Vitamin D and Marine Omega-3 Fatty Acid Supplementation and Incident Autoimmune Disease in the VITAL Randomized Controlled Trial: Supplement 1

The information provided below is a summary of previously published information [1,2], provided here for the convenience of the reader. Additional details, including questionnaires, may be found at https://www.vitalstudy.org/Investigators.html.

Trial Population and Recruitment

VITAL participants were recruited from November 2011 through March 2014 as follows: (1) from participants in previously completed trials conducted by the principal investigators; (2) from a master mailing tape of names and addresses assembled from commercially available U.S. mailing lists of organizations and subscription lists of magazines; (3) through direct appeals in articles and advertisements in newspapers and magazines; and (4) through targeted recruitment in black communities. 401,605 persons were screened for eligibility, and 25,871 persons underwent randomization.

Eligible participants were randomized into the trial if during the three month run-in they reported no new history of cardiovascular diseases or procedures, cancer (except non-melanoma skin cancer), hypercalcemia, sarcoidosis, or other serious illness; if they were willing to comply with limits on non-study used of supplemental calcium, omega-3 fatty acids, and vitamin D; and if they complied with pill taking (defined as taking $\geq$2/3 of the study pills during the run-in). Individuals were randomized in blocks of eight, within sex, race, and 5-year age groups, with two individuals assigned to each of the four treatment combinations.

Participants took two pills each day, supplied in a calendar pack: one for the vitamin D arm (either vitamin D or placebo), and one for the omega-3 fatty acid arm (either omega-3 fatty acid or placebo).

Safety and Efficacy

The VITAL trial tested the effects of a single dose of vitamin D, chosen (as detailed in [2]) for the best balance of safety and efficacy based on previous findings from the Women's Health Initiative and other studies. Efficacy was based on extrapolating previous findings from the Women's Health Initiative, a population similar to the VITAL population, along with a study by Aloia et al. [3] that showed a nonlinear dose-response relation between serum 25-hydroxyvitamin D [25(OH)D] and vitamin D intake, such that an increase in serum levels slowed at higher intake levels. Because individuals were excluded from the VITAL trial if they reported supplemental vitamin D intakes of more than 800 IU/day, at a dose of 2000 IU/d participants assigned to the vitamin D group would be consuming a dose well below the Institute of Medicine tolerable upper intake level of 4000 IU/day. The dose of 2000 IU was expected to result in about 50 nmol/L difference between the supplemented and the placebo groups. Because individuals were excluded from the trial if they reported supplemental vitamin D intakes of more than 800 IU/day, participants assigned to active vitamin D group would be consuming a dose well below the IOM tolerable upper intake level of 4000 IU/day. In addition, because participants were allowed to take supplements at RDA levels (up to 800 IU/day), those in the placebo group were not at increased risk of becoming vitamin D-deficient.

The single dose of 1g/day for omega-3 fatty acids was that recommended by the American Heart Association (AHA), and was found in a large secondary prevention trial [4] to be beneficial, with minimal side effects. Because at the time of trial inception the optimal ratio of EPA to DHA was unknown, an EPA-to-DHA ratio close to 1-to-1 (specifically, 1.3- to-1) was chosen. The dose chosen fell well within the FDA's guidelines that marine omega-3 fatty acid doses of up to 3 g/day were "Generally Recognized as Safe".

The omega-3 fatty acid supplement, Omacor® fish oil, was chosen as it was free of environmental toxins (e.g., polychlorinated biphenyls, methylmercury, and dioxins) found in some fish, and it contained no vitamin D.

Blood collection

Fasting blood samples were collected at baseline (prior to randomization) from 16,956 individuals; as well as at trial years 1, 2, and 4 from all participants who provided baseline samples and returned a follow up blood sample. Baseline blood samples were assessed for levels of 25(OH)D and EPA+DHA. Follow-up blood samples were used to assess for pill-taking compliance, changes in biomarkers, and changing trends in background intakes of vitamin D and marine omega-3 fatty acids (tested in the placebo groups).

For each blood collection, participants were mailed a blood collection kit, and were instructed to return the blood sample in a provided freezer pack within 24 hours of venipuncture. Upon return, samples were centrifuged to separate plasma, serum, red blood cells, and buffy coat, and components were stored in nitrogen freezers (−170 °C) within 30-36 hours of venipuncture.

Serum 25(OH)D was assayed by radioimmunoassay at the Clinical and Translational Science Center (CTSC) laboratory at Harvard, using reagents (DiaSorin Corporation, Stillwater, NM) that recognize and quantify 25(OH)D_2 and 25(OH)D_3 equally.

Omega-3 fatty acids in red blood cells (RBC) were assayed by gas chromatography in the laboratory of Dr. William Harris at the University of South Dakota. The coefficient of variation for EPA +DHA as a percent of total RBC fatty acids was 5.0% for a mean value of 10.9% (SD 0.5%) and 5.3% for a mean value of 3.8% (SD 0.2%).

Participant Characteristics, Adverse Reactions, and Adherence

As detailed in [1], data on baseline characteristics were collected from questionnaires answered before randomization, and included demographic characteristics (sex, age, race/ethnicity, geographic region of residence, education, income), health history (body mass index, obesity, hypertension, ever use of anti-hypertensive medication, current use of cholesterol-lowering medication, diabetes, current use of anti-diabetic medication, parental history of premature MI, and history of cancer in first-degree relatives), and behavioral characteristics (including smoking status, weekly energy expenditure in leisure time physical activities and stair climbing, alcohol use, aspirin use, postmenopausal hormone use in women, and a modified version of the Harvard Food Frequency Questionnaire, which assessed daily or weekly intake of foods related to vitamin D and/or omega-3 fatty acids).

Questionnaires at 6 months and 1 year after randomization, and then annually, had items querying adverse events related to vitamin D or omega-3 fatty acid supplementation. For vitamin D, this included GI symptoms (nausea, diarrhea, or constipation), and physician diagnosis of hypercalcemia or kidney stones. For omega-3 fatty acids, this included GI upset or bleeding, skin eruptions, and physician diagnosis of atrial fibrillation or other irregular heart rhythms. The incidence of adverse events did not differ significantly between the treatment and placebo group for either vitamin D or omega-3 fatty acids.

In addition to repeated questionnaire items asking about adherence, blood levels were assessed in a random subgroup of participants over time. In a subgroup of 1644 participants with repeat measurements of 25-hydroxyvitamin D after 1 year, mean levels increased from 29.8 ng/mL (74 nmol/L) at baseline to 41.8 ng/mL (104 nmol/L) at 1 year (a 40% increase) in the vitamin D group and changed minimally [mean, −0.7 ng/mL (−2 nmol/L)] in the placebo group. Among the 1583 participants who provided a blood sample at 1 year that could be analyzed for

EPA+DHA, the mean n−3 index rose to 4.1% (increase of 54.7%) in the n−3 group and changed by less than 2% in the placebo group.

References

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