Title: Intervention With Vitamin D and Omega-3 Supplements and Incident Heart Failure

NCT #: NCT02271230

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Study Protocol and Statistical Analysis Plan

1. Overview of parent *VITAL*

VITAL is an ongoing and NIH-funded (R01 CA138962) randomized, double-blind, placebo-controlled trial using a 2x2 factorial design to test the effects of 2,000 IU/d of vitamin D and 1 g/d of EPA/DHA (EPA: DHA ratio of 1.3:1) supplements in the primary prevention of cardiovascular disease and cancer among 25,871 men and women during 5 years of treatment and follow-up.

Enrollment, eligibility, run-in, and randomization. For enrollment into VITAL, 1.2 million ethnically and racially diverse people, including health professionals, AARP members, *Essence* subscribers, professionals, college-educated individuals, and Black business professionals, were mailed a detailed description of VITAL's objectives, an informed consent form, a brief questionnaire, and a self-addressed, pre-paid envelope for returning the forms. Eligibility criteria for VITAL included (1) age ≥50 (men) or ≥55 (women); (2) no history of cancer (except non-melanoma skin cancer), myocardial infarction, stroke, transient ischemic attack, angina pectoris, coronary artery bypass graft, or percutaneous coronary intervention; (3) no history of safety exclusions: kidney stones, renal failure or dialysis, hypercalcemia, hypo- or hyperparathyroidism, severe liver disease, sarcoidosis, active chronic tuberculosis, or Wegener's granulomatosis; (4) no allergy to fish (for EPA+DHA); (5) no other serious illness that would preclude participation; (6) consumption of <800 IU vitamin D from all supplemental sources combined; (7) current intake of <1.2 g/d of calcium from all supplemental sources combined; (8) not taking fish oil supplements; and (9) ability to sign the informed consent form. **Table 1** shows baseline characteristics of VITAL participants.

A **run-in** phase was designed to eliminate poor compliers prior to randomization to increase statistical power. Willing and eligible participants were mailed placebo for vitamin D and EPA/DHA to assess potential compliance and side effects. After 3 months, a follow-up questionnaire was used to query about continued eligibility, willingness, compliance, and potential side effects. Non-responders were mailed 2nd and 3rd requests.

For **randomization** into VITAL, participants are required to be: (i) compliant (taking two-thirds or more of the study pills during run-in); (ii) willing to continue; (iii) eligible based upon the above criteria; and (iv) willing to limit supplemental vitamin D intake and forego fish oil supplements. Randomization is being stratified by 5-year age groups, and in blocks of 8 individuals, with 2 individuals in each of the 4 treatment combinations. Pilot mailings indicated that 40,000 individuals, including >10,000 minority participants, were willing and eligible for the run-in, of whom 65% (n = ~26,000) were compliant and remained willing and eligible for randomization.

Table 1. Baseline characteristics of VITAL cohort (n=25,874)

Male	50%
Mean age (±SD) -Years	67±7
Mean BMI (±) - kg/m^2	29±6
Non-Hispanic White	71%
African Americans	20%
Age ≥ 65	63%
≥High school graduate	99%
Exercise (MET/week)	15
Any alcohol use	69%
Aspirin use	45%
Income <$15,000	6%
Use of multivitamin	44%
Current smoker	4%
History of diabetes	13%
Prevalent hypertension	53%
Current statin use	37%

Baseline questionnaire and food frequency questionnaire (FFQ): Each VITAL participant completed a comprehensive baseline questionnaire to collect information on demographics, medical history, medication use, vitamin D, fish oil, and other supplement use, lifestyle and clinical risk factors, and potential effect modifiers of vitamin D such as skin pigmentation and sunlight exposure. A self-administered FFQ was also mailed to participants during the run-in. The FFQ has been shown to be an efficient and valid instrument to assess individuals' diet and to derive nutrients. To capture changes in dietary habits over time, a second FFQ will be administered to all VITAL participants at 24 months of follow up. Average dietary intake of vitamin D, EPA, and DHA and other important nutrients will be estimated from the FFQ for proposed analyses.

Baseline and follow-up blood collection: Blood collection kits were sent to willing subjects during the run-in period to collect fasting blood samples; subjects were asked to record the time of venipuncture and their last meal. Collected blood samples are being sent back to our laboratory in freezer packs within 24 hours of blood collection. Upon receipt, we process and aliquot these specimens into multiple vials that are then kept at -170º Celsius in liquid nitrogen. Blood samples are processed within hours of receipt to ensure that samples are frozen within 30-36 hours after venipuncture. In addition, a random sample of 2,000 individuals will have follow-up blood drawn after 24 and 48 months of follow-up at the Boston Clinical and Translational Science Center

(CTSC) to assess compliance and changing trends in background fortification with vitamin D and marine omega-3 fatty acids, as well as to evaluate physiologic changes in selected biomarkers. To date, we have collected baseline blood collection 16,361 VITAL participants.

Quality assurance and follow up. Quality assurance testing ensures that the intervention (vitamin D and EPA/DHA) contains the correct doses and is free of contaminants. At 6 months and at each anniversary date of randomization, each participant will receive a follow-up questionnaire and a re-supply of study treatments. Each annual follow-up questionnaire assesses compliance, use of non-trial vitamin D and fish oil, dietary intake of vitamin D and fish, information on interim medication use, physical activity, weight, comorbidity including HF, etc. Data on potential side effects are being collected and tracked by a Data and Safety Monitoring Board. Non-respondents receive multiple mailed requests, followed by a telephone call to collect data. For non-respondents, vital status will be ascertained. To further test compliance, 250 VITAL subjects residing in New England will receive unannounced visits to draw bloods to measure plasma vitamin D and EPA/DHA.

2. Follow-up and adjudication procedures for HF (requiring hospitalization) and recurrent HF hospitalizations.

The main method of follow-up is mailed questionnaires and review of medical records to confirm study endpoints. Participants receive follow-up questionnaires at 6 months and 1 year after randomization and annually thereafter. A new supply of study pills is mailed with each annual questionnaire. The questionnaires ask about compliance with randomized treatments, use of non-study supplements of vitamin D and marine omega-3 fatty acids, development of major illnesses including HF, cancer and CVD, and potential side effects of the study agents. Non-responders receive two additional requests by mail and are then telephoned to collect study data. At a minimum, vital status is ascertained.

Participants who report a HF diagnosis are asked to sign a medical release for relevant medical records. An Endpoints Committee of physicians blinded to treatment assignment (including Drs. Djousse, Gaziano, and Albert) will review the records to confirm or disconfirm the diagnosis of HF. Deaths are confirmed by convincing evidence from available sources, including death certificates, hospital records, autopsy reports, and for deaths outside the hospital, observer accounts. For deaths reported by family members, the next-of-kin is asked to provide medical records and a copy of the death certificate. If the latter is not provided, a copy is obtained from the appropriate state vital records bureau. If records are not available (or if participants are lost to follow-up), we search the National Death Index Plus to obtain an International Classification of Disease-coded cause of death based on death-certificate information.

We will **use criteria outlined by the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards** to define HF. This definition requires **ALL** of the following **four elements** to be met:

1. Patient's admission to the hospital with a primary diagnosis of HF
2. New or worsening symptoms due to HF on presentation including at least **ONE** of the following: dyspnea, worsening peripheral edema, decreased exercise tolerance, fatigue, and other symptoms of worsened end-organ perfusion or volume overload
3. At least **TWO physical signs** (including rapid weight gain, pulmonary rales, increased jugular venous pressures and/or hepatojugular reflux, peripheral edema, increasing abdominal distension or ascites in the absence of primary liver disease, and S3 gallop) or **ONE physical sign plus at least ONE laboratory criterion** obtained within 24 hours of presentation (increased B-natriuretic peptide, radiologic evidence of pulmonary congestion, and non-invasive or invasive diagnostic evidence of elevated left- or right-sided ventricular filling pressure or low cardiac output)
4. Patient receives initiation or intensification of HF treatment including at least **ONE** of the following [(i) significant augmentation in oral diuretic therapy, (ii) intravenous diuretic, inotrope, or vasodilator therapy, and (iii) mechanical or surgical intervention such as mechanical circulatory support or mechanical fluid removal].

The same definition will be used to adjudicate recurrent HF hospitalizations. Once pertinent information has been abstracted from the medical records using the data form, two physician adjudicators will review each chart independently, followed by a joint meeting of the entire HF Endpoint Committee to discuss cases where disagreement exists among the two reviewers. Here, we will adopt a simple majority vote. Each event will be

evaluated for presence of HFpEF (EF≥ 50%) or HFrEF (EF<50%) based on ejection fraction (EF) obtained during the index hospitalization. Whenever possible, we will assign the etiology of HF (i.e., valvular, arrhythmia, coronary artery disease, etc).

Linkage to the Centers for Medicare & Medicaid Services (CMS)

To supplement the number of self-reported HF from follow-up questionnaires, we will also identify VITAL participants (mean age 67 y) with incident HF by linking our data with the CMS database. We will use HF diagnoses (ICD-9 codes 428) for such linkage. Costs associated with CMS linkage are covered by the parent VITAL trial. Each year, we will obtain Medicare Data Files through ResDAC, a CMS contractor for academic researchers. Although CMS might be more likely to capture older subjects (65+ years), women, and white participants, we anticipate equal proportion of subjects randomized to either intervention or corresponding placebo given randomization and a large sample size. Hence, the difference between intervention and placebo should remain constant.

3. Research Design and Methods

Measurement of other important variables

Information on demographics, anthropometrics, lifestyle factors, family history of coronary artery disease, and comorbidity will be obtained at baseline and during follow-up via study questionnaires.

Data management and cleaning

Our team has developed a sophisticated computer system to create and maintain data. A computing system tracks each participant's stage and level of participation in VITAL by automatically generating letters, questionnaires, and phone call reminders. Questionnaires are being optically scanned using TELEform and Alchemy (Cardiff Software) programs, which have been successfully used for our other mail-based clinical trials (PHS, WHS). Out-of-range, internally inconsistent, and unclear data are reviewed and corrected as necessary. Unscannable forms and name or address changes are double entered into the system. All data undergo additional within-form and across-time checks to verify accuracy. The VITAL database will be maintained on a UNIX server that is backed up nightly, ensuring at least two current copies at all times.

Data entry is performed using formatted screens with built-in error checking. Each follow-up questionnaire is entered by two different persons. The two generated files are compared using an interactive verification program that allows for immediate correction of any discrepancies. Before being added to the database, the data undergo further within-form and across-time verifications to assure completeness, accuracy, and participant identification number. Any errors in coding or keying are corrected promptly.

Statistical analyses by specific Aim

Primary Aim 1 Quantify the effects of vitamin D3 intervention vs. placebo on incidence rate of HF.

***Hypothesis**: Intervention with vitamin D3 reduces the incidence rate of HF among VITAL participants.*

Study design: Randomized double-blind, placebo-controlled, 2x2 factorial trial of vitamin D3 and EPA/DHA.

Study subjects: Participants included in the VITAL (n=25,871).

Primary outcome: Incidence of HF hospitalization

Statistical model: We will exclude any prevalent HF at baseline and use Cox proportional hazards model to estimate effects. Time-to-event will be calculated as the interval between time of randomization and the earliest of: 1) HF incidence, 2) death, or 3) end of the study. 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 HF, such as age; gender; race/ethnicity; body mass index; smoking; alcohol use; physical activity; medical conditions such as hypertension, hyperlipidemia, diabetes, and family history of coronary disease; and baseline vitamin D and omega-3 fatty acid levels as assessed by FFQ. 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

In this 2x2 factorial design, the primary aim is to compare the main effects of intent-to-treat with vitamin D3 on incidence rate of HF. We will use the Cox proportional hazards model to allow for variable follow-up lengths and will estimate the hazard ratio for vitamin D3 intervention using indicators for treatment exposure, controlling for EPA/DHA intervention, age, and gender. Because the cohort will consist of older individuals,

competing risks due to deaths from other causes will be considered. The primary analysis will estimate the cause-specific hazard and the hazard ratio comparing intervention groups for HF by censoring individuals with deaths due to competing causes. To estimate the cumulative incidence function, the subdistribution of HF will be plotted over time. The alternative Fine and Gray approach models the effect of treatment on the subdistribution hazard or directly on the cumulative incidence function. While we will consider this and compare its fit to the model-free cumulative incidence curves, the proportional hazards approach will be our primary analysis. Beyond the primary analyses, we will examine effect modification by EPA/DHA intervention, baseline risk factors, and time.

Statistical power: We computed statistical power assuming a) 2x2 factorial trial of 25,871 subjects at risk; b) equal and independent treatment allocation; c) mean follow-up of 5 years with minimal loss to follow-up; d) incidence rate of 8.29 cases per 1,000 person-years (40% of the rate reported in the ARIC and CHS studies (21/1000 person-years) in order to account for healthy volunteer effect in VITAL) , and e) a non-compliance rate of 20%. We also used 30% [6.19 cases /1,000 person-years] of the rates seen in the ARIC and CHS to be more conservative. We used a log-rank analysis with a 2-tailed p-value of 0.05. We have 80% power to detect a 18% reduction in the incidence of HF (**Table 2**).

Table 2. Statistical power (%) to detect vitamin D3 effect on HF incidence

Observed RR*	0.82	0.81	0.80	0.79	0.78	0.77	0.76	0.75	0.74	0.73	0.70
True RR†	0.78	0.76	0.75	0.74	0.73	0.71	0.70	0.69	0.68	0.66	0.63
Power (rate 8.29/1000)	80	84	87	91	93	95	96	98	98	99	>99
Power (rate 6.19/1000)	67	72	76	81	84	87	90	92	94	96	99

*Observed RR=intent-to-treat relative risk with 80% compliance; †True RR=Relative risk under perfect compliance

Primary Aim 2: ***Effects of EPA/DHA intervention vs. placebo on incidence rate of HF.***
Study design: Randomized, placebo-controlled, double-blind, 2x2 factorial trial of vitamin D and EPA/DHA.
Hypothesis*: Intervention with 1g/d of EPA/DHA supplements vs. placebo reduces the incidence rate of HF.*
Study subjects: A total of 25,871 VITAL participants.
Statistical model: Cox proportional hazards model as detailed above for the primary aim.
To determine whether treatment effects vary over time, we will use time-dependent Cox models to update covariates over time (i.e., dietary EPA/DHA and other nutrients, anthropometric, comorbidity, and lifestyle factors collected repeatedly during the trial).

Statistical power: For primary analyses for EPA/DHA, we will have identical statistical power presented in Table 2 above.

Secondary Aim: ***Effects of vitamin D3 and EPA/DHA on incidence rate of recurrent HF hospitalizations***.
Study design: Randomized, placebo-controlled, double-blind, 2x2 factorial trial of vitamin D3 and EPA/DHA.
Hypothesis*: Intervention with either vitamin D3 or EPA/DHA reduces the rate of recurrent HF hospitalization.*
Study subjects: All 25,871 VITAL participants.
Statistical model: We will analyze recurrent HF hospitalizations using the Andersen-Gill model, which allows for varying numbers of events per person with different time between events. It can accommodate correlated observations within individuals by using robust covariance estimates. This method has been found to produce similar results to Poisson regression models allowing for over-dispersion***.***

Statistical power: To estimate the statistical power for recurrent HF hospitalization, we assumed a Poisson distribution with over-dispersion, following Cook et al. Rates of recurrence were based on those seen in the Olmstead County data with 16.5% recurrent hospitalization rate (0.144 per person-year). We will have 80% power to detect 16% reduction in recurrent HF hospitalization (RR=0.84) if we assume an incidence rate of 8.29/1000 person-years and 83% power to detect 19% reduction in recurrent HF hospitalization (RR=0.81) assuming a HF rate of 6.19/1000 person-years. We expect 280 recurrent HF hospitalizations with latter rate.