance problems.

Each of the 4 participating CTSCs has a project director and additional staff to carry out the protocol that may include recruitment coordinators, physicians, nurses, data collectors, and others. The project director will be appointed among local clinicians by the CTSC Director at each site and will have broad oversight for our ancillary studies, inter-institutional collaborations, and will bear ultimate responsibility for the operations of the substudy (Three of four CTSC project directors have been appointed and their letters of support are provided in Appendix C). The project director at each of the four CTSC will be responsible for monitoring each of the proposed ancillary studies, supervising the staff at the CTSC who will be conducting the various evaluations, and interfacing with the Project Coordinator and Principal Investigator in Boston. They will oversee the implementation of the CTSC protocol (including the oral glucose tolerance testing [OGTT]) at each site. The Principal Investigator of this ancillary study will oversee the entire ancillary project and will coordinate each project director of 4 CTSCs by producing and modifying standard protocols or operating procedures, monitoring the recruitment, reviewing and approving requests for data access and database proposals, monitoring human protection, confidentiality, and security procedures, scheduling regular meetings, and creating opportunities to enhance collaboration within and across CTSCs. Under the supervision of the Principal Investigator, the Project Coordinator will work closely with four project directors and other CTSC staff to ensure that the work is completed in an accurate and timely fashion. To maximize the consistency and quality control in the performance of the parent and ancillary studies across the CTSCs, staff at each site will be trained in the protocol for the VITAL CTSC component and will undergo regular site visits to evaluate compliance with the protocol and to identify any quality assurance problems. Data collected for this study will comply with the standardized protocol established by the ancillary study. Our project coordinator will provide research and administrative support as needed by the Principal Investigator and research team. He/she will work with CTSC staff from 4 sites to collect the data in a consistent manner, and to conduct site visits to ensure compliance.

As potentially willing and eligible participants are enrolled into VITAL, we will identify 1,000 participants without previously diagnosed T2D who are eligible respondents to the VITAL questionnaires in the greater metropolitan areas of Boston, Chicago, San Francisco, and Houston, and also agree to participate in ancillary studies (funded through other grants) that will make use of CTSCs for clinical examinations and blood collections. Approximately 250 participants will be recruited at each of 4 CTSC sites. In addition to prespecified inclusion criteria in the VITAL trial, all eligible participants should be in generally good health, mobile, and able to give complete informed consent. A copy of the consent form will be sent home and all participants will provide written informed consent. At both the baseline and 2-year follow-up CTSC clinical visits, several standard clinical assessments will be performed, including height, weight, waist/hip circumference, and blood

pressure. Participants will be compensated for their time and travel. This CTSC subcohort is expected to have the same racial/ethnic composition as the overall VITAL cohort and will be randomized equally into the 4 treatment groups (1000 overall; 250 participants in each of the 4 groups: vitamin D alone, omega-3 alone, both agents, or both placebos).

These 1,000 participants will also have blood drawn at baseline and after 2 years of follow-up (matched by season, by month). During the CTSC visit, blood will be drawn, centrifuged and processed locally, and the plasma, serum, red blood cells, and buffy coats (including multiple vials of EDTA, heparin, and citrate plasma, and one vial each for EDTA red blood cells, EDTA buffy coat, and citrate buffy coat) will be frozen and stored temporarily at the CTSC laboratory. At regular intervals, the CTSC will send overnight shipments of all frozen samples to the blood laboratory at the Division of Preventive Medicine, Brigham and Women's Hospital for storage in liquid nitrogen freezers. Each freezer has a back-up power system connected to an electronic alarm that has protected stored samples from inadvertent thawing or warming for nearly 20 years. This resource will also be extremely valuable for future studies to explore other genetic and biochemical hypotheses in this well-characterized cohort. A nested case-control or case-cohort approach can be used, enabling the study of many promising biochemical and genetic markers (e.g., VDR polymorphisms) as potential predictors of disease risk and modifiers of intervention effects at very low incremental cost.

D.2.2. Assessment of glucose and insulin homeostasis

We propose to perform an oral glucose (75 g) tolerance test (OGTT) (2 hours) to accurately screen for T2D in our subsample of 1,000 participants at study entry and after randomization. Our aims in this effort will be to: 1) obtain more accurate assessment of insulin and glucose homeostasis using OGTT; and 2) assess the effect of vitamin D and omega-3 supplementation on objective measures of insulin sensitivity and insulin secretion by OGTT, plasma HbA1c levels, and HOMA-IR and HOMA-%B. This approach represents an extremely cost-effective trial considering its size and potential to answer critical questions. Participants in this subset will be stratified by randomized assignments in a manner that will provide important information for objective estimates of insulin sensitivity and secretion. The data collected in this subset will complement the data collected in the entire trial by providing more important information on the individual effects of vitamin D and omega-3 fatty acids in intermediate metabolic phenotypes.

Following standardized protocol, an OGTT using an oral dose of 75 g glucose will be performed in the morning at the same time for each participant after an overnight fast. Blood samples will be taken at baseline (-5 minutes) and after 30, 60, and 120 minutes for measurements of glucose and insulin. Diabetes will be defined according to the 2003 criteria of the American Diabetes Association[186]: a plasma glucose value of $\geq$ 126 mg/dL (7 mmol/L) in the fasting state or a 2-hour postprandial glucose $\geq$ 200 mg/dl (11.1 mmol/L) after taking a 75-g oral glucose load. The following parameters will be measured: (1) Insulin sensitivity and β-cell function. Insulin sensitivity will be estimated using the whole-body insulin sensitivity index (ISI) derived from the OGTT as proposed by Matsuda and DeFronzo[190]. Insulin secretion will be estimated using the insulinogenic index, which is a commonly used index of β-cell function defined as the ratio of the increment of plasma insulin (μIU/ml) to that of plasma glucose 30 min (mmol/ml) after glucose loading. (2) Glucose tolerance or glycemic status. In addition to fasting glucose and insulin levels, HbA1c will be monitored to reflect recent average glycemia during the previous 2 to 3 months and to assess its changes in response to the assigned interventions. (3) Other indices of glucose and insulin homeostasis. The fasting plasma glucose and insulin levels and their derived HOMA-IR and HOMA-B will be analyzed as a secondary variable. HOMA-IR will be computed as follows: fasting insulin (μIU/ml)$\times$ fasting glucose (mmol/ml) /22.5 and HOMA-%B will be calculated using the following formula: 20 $\times$ fasting insulin (μIU/ml)/ fasting glucose (mmol/ml)-3.5. Glucose will be measured enzymatically on the Hitachi 911 analyzer using Roche Diagnostics reagents (Indianapolis, IN). Insulin will be measured by an ultra-sensitive ELISA assay from ALPCO Diagnostics (Windham, NH). HbA1c will be measured using turbidometric immunoinhibition, classically using whole blood hemolysates (Boehringer Manheim). **We will store all the samples collected at baseline and 2-year follow-up for batching at the blood laboratory at the Division of Preventive Medicine, Brigham and Women's Hospital in Boston, MA. To minimize biases due to intra- and inter-assay variability across CTSCs and/or over time, no assays including OGTT will be done by the Central Lab, the Biochemistry Laboratory of Dr. Nader Rifai at Children's Hospital in Boston (MA), until the end of follow-up for this substudy.**

D.2.3. Measurements of plasma 25(OH)D levels:

As part of the parent VITAL grant (NIH 1 U01 CA138962), plasma levels of 25(OH)D will be assayed and available for our proposal in 2000 paired blood samples (baseline and 2 year follow-up). Plasma 25(OH)D will be measured by Dr. Bruce Hollis at the Medical University of South Carolina. Circulating 25(OH)D will determined by radioimmunoassay, which uses reagents from the DiaSorin Corporation (Stillwater, NM), recognizes and quantitates $25(OH)D_2$ and $25(OH)D_3$ equally. This assay has been in widespread clinical use for nearly 20 years, and has also been employed for research use in numerous research studies. In Dr. Hollis' laboratory, the intra- and inter-assay coefficients of variation are <10% for 25(OH)D[191-193].

Despite the widespread use of this radioimmunoassay, we are cognizant that there are multiple other measurement techniques currently employed around the world, and that there is substantial intra- and interassay variation in reporting of 25(OH)D levels among these techniques (i.e., laboratory drift over time plus lack of agreement among different techniques). As a consequence, the VITAL study will purchase and utilize Standard Reference Material (SRM) for $25(OH)D_2$ and $25(OH)D_3$ measurements (SRM 972); these samples will soon be available from the National Institute of Standards and Technology (NIST). Dr. Hollis will report his assay performance based on this SRM, and we will incorporate the results in all appropriate manuscripts. In addition, the VITAL study will participate in the Office of Dietary Supplements (ODS) funded NST quality assurance program for measurement of $25(OH)D_2$ and $25(OH)D_3$ using human blood samples. Use of SRM 972 will enhance the interpretability and validity of our study, and participation in the quality assurance program will enhance the overall performance of our lab over time.

D.3. Trial Data Management and Monitoring

For the main VITAL study of 20,000 participants, our computing system, which was developed and fine-tuned in our previous trials, will track each participant's stage and level of participation in VITAL by automatically generating letters, questionnaires, and phone call reminders at the appropriate times. Questionnaire data will be optically scanned into the computer using TELEform and Alchemy (Cardiff Software) programs that have been successfully used for our other mail-based clinical trials (WHS, PHS). Out-of-range, internally inconsistent, and unclear data are reviewed by a verifier who corrects misread variables. Forms that cannot be scanned, and name and address changes, are entered using traditional double-entry procedures. All data undergo additional within-form and across-time checks to verify accuracy. The database will be maintained on a UNIX server. All data files will be backed up nightly, ensuring at least two current copies at all times. Each month, a set of data files will be taken off-site for long-term storage.

The Principal Investigator will be responsible for the observance of all conditions of use and for establishment and maintenance of appropriate administrative, technical and physical security safeguards to prevent unauthorized use and to protect the confidentiality of the data. To maintain confidentiality, we will substitute codes for participant identifiers, remove face sheets containing patient demographic information, properly dispose of participant identifying documents, limit access to identified data and store research records in locked cabinets. All participant samples obtained will be maintained in the Division of Preventive Medicine at Brigham and Women's Hospital. Information obtained from the results of our study, if published, will be presented collectively and without participant identifiers. In addition, our project programmer will analyze the data and create summary-level reports for each CTSC site to inform the Principal Investigator of the results of the project. Data reports will also be produced to comply with the requirements of the grant. For the CTSC subcohort of 1,000 VITAL participants, study personnel at each CTSC site will collect the data from the proposed clinical visits using optically scannable forms. These forms will be optically scanned at each CTSC site and sent to the Division of Preventive Medicine for additional reviews, verification, and data checks to identify out-of-range values and maximize accuracy. **All the blood samples collected at baseline and 2-year follow-up for the substudy will be stored for batching until the end of follow-up. If available, the testing results including OGTT will be reported to local health care providers. The investigators will refer participants to their primary care physicians when diabetes is diagnosed or when test results require follow-up.**

With regard to the risks of the study agents, information on potential side effects discussed above will be elicited on each follow-up questionnaire for monitoring by the Data and Safety Monitoring Board. We will compare the incidence of potential side effects in the active versus placebo groups for each agent, including the incidence of kidney stones with vitamin D assignment and the incidence of GI symptoms, skin abnormalities, and bleeding with fish oil assignment. The potential risk of disclosure of confidential information is guarded against by maintaining completed questionnaires and blood test results in locked files accessible by authorized personnel only. 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. Each dataset will require documentation. Documentation provides information about the methodology and procedures used to collect the data, details about codes, definitions of variables, variable field locations, frequencies, and the like. The precise content of documentation will vary by scientific area, study design, the type of data collected, and characteristics of the dataset. The data will be available only to authorized staff members. Our existing computer and security systems, which balance these considerations, will be used in VITAL. 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.

D.4. Statistical Analysis

D.4.1. Primary analysis plan for all incident T2D in the parent trial

All analyses will be conducted with SAS version 9 (SAS Institute, Cary, NC), and a 2-sided test with a significance level of α=0.05 ($p<0.05$) will be used. Analyses of treatment effects will be based on the intent-to-treat principle. All prevalent T2D cases at baseline will be excluded. The first analysis will compare baseline characteristics by randomized treatment assignment to ensure that balance is achieved by the randomization. Baseline characteristics will be compared by randomized groups using 2-sample t tests for continuous variables and χ^2 statistics for categorical variables. Characteristics to be examined include known diabetes risk factors, including age; gender; race/ethnicity; BMI; smoking; alcohol use; physical activity; medical conditions such as hypertension, hyperlipidemia, and family history of diabetes mellitus; and baseline vitamin D and omega-3 fatty acid intakes as assessed by dietary questionnaires of all participants. 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.

The primary aim is to compare the main effects of intention-to-treat with vitamin D and with fish oil on the incidence of T2D. Kaplan-Meier survival curves will be used to estimate the overall cumulative incidence over time for each active treatment group and its corresponding placebo group. The log-rank test will be computed to compare the curves. We will then use the Cox proportional hazards model to allow for variable follow-up lengths and will estimate the hazard ratio for each intervention using indicators for treatment exposure[194], controlling for age and gender. To test the proportionality assumption (i.e., that of non-changing hazards ratios over time), we will include an interaction term for treatment with the logarithm of time in the Cox models. Beyond the primary analyses, we will examine effect modification by two randomized interventions, sex, and race. Similar analyses will be conducted in subgroups stratified by sex and race (Whites and Non-Whites). We also have a prior interest in the effects of the vitamin D intervention within groups defined by race/ethnicity, sunlight exposure, geographic regions, and BMI. In addition, we will evaluate effect modification by vitamin D, long-chain polyunsaturated fatty acids, calcium and phosphorus intakes from the FFQ (as these nutrients affect vitamin D bioavailability[41]), as well as other baseline risk factors for T2D. We will formally assess the statistical significance of effect modification using interaction terms between subgroup indicators and randomized assignment, testing for trend when subgroup categories are ordinal. We will also test interactions of the 2 agents using interaction terms in the Cox model.

Multiple sensitivity analyses will be carried out according to compliance. Participants will be censored if and when they stop taking at least two-thirds of their study pills, report taking outside supplements containing study agents, or are missing compliance information. Considering latency period for diabetes prevention, we will also subdivide the period of risk into years 1 and 2 combined and year 3 onward combined. We will examine whether treatment effects vary over time and duration of treatment by examining survival plots and interactions with time as described above in the test for non-proportional hazards. **To further safeguard the health of the study participants, all the test results including OGTT in the subset study will be reported to local health care providers. Because intervention approaches provided by physicians among diabetes patients or pre-diabetic individuals (i.e. IFG or IGT) will possibly dilute the effect estimates from the primary intention to treat analysis in the entire trial, future secondary analyses by excluding the substudy samples (5%) will be performed.**

D.4.2. Analysis plan for longitudinal changes in intermediate phenotypes in our subset study

For all continuous measurements (e.g. HbA1c, OGTT parameters) at one time point (pre-randomization and post-randomization), we will first examine the distributions and use transformations to achieve normality when necessary. We will compare baseline values to ensure balance by randomized treatment assignment, both overall and by CTSC site. The intraclass correlation coefficient will be calculated to determine the association between two separate measurements for each parameter over time in each intervention group for descriptive purposes.

The primary analysis in our proposed substudy is to determine the effects of randomized vitamin D or omega-3 treatments on the changes in surrogate measures of glucose tolerance, insulin sensitivity and insulin secretion between baseline and the end of 2-year intervention period. These continuous measurements will be examined by analysis of covariance, including terms for baseline value, treatment, and CTSC site. For each continuous primary outcome (surrogate indices of insulin sensitivity and secretion and HbA1c levels), we will use a linear mixed-effects model for these repeated measures over time, including two measurements for each outcome as the dependent variables, with random intercepts and slopes as a function of time and fixed effects for CTSC sites, treatment, time, and interaction between time and treatment, accounting for the correlation among repeated measures in the same participant. Within the mixed model, we will estimate 95% CIs and P values for the two main intervention effects (vitamin D versus placebo and omega-3 fatty acids versus placebo) for changes in those indices between baseline and 2 years of follow up within each group.

With multiplicative interaction terms, we will study whether treatment effects differ by the other intervention or across subgroups. These prespecified analyses will be performed for age (<75 and ≥75 years), sex, race (Whites or non-Whites), BMI (<25 and ≥25 kg/m^2), baseline levels of 25(OH)D, and baseline intake of dietary long-chain omega-3 fatty acids (above and below the median). Additional analyses will be performed with adjustment for age and BMI at baseline and sex and race. For continuous secondary outcomes (HOMA-IR, HOMA-B and fasting glucose and insulin levels), we will use the same procedures as in the primary analysis. The regression parameters in our linear mixed-effects models will be estimated with the Proc Mixed program of the SAS software package (version 9, SAS Institute, Cary, N.C.). Testing will be two-sided and conducted with a 5% type I error rate.

In addition, to increase power, we will analyze the change in measures from baseline to follow-up conditioning on the baseline value. Because of the randomization, this is unlikely to be affected by regression to the mean, and should provide a more powerful analysis, depending on the correlation of the measures over time[195]. We will use linear regression analysis to adjust for any imbalance in key variables at baseline, and to adjust for study site in the CTSC data. If follow-up to the 2-year evaluation is less than complete, we will conduct sensitivity analyses to estimate the effect under intention-to-treat. For example, we will impute the mean change in effect found in the placebo group, adding a random error to allow for within-person variation. We will use multiple imputation to correct the error term[196, 197]. While this should drive the estimated treatment effect closer to the null, those who do not appear for follow-up visits are less likely to be continuing to take study agents.

D.5. Power Calculations

We calculated the power of our primary trial and substudy separately by using a Stata program developed by Royston and Babiker for multi-arm clinical trial designs[198, 199]. The power computation is based on the log-rank test and is performed according to the asymptotic distribution of the log-rank test statistic, adjusted appropriately for the design features such as arbitrary time-to-event distribution, nonproportional hazards, non-uniform rates of patient entry, loss to follow-up, and treatment changes from allocated treatment [198]. This approach was performed using Intercooled Stata version 8.2 (Stata Corporation, College Station, TX).

D.5.1. Type 2 diabetes risk in the trial. Based on the age-specific rates of prevalence and incidence of T2D in the U.S. general population (non-Hispanic whites and African Americans), we calculated the power to detect a clinically meaningful 10-40% reduction in the incidence of T2D among the participants without self-reported T2D at baseline. Assuming that the prevalence of T2D for the U.S. elderly population (≥60 years) is

Table 3. Power for effects of a single agent on incident T2D in the parent VITAL trial with 10,000 men aged≥60 and 10,000 women aged≥65 with 5 years of follow-up

RR†	Total	Women	Men	White	Minority
	16,770 ‡	9,210 ‡	9,270 ‡	14,100 ‡	4,305 ‡
0.90	79.4%	42.2%	42.4%	58.8%	22.5%
0.85	98.8%	76.5%	76.8%	91.3%	45.0%
0.80	>99.9%	95.3%	95.4%	99.4%	69.9%
0.75	>99.9%	99.6%	99.6%	>99.9%	88.3%
0.70	>99.9%	>99.9%	>99.9%	>99.9%	97.0%
0.65	>99.9%	>99.9%	>99.9%	>99.9%	99.5%
0.60	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%

†Expected RR; ‡ Expected total number of non-diabetic participants.

16.0%[31], we will have approximately 4,200 participants for each randomized treatment. With an annual incidence rate of T2D of 2.7% for the U.S. elderly population (≥60 years)[32] and a significance level of 5% for a 2-sided test, we will have about 80% power to detect a 10% reduction in T2D during 5 years of treatment and follow-up (allowing for a 80% compliance rate in each arm) (**Table 3**). As expected, power to explore the sex-specific or ethnic-specific effects in the cohort will be lower due to decreasing sample sizes. We would have enough power to detect a 15-20% risk reduction for women and men, separately. Since 25% of the total population will be non-white minority group, we would have 80% power to detect a risk reduction of approximately 25% in this group.

If both agents are effective in preventing T2D 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. By assuming a 2-sided α level at 0.05, we calculated power over a range of one single agent effect from 0.80 to 0.75, and the ratio of RRs for interaction ranging from 1.00 (only an additive effect) to 0.70 (with an additional 30% reduction). As shown in Table 4, using the total samples of non-diabetic participants (n=4,200 participants for each randomized treatment given approximately 16% prevalent T2D in the U.S. elderly population)[31], we will have enough power (>80%) to detect important interactions with a ratio of RR ≤0.90. Should the agents act synergistically, power would increase, as illustrated in **Table 4** for main effects for the outcome of T2D.

Table 4. Power for interaction effects on incident T2D in the VITAL trial of 10,000 men aged ≥60 and 10,000 women aged ≥65 with 5-year follow-up

RR Single agent†	Interaction †	RR Both agents	Power Main Effect	Power Interaction
0.90	**1.0**	**0.81**	73.3%	
	0.9	**0.73**	96.1%	24.4%
	0.8	**0.65**	99.8%	74.8%
	0.7	**0.57**	>99.9%	98.4%
0.85	**1.0**	**0.72**	97.1%	
	0.9	**0.65**	99.8%	23.1%
	0.8	**0.58**	>99.9%	71.7%
	0.7	**0.51**	>99.9%	97.7%

†RR = expected RR, including only compliant participants (assuming 80% compliance). Represents the effect among those not assigned to the other intervention and assumes the same effect for both agents. The interaction is the RR for the combined group divided by the product of risks for the two separate groups—i.e., RRint=RRboth/(RR vit D alone* RR fish oil alone). An interaction=1 implies additive effects (no interaction).

D.5.2. <u>Continuous outcomes in the substudy</u>. To compute statistical power for changes in each of continuous outcomes collected in the subset study, we must obtain the expected mean difference and its standard deviation based on previous clinical randomized trials which ascertained the effect of fish oil supplements (DHA and EPA) and vitamin D3 on glucose and/or insulin homeostasis. Given numerous small-scale trials of fish oil supplementation, we focused on the pooled weighted mean differences or net mean differences from previous meta-analyses rather than individual trials. We identified 4 meta-analyses of randomized trials of fish oil supplements on glycemic biomarkers in which 15 or 21 randomized trials were included with trial durations of 2 to 52 weeks (**Table 2**)[124, 126, 158, 159]. We also identified 4 randomized trials with a total of 1,560 non-diabetic participants and durations from 6 weeks to 7 years (median=24 months) that specifically examined vitamin D3 supplementation (doses ranged from 400 to 8,572 IU/day; the median dose=2000 IU) (**Table 1**)[67, 68, 92, 93]. These previous clinical trials or meta-analysis of randomized trials provided sufficient information to compute the standard deviations of the outcome change for at least one of main outcomes. Based on the respective median values (to avoid unstable outliers), we finally assumed standard deviations of 2.28 for ISI, 12.0 for IGI, 0.72 for HbA1c, 0.32 for HOMA-IR, and 16 for HOMA-B, respectively.

Table 5. Power calculation for intervention effects on surrogate measures as continuous outcome variables in our subset study of 1000 non-diabetic participants †

ISI	Power	IGI	Power	HbA1c	Power	HOMA-IR	Power	HOMA-B	Power
0.15	41.2%	1.5	91.1%	0.05	45.0%	0.05	98.5%	2.0	91.1%
0.25	82.6%	2.5	>99.9%	0.10	95.7%	0.10	>99.9%	2.5	98.5%
0.35	98.2%	3.5	>99.9%	0.15	>99.9%	0.15	>99.9%	3.0	99.8%
0.45	99.9%	4.5	>99.9%	0.20	>99.9%	0.20	>99.9%	3.5	>99.9%

ISI, Insulin Sensitivity Index (mg/kg/min); IGI, Insulinogenic index for Insulin Secretion (pmol/mmol); HbA1c, glycated hemoglobin A1c (%); HOMA-IR, the homeostasis model assessment of insulin resistance; HOMA-B, the HOMA of beta-cell function.

†The expected effects were expressed as the mean difference of the outcome change between baseline and after 2 years of follow-up (the treatment group versus the placebo group).

Therefore, to calculate power, the following assumptions were made: (1) a 2x2 factorial trial in 1,000 participants without T2D at baseline; (2) independent and equal allocation of participants to each treatment; (3) an estimated 13% undiagnosed T2D identified by OGTT; and (4) a dropout rate of 20%. Using these estimates, a total of 700 non-diabetic participants will provide us sufficient power to detect various changes in these intermediate measures between baseline and after 2 years of follow-up by interventions. As noted in **Table 5**, overall, we will have adequate power (>80%) to detect the expected 2-year changes in our

primary outcome measures (ISI, IGI, and HbA1c) and secondary measures (HOMA-IR and HOMA-B), although we will not have enough power to detect sex- or ethnicity-specific effects in this subset study.

D.6. Strengths and Limitations

This ancillary study will test two very promising nutritional agents (vitamin D and fish oil) for the prevention of T2D in an extremely cost-effective fashion—i.e., utilizing the resource provided by the parent VITAL trial. Strengths of this ancillary study include the unique resource provided by the parent trial, large numbers of participants at high risk for T2D, long follow-up, high-quality covariate data, and exceptional cost-efficiency. The dose for each agent was chosen on the basis of an extensive and careful review of available evidence, with the goal of optimizing the balance of safety and efficacy. We will develop and implement an inexpensive and efficient strategy to validate all self-reported incident T2D cases in the entire trial population as a primary outcome and to collect pre- and post-intervention measures of insulin and glucose homeostasis in a subset of the participants as secondary outcomes. The proposed study also has some potential limitations.

Misclassification due to undiagnosed T2D: As a continuous progressive metabolic disorder, T2D often begins with years of an asymptomatic state of insulin resistance (including hyperglycemia and compensatory hyperinsulinemia) before resulting in an irreversible "exhaustion" of pancreatic β-cells that ultimately leads to an irreversible state of T2D. As in any large-scale epidemiological study, classification will not be perfect and will involve some trade-offs. Because the cost for screening 20,000 participants who currently reside in different parts of the country would be prohibitively high and the gain in data quality negligible, the strategy we propose to assess T2D is a cost-efficient and sufficiently accurate to achieve the outlined scientific goals. We believe undiagnosed diabetes is likely to be evenly distributed in each comparison group as a result of randomization in a large population. Furthermore, any misclassification on this basis would only lead to an underestimation of the intervention effect in a prospective setting; it should not produce spurious positive results. We also plan to retrieve additional data on the occurrence of any diabetes-related hospitalizations, and use of oral hypoglycemic agents or insulin by linking the participants with Medicare claims data from the CMS database. Based on results from our previous validation studies and our extensive experience, we believe that combined information from annual self-administered questionnaires and medical information provided by physicians through medical record, phone interview, or supplementary questionnaire, coupled with additional medical record data from the CMS linkage database, will be a highly reliable and cost-effective approach to identify T2D in the cohort and minimize the proportion of undiagnosed cases.

Fixed doses set by the parent study: The goal of our ancillary study is to complement but not overlap or interfere with the primary and secondary objectives of the parent trial. Our proposed diabetes research will test only one dose of each agent rather than examining multiple doses to determine the dose-response relationship. The dose for each agent was chosen on the basis of an extensive and careful review of available evidence, with the goal of optimizing the balance of safety and efficacy. The dose of vitamin D (2,000 IU/day) is sufficient to produce optimal plasma concentrations of 25(OH)D but below the tolerable upper intake level of vitamin D ingestion in adults. On the basis of pharmacokinetic and epidemiologic data, it may be possible to conclude that vitamin D supplementation is effective without an additional trial using multiple doses. The proposed intervention of 1 g/d EPA/DHA supplement would be expected to have the effect of achieving the purported optimal levels associated with health benefits in previous studies but with minimal risks.

Multiple comparisons: There is a concern of false positive results due to multiple testing especially in our proposed subgroup analyses. However, our stratified analyses are essentially fully pre-specified based on prior clinical data and are exploratory in nature. Although we do not plan to formally implement statistical adjustments for multiple comparisons, we will evaluate any observed effects in light of prior human and animal data, as well as the plausibility of the biological mechanisms.

Generalizability: Because our participants are not a random sample of US adults, we will not generalize our findings to the US population without carefully considering lifestyle and genetic characteristics. Because the trial population is older, the results may not be generalizable to younger men and women. However, older populations have the highest rates of T2D, allowing the trial to be completed in a shorter time period, at greater cost efficiency.