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Vitamin D, Marine n-3 Fatty Acids, and Primary Prevention of Cardiovascular Disease: Current Evidence

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Abstract

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Whether marine omega-3 fatty acid (n-3 FA) or vitamin D supplementation can prevent cardiovascular disease (CVD) in general populations at usual risk for this outcome is unknown. A major goal of the *VIT*amin D and Omeg*A*-3 Tria*L* (VITAL) was to fill this knowledge gap. In this article, we review the results of VITAL, discuss relevant mechanistic studies regarding n-3 FAs, vitamin D, and vascular disease, and summarize recent meta-analyses of the randomized trial evidence on these agents. VITAL was a nationwide, randomized, placebo-controlled, 2×2 factorial trial of marine n-3 FAs (1 g/d) and vitamin D_3 (2000 IU/d) in the primary prevention of CVD and cancer among 25,871 U.S. men aged ≥50 and women aged ≥55, including 5,106 African Americans. Median treatment duration was 5.3 years. Supplemental n-3 FAs did not significantly reduce the primary cardiovascular endpoint of major CVD events (composite of myocardial infarction [MI], stroke, and CVD mortality; hazard ratio [HR]=0.92 [95% confidence interval 0.80–1.06]) but was associated with significant reductions in total MI (HR=0.72 [0.59–0.90]), percutaneous coronary intervention (HR=0.78 [0.63–0.95]), and fatal MI (HR=0.50 [0.26–0.97]) but not stroke or other cardiovascular endpoints. For major CVD events, a treatment benefit was seen in those with dietary fish intake below the cohort median of 1.5 servings/week (HR=0.81 [0.67–0.98]) but not in those above (p, interaction=0.045). For MI, the greatest risk reductions were in African Americans (HR=0.23 [0.11–0.47]; p, interaction by race=0.001). Vitamin D supplementation did not reduce major CVD events (HR=0.97 [0.85–1.12]) or other cardiovascular endpoints. Updated meta-analyses that include VITAL and other recent trials document coronary risk reduction from supplemental marine n-3 FAs but no clear CVD risk reduction from supplemental vitamin D. Additional research is needed to determine which individuals may be most likely to derive net benefit from supplementation.

(VITAL (clincialtrial.gov identifier:)

Keywords

cardiovascular disease; diet and nutrition; epidemiology; race and ethnicity; primary prevention; marine n-3 fatty acids; randomized controlled trial; vitamin D

INTRODUCTION

Marine n-3 FA supplementation has been recommended for heart health in patients with coronary heart disease (CHD) who do not meet target intakes for n-3 FA-rich fatty fish,[1, 2] and vitamin D supplementation is an established intervention for the prevention and treatment of bone disorders.[3] A decade ago, these supplements were also increasingly being used for the possible prevention of a first cardiovascular event or cancer, and their U.S. sales soared.[4–6] Indeed, n-3 FAs and vitamin D remain among the most widely used supplements today.[7, 8]

Ecologic, laboratory, and observational study data supporting these potential new indications were promising but inconclusive and insufficient to establish causality.[3, 9, 10] For n-3 FAs, some[11–13] though not all[14–16] trials in secondary prevention or high-risk settings had found cardiovascular disease (CVD) risk reductions, but no large trial in a general population unselected for elevated cardiovascular risk had been conducted. For vitamin D, trials that had assessed CVD or cancer outcomes in secondary or *post hoc* analyses had shown

generally null results, but low doses, inadequate statistical power, short treatment durations, and/or lack of rigorous endpoint adjudication precluded definitive assessments.[3] Large trials of vitamin D in doses adequate to produce meaningful changes in 25-hydroxyvitamin D (25(OH)D) levels and designed to assess CVD or cancer as primary outcomes were lacking. The Institute of Medicine[3] in 2011 concluded that the effectiveness and benefit-risk balance of vitamin D supplementation for CVD or cancer prevention could not be established with existing data (as did the U.S. Preventive Services Task Force[17] in 2014) and highlighted the need for trials of vitamin D in doses at least twice the current recommended dietary allowance of 600–800 IU/d for bone health to understand the benefit-risk balance, including in diverse populations.

We conducted the *VIT*amin D and Omeg*A*-3 Tria*L* (VITAL), an investigator-initiated study, to fill these knowledge gaps. To date, VITAL is the only trial of n-3 FA supplementation for prevention of CVD in a general population selected only on age and *not* on high risk for CVD. VITAL is also the first of only two large (N≥10,000) trials of moderate- to high-dose vitamin D for the prevention of CVD and cancer, and the only such trial with a significant number of black individuals, for whom these issues may be particularly salient because of their lower cutaneous synthesis of vitamin D in response to solar radiation.[18] In this article, we summarize the design and primary results[19, 20] of the trial; discuss the findings in the context of relevant research; report on recent meta-analyses of clinical trial evidence; summarize mechanistic data; and suggest avenues for further investigation. The focus is on the trial's cardiovascular findings, but cancer and all-cause mortality are also discussed as these outcomes are important contributors to the benefit-risk balance of supplementation.

OVERVIEW OF VITAL

VITAL was a randomized, double-blind, placebo-controlled trial of the benefits and risks of supplemental marine n-3 FAs (1 g/d Omacor® fish-oil capsule with 840 mg of n-3 FAs, including eicosapentaenoic acid [EPA, 460 mg] + docosahexaenoic acid [DHA, 380 mg]) and vitamin D_3 (2000 IU/d) in the primary prevention of CVD and cancer among 25,871 men and women, aged ≥50 and ≥55, respectively.[9, 19–21] Participants were recruited throughout the U.S., balanced by sex, and with oversampling of African Americans (n=5,106). Eligible participants had no prior myocardial infarction (MI), stroke, transient ischemic attack, coronary revascularization, or cancer (except non-melanoma skin cancer) at study entry. They needed to agree to forego the use of fish-oil supplements and to limit their daily intake of vitamin D and calcium from all supplemental sources, including multivitamins, to no more than 800 IU and 1200 mg, respectively (the recommended dietary allowances [RDAs] for older adults[3]). Safety exclusions included renal failure or dialysis, severe liver disease (cirrhosis), use of anticoagulants, history of hypercalcemia or parathyroid disorders, or other conditions that would preclude participation. After successful completion of a 3-month placebo run-in, participants were randomized in a 2×2 factorial design to vitamin D, n-3 FAs, both active agents, or both placebos (Figure 1). Randomization took place from November 2011 to March 2014. Randomized treatment ended as planned on December 31, 2017, yielding a median intervention period of 5.3 years (range 3.8–6.1 years).

Baseline questionnaires collected data on clinical and lifestyle risk factors for CVD, cancer, and other conditions; a food frequency questionnaire ascertained intake of fish, dairy products, and other foods. Annual follow-up questionnaires assessed treatment compliance and side effects, risk factor updates, and endpoint occurrence. Participant-reported endpoints were confirmed/disconfirmed by medical record review using accepted criteria[22–24] by physicians blinded to treatment assignment, and deaths were ascertained through the National Death Index-Plus and other sources. Baseline blood samples were collected during the run-in from all willing participants (16,956 of 25,871 randomized participants [66%]), and follow-up samples were collected in years 1–5 from ~6000 participants in ancillary studies. Some Boston-area participants (n=1,054) underwent detailed in-clinic assessments at a local Clinical and Translational Science Center (CTSC) clinic at baseline and years 1, 2, and 4. Except for the CTSC component and selected ancillary studies, VITAL has been conducted primarily by postal and electronic communication.

Baseline characteristics of the study population are in Table 1. During the 5.3-year intervention period, response rates to yearly questionnaires averaged 93%, and mortality follow-up was >98%.[19] Adherence to randomized treatment was high.[19, 20] Among the ~15,500 participants with analyzable baseline blood samples, the mean plasma n-3 index (EPA+DHA as a percent of total fatty acids[25]) was 2.6% (SD, 0.9%) and the mean serum total 25(OH)D level was 30.8 ng/mL (SD, 10.0) ng/mL, with 12.7% and 32.2% having levels <20 ng/mL and 20-<30 ng/mL, respectively. There was a large post-randomization difference in the n-3 index between the active n-3 FA and placebo groups (55% increase) and in 25(OH)D levels between the active vitamin D and placebo groups (40% increase), which was evident throughout the trial. In the subgroup with follow-up measurements, the achieved plasma n-3 index with active n-3 FA was ~4.1% and the achieved serum 25(OH)D with active vitamin D exceeded 40 ng/mL (100 nmol/L), without changes over time in the placebo groups.

SUPPLEMENTAL n-3 FAs

VITAL Results

Supplemental n-3 FAs did not significantly reduce the primary endpoint of major CVD events (a composite of MI, stroke, and CVD mortality; hazard ratio=0.92 [95% confidence interval 0.80–1.06]) but did reduce total MI (a prespecified secondary endpoint) by a significant 28% (HR=0.72 [0.59–0.90]) (Table 2). This benefit emerged after the first year and persisted throughout the trial (Figure 2). Significant reductions in risk of percutaneous coronary intervention (PCI; HR=0.78 [0.63–0.95]), fatal MI (HR=0.50 [0.26–0.97]), and total CHD (HR=0.83 [0.71–0.97]) were also found. In contrast, there were no significant reductions in risk of coronary artery bypass grafting (CABG), stroke, CVD mortality, or an expanded CVD endpoint (major CVD events plus coronary revascularization [PCI or CABG]). In analyses that excluded the first 2 years of follow-up, the HR for major CVD events was 0.89 (0.76–1.05). In analyses that censored for noncompliance, the HRs were similar to those in intention-to-treat analyses.

With respect to noncardiovascular endpoints, n-3 FA supplementation was not associated with incidence of total cancer (co-primary endpoint) or breast, prostate, or colorectal

cancers; cancer mortality; or all-cause mortality in analyses of the full trial period (Table 2). However, a signal for increased cancer incidence (HR=1.13 [1.00–1.28]), though not for cancer mortality (HR=0.93 [0.73–1.19]) or all-cause mortality (HR=0.97 [0.84–1.12]), emerged in analyses that excluded the first two years of follow-up. With regard to side effects, the n-3 FA intervention was well tolerated, with no treatment-associated increase in bleeding or gastrointestinal symptoms.

The following characteristics, assessed at baseline, were examined as potential modifiers of n-3 FA treatment effects: age; sex; race/ethnicity; traditional cardiovascular risk factors; aspirin use; statin use; dietary fish intake, plasma n-3 index, and concurrent randomization to the active vitamin D group. Of these, only baseline dietary fish intake significantly influenced the effect of n-3 FA supplementation on both major CVD events (Online Figure I) and total MI (Figure 3) (p, interaction<0.05 for each endpoint). This characteristic also significantly modified the intervention's effect on all-cause mortality (p, interaction=0.02) and tended to modify its effect on cancer (p, interaction=0.09). In individuals with low fish intake (below the median of 1½ servings/week), n-3 FA supplementation was associated with a 19% reduction in major CVD events (HR=0.81 [0.67–0.98]), including a 40% reduction in MI (HR=0.60 [0.45–0.81]), and a trend toward a reduction in all-cause mortality (HR=0.87 [0.73–1.04]) and no indication of increased cancer risk (HR=0.96 [0.84–1.09]). In contrast, for individuals with higher fish intake (≥1½ servings/week), n-3 FAs offered no protection against major CVD events, MI, all-cause mortality, or cancer. No other characteristic significantly affected the association between the intervention and risk of major CVD events or all-cause mortality. For MI, however, race/ethnicity and the presence of traditional cardiovascular risk factors also significantly modified the effect of n-3 FA supplementation. African Americans experienced a significant 77% treatment-associated reduction in MI (HR=0.23 [0.11–0.47]) while other racial/ethnic groups had smaller reductions (p, interaction=0.001) (Figure 3). Of note, African Americans derived an MI benefit irrespective of fish intake, but non-Hispanic whites did so only when fish intake was low. African Americans also had significant treatment-associated reductions in coronary revascularization (HR=0.51 [0.28–0.92]) and total CHD (HR=0.61 [0.43–0.88]) and no treatment-associated increase in cancer risk (HR=1.02 [0.79–1.33]) or all-cause mortality (HR=0.84 [0.64–1.11]). Participants with a larger number of traditional cardiovascular risk factors also derived an MI benefit from n-3 FA supplementation, but those without these risk factors did not (p, interaction=0.047) (Figure 3). The aforementioned treatment benefit for MI in African Americans did not diminish after adjustment for cardiovascular risk factors (HR=0.19 [0.07–0.50]) and was more apparent than in non-Hispanic whites across all cardiovascular risk-factor strata. For all-cause mortality, no factor other than dietary fish intake significantly modified the treatment effect.

We recently analyzed Intervention effects on lipids and inflammatory markers in participants with 1-year follow-up blood samples. n-3 FA supplementation was associated with a small but significant reduction in triglycerides but had no significant effect on other lipids. Treatment-associated reductions in high-sensitivity C-reactive protein levels were greater in those with low fish intake (p, interaction=0.06), supporting the finding that such individuals were more likely to experience reduced risk of major CVD events with n-3 FAs.

Discussion and Context of Other Research

VITAL is the only large trial of n-3 FAs for the primary prevention of CVD in a usual-risk population. However, several earlier n-3 FA trials have been conducted in patients with or at high risk for CVD. Various meta-analyses have aggregated these findings, with some reporting benefit[26] but most[27–29] concluding that supplementation has no or at most a weak preventive effect limited to coronary death but not stroke or other cardiovascular outcomes. In a 2018 meta-analysis by the Omega-3 Treatment Trialists' Collaboration of 10 trials with 12,001 incident major vascular events among 77,917 participants, n-3 FA supplementation (EPA dose range, 226–1800 mg/d; all but one trial[12] tested an EPA-DHA combination) for a mean of 4.4 years (range, 1.0–6.2 years) did not reduce the incidence of major vascular events, major CHD events, stroke, or revascularization,[28] although the subdivision of major CHD events into nonfatal MI and CHD death revealed a suggestive 7% reduction in the latter outcome (relative risk [RR]=0.93 ([99% confidence interval 0.83–1.03]; p=0.053).[30] Although the 2018 meta-analysis did not consider total MI, an earlier meta-analysis[27] of 13 trials found a HR of 0.89 (0.76–1.04) for this endpoint, and a HR of 0.91 (0.85–0.98) for cardiac death. The results of the ASCEND trial, which tested a median of 7.4 years of supplementation with a 1 g/d fish-oil capsule (containing the same EPA-DHA dose and ratio as in VITAL) in 15,480 UK adults with diabetes, were published shortly before those of VITAL. ASCEND investigators reported null results for the primary endpoint of serious vascular events (HR=0.97 [0.87–1.08]).[31] With regard to coronary outcomes, treatment-associated HRs for coronary death, nonfatal MI, and the composite of these two endpoints were 0.79 (0.61–1.02), 0.93 (0.76–1.14), and 0.89 (0.75–1.04), respectively. For vascular death, the HR was 0.81 (0.67–0.99). Although the marked treatment benefit on MI and PCI in VITAL contrasts with the more modest coronary benefits suggested by the collective findings of these secondary-prevention or high-risk trials, neither VITAL nor these other trials indicate a consistent benefit for stroke or composite cardiovascular endpoints, suggesting that future investigations should be designed and powered to analyze potential effects of n-3 FAs on coronary outcomes as distinct endpoints. That said, recent results from the 4.9-year REDUCE-IT trial, which tested high-dose synthetic EPA (icosapent ethyl [4 g/d]) in 8,179 statin users with elevated triglyceride levels (70% with prior CVD, and the rest with diabetes plus other cardiovascular risk factors) indicate a significant 26% reduction in major CVD events, including significant reductions of 31%, 28%, and 20% in total MI, total stroke, and cardiovascular death, respectively.[32] Whether these greater risk reductions are due to the higher dose, specific formulation, and/or other factors requires further study.

There are several potential explanations for the apparently stronger coronary benefits of n-3 FAs in VITAL than those seen in aggregated analyses of secondary prevention trials. Two early open-label trials—GISSI[11] in Italy, which tested 3.5 years of EPA+DHA (1 g/d) in 11,324 recent MI patients, and JELIS[12] in Japan, which tested 4.6 years of EPA (1.8 g/d) in 18,645 hypercholesterolemic patients on statins—found significant coronary protection. However, all but one[13] (two, if REDUCE-IT[32] is included) subsequent placebo-controlled trials[13–16, 31, 33, 34] (some with smaller sample sizes[13–16] and some testing lower doses[14, 15]) failed to find benefit. The divergent results may be partly attributable to differences in these design parameters. In addition, the use of cardiovascular medications, including statins, β-blockers, anticoagulants, and angiotensin-converting enzyme inhibitors, was more prevalent

among participants in recent trials than among those in earlier trials, perhaps reducing the opportunity for n-3 FAs to provide incremental benefit. The Omega-3 Treatment Trialists' meta-analysis, ASCEND, and VITAL found no variation in results by statin use, but attenuation of a potential n-3 FA effect by other medications remains a possibility. This attenuation would likely be greater in secondary prevention settings, where medication use is more prevalent. (In REDUCE-IT, where statin use was an entry requirement, there was a significant protective effect of the icosapent ethyl intervention in participants with high- or moderate-intensity statin use but not in those with low-intensity use [p, interaction=0.12]. However, interpretation of the findings is complicated by the trial's use of a mineral-oil placebo, which may have interfered with the absorption of, and thus the salutary effects of, statins.[35]) Another possible explanation for different effects in primary vs. secondary prevention is the more advanced atherosclerotic disease in the latter, which may require more powerful interventions than n-3 FAs—or as suggested by REDUCE-IT, significantly higher doses of n-3 FAs—to forestall clinical events. Indeed, in the Omega-3 Treatment Trialists' meta-analysis,[28] a greater n-3 FA benefit for major vascular events was found in participants without (HR=0.92 [0.84–1.01]) than with (HR=1.07 [0.95–1.20]) prior stroke (p, interaction=0.06). Similarly, the Age-Related Eye Disease Study 2,[36] a trial of n-3 FAs for progression of macular degeneration or vision loss in ophthalmology patients, reported a greater treatment benefit for CVD risk reduction in participants without (HR=0.81 [0.62–1.06]) than in those with (RR=1.25 [0.91–1.72]) prior CVD (p, interaction=0.04). Differences in dietary fish intakes across study populations may have also influenced findings. The VITAL finding of a substantial treatment benefit on MI (and also a reduction in the primary composite CVD endpoint) in those with low fish intake is a novel finding requiring confirmation (few trials have examined this variable as a potential effect modifier) and suggests that larger benefits may be observed in populations with very low n-3 FA intakes. Finally, the secondary prevention trials enrolled few black participants, who as a group derived a greater coronary benefit from n-3 FA supplementation than members of other racial/ethnic groups in VITAL.

A 2019 meta-analysis of 30 n-3 FA trials with a total of >130,000 participants that included VITAL and ASCEND, but not REDUCE-IT, reported modest but statistically significant risk reductions for MI (RR=0.92 [0.85–0.99]) and total CHD (RR=0.93 [0.89–0.98]); a nonsignificant reduction in CVD mortality (RR=0.93 [0.86–1.01]); and no reduction in stroke (RR=1.05 [0.97–1.13]).[37] Inclusion of the REDUCE-IT results would be expected to magnify the risk reductions. However, results of meta-analyses that combine VITAL with trials in higher-risk cohorts may be of limited use in formulating public-health guidelines regarding n-3 FA supplementation in usual-risk populations. Moreover, VITAL's promising findings in African Americans are of interest due to the potential of n-3 FAs for reducing health disparities.

Coronary benefits of supplemental n-3 FAs are consistent with data from laboratory investigations, animal studies, and/or small trials of intermediate cardiovascular endpoints in humans, which suggest that n-3 FAs reduce inflammation, low-density lipoprotein oxidation, triglycerides, blood pressure, heart rate, thrombosis, and atherosclerotic plaque growth and instability; and improve endothelial function (Figure 4).[9, 38–44] Experimental studies indicate relevant molecular and gene-regulatory effects.[38] The dose-response curve for some

effects appears to plateau at n-3 FA doses of 1 g/d or lower.[45] Observational studies indicate that healthy individuals who regularly eat fish, a rich dietary source of marine n-3 FAs, experience significant reductions in fatal CHD and possibly nonfatal MI.[46] Such studies also report significant inverse associations between dietary intakes of EPA+DHA from food or supplements and incident CHD events,[26, 47] and between selected n-3 FA biomarkers and CHD mortality,[48] nonfatal MI,[48] and total CHD.[47] The data for stroke are less compelling; high fish intakes[49, 50] show more consistent inverse associations with ischemic stroke than do high levels of n-3 FA biomarkers.[50–53]

For cancer, the VITAL findings agree with those from trials of n-3 FAs for secondary prevention of CVD, which have reported neutral effects or slight (but nonsignificant) elevations in cancer incidence[28, 54] and neutral effects or borderline significant reductions in cancer mortality.[13, 31, 55] For all-cause mortality, the absence of a significant treatment effect in VITAL agrees with results of meta-analyses of earlier trials[27, 29] and ASCEND.[31] A 2019 meta-analysis of n-3 FA trials (41 trials, 10,707 deaths, 134,034 participants) that included VITAL and ASCEND reported a RR of 0.98 (0.93–1.02) for this endpoint.[37] However, longer follow-up may be needed to detect a benefit, should such an effect exist.

Future Directions

Racial considerations.—We did not anticipate that African American participants in VITAL would experience a greater treatment-associated reduction in coronary risk than non-Hispanic whites, given that both groups entered the trial with comparable EPA+DHA blood levels and fish intakes. This finding requires replication in future trials. As noted above, n-3 FA supplementation trials for CVD prevention have, with few exceptions,[12] been conducted in non-Hispanic white populations, precluding an assessment of treatment effects by race.[28] However, a recent pooling project of 19 observational cohorts from 16 countries reported racial variation in associations of marine- and plant-derived n-3 FA biomarkers with incident coronary disease, including a significantly stronger inverse relationship between α-linolenic acid and nonfatal MI in blacks than whites.[48] Observational studies also suggest that genetic variation in genes encoding key enzymes in FA metabolism, including the FA desaturase genes *FADS1* and *FADS2*, the 5-lipooxygenase gene *ALOX5*, the 5-lipoxygenase activating protein gene *ALOX5AP*, and the cyclooxygenase *COX-2* gene, may interact with dietary FA intakes to influence risk of CHD and other health outcomes,[56–58] and that people with African ancestry differ from those with European ancestry with respect to *FADS* variants.[58–60] Clarifying the role of genetic factors may be key to understanding the significant n-3 FA-associated reduction in MI in African American participants. In addition, although treatment-associated benefits remained more pronounced in African Americans than in non-Hispanic whites across risk strata defined by the presence or absence of traditional cardiovascular risk factors, it is possible that racial/ethnic differences in other clinical, dietary, or socioenvironmental factors may help to explain the comparatively stronger benefit in African Americans. For example, it has been postulated that n-3 FAs may ameliorate the adverse impact of air pollution,[61] an exposure that disproportionately affects African Americans[62] and increases cardiovascular risk.[63] Elucidating the reasons for possible racial differences in n-3 FA supplementation effects is of interest.

Dose, formulation considerations.—VITAL tested only one n-3 FA dose and formulation and thus could not assess whether the effectiveness of supplementation varies according to these parameters. However, the tested dose has been recommended by the American Heart Association for cardioprotection in patients with prior CHD[1, 2] and, based on fish consumption (1–2 servings/week), is at least twice the dose recommended by this organization for cardiovascular protection in healthy individuals.[2, 46] That coronary benefits of n-3 FA supplementation were limited to participants with low baseline fish intake (in non-Hispanic white individuals and in the total cohort) suggests that further benefits may not accrue beyond a threshold dose. However, as noted above, REDUCE-IT found significant benefits with a higher-dose formulation in patients with CVD or at high risk for it. Results from the ongoing STRENGTH trial,[64] which is testing whether 3 to 5 years of high-dose n-3 FAs (n-3 carboxylic acids [4 g/d], DHA-EPA ratio of 1:2.75) reduce major CVD events in 13,000 statin users with hypertriglyceridemia and low high-density lipoprotein cholesterol and who have established atherosclerotic disease or are at high risk for CVD, will be useful for further assessing the efficacy of a high-dose intervention. However, the generalizability of these findings to primary prevention populations is unknown. Future trials of higher doses and/or alternative formulations of supplemental n-3 FAs in primary prevention settings are warranted.

Other cardiovascular endpoints.—In VITAL, results of ancillary studies addressing effects of n-3 FA supplementation on heart failure, cardiac structure and function (2D-echocardiograms), atrial fibrillation, hypertension, diabetes, and other endpoints will soon be available to provide a fuller picture of the impact of n-3 FA supplementation on cardiovascular health and perhaps to help clarify underlying mechanisms for protective coronary effects. With respect to heart failure, n-3 FAs may be effective in reducing the risk of this condition or its cardiovascular sequelae. The placebo-controlled GISSI-HF trial, conducted among 6,975 patients with heart failure (>90% with reduced ejection fraction), found that supplemental n-3 FAs (1 g/d), added to standard therapy, had a favorable effect on clinical outcomes, reducing cardiovascular-related hospitalizations and CVD mortality by 7% (1–13%) and 10% (1%−19%), respectively, over a median follow-up of 3.9 years.[13] Meta-analyses of small, short-term trials of n-3 FA supplementation in patients with heart failure[65, 66], showed favorable changes in cardiac function, ventricular remodeling, inflammatory markers, and/or fibrosis, as did a 6-month trial in 358 post-MI patients.[67] Supplemental EPA prevented contractile dysfunction and fibrosis in a heart-failure mouse model.[68, 69] Among a racially/ethnically diverse cohort of 6,562 US adults aged 45–84 followed for 13 years in the observational Multi-Ethnic Study of Atherosclerosis, high baseline plasma EPA levels were associated with a reduced incidence of heart failure.[70] However, in REDUCE-IT, high-dose EPA supplementation did not significantly lower heart failure risk (HR=0.95 [0.77–1.17]).[32] VITAL is the first large trial of n-3 FAs for prevention of this endpoint in a population unselected for elevated cardiovascular risk. Regarding atrial fibrillation, observational studies of fish or n-3 FA intakes or n-3 FA biomarkers in relation to this endpoint in initially healthy populations have yielded generally neutral results.[71–75] Trials of n-3 FAs have not found benefit for the prevention of recurrent atrial fibrillation[76–78] or postoperative atrial fibrillation in cardiac surgery patients.[79, 80] In REDUCE-IT, n-3 FA supplementation was associated with a significant increase in risk of atrial fibrillation as

compared with placebo (5.3 vs. 3.9%, p=0.003).[32] With respect to hypertension, a 2014 meta-analysis[40] of small, short-term n-3 FA trials found that the interventions significantly reduced blood pressure, with the greatest reductions in participants with untreated hypertension. Regarding diabetes, meta-analyses of small, short-term n-3 FA trials in patients with this condition[81] or healthy individuals[82] have shown neutral or inconsistent treatment effects on glucose or insulin-related biomarkers. A 2012 meta-analysis of 18 observational studies in generally healthy populations found that neither fish, seafood, nor EPA+DHA intake, nor circulating EPA+DHA levels, predicted incident diabetes.[83]

Benefit-risk balance.—Forthcoming results of VITAL ancillary studies of noncardiovascular outcomes, including cognition, depression, macular degeneration, infections, pulmonary health, autoimmune disorders, fractures, and falls, will help to inform the overall benefit-risk balance of n-3 FA supplementation. Post-intervention follow-up of the VITAL cohort is ongoing to capture potential latent and long-term treatment effects and to increase statistical power, especially for assessment of secondary endpoints and subgroup effects.

SUPPLEMENTAL VITAMIN D

VITAL Results

Vitamin D supplementation did not reduce the primary composite endpoint of major CVD events (HR=0.97 [0.85–1.12]), nor did it affect the risk of secondary cardiovascular endpoints or all-cause mortality (Table 3). Analyses that excluded the first year or two years of follow-up or that censored for noncompliance did not materially change these results. Vitamin D had no effect on 1-year changes in lipid profiles or inflammatory markers.

Vitamin D did not reduce total cancer incidence (HR=0.96 [95% 0.88–1.06]) but showed a promising signal for reduction in cancer mortality (HR=0.83 [0.67–1.02]), especially in analyses accounting for latency by excluding the first year (HR=0.79 [0.63–0.99]) or first two years (HR=0.75 [0.59–0.96]) of follow-up. The intervention was well tolerated, with no significant treatment-associated increases in risk of hypercalcemia, kidney stones, or gastrointestinal symptoms.

The association between vitamin D and risk of CVD endpoints or all-cause mortality did not significantly vary by age, sex, race/ethnicity, cardiovascular risk factors, serum 25(OH)D level, or concurrent randomization to n-3 FAs (results of the latter two analyses are in Online Table I); nor did vitamin D significantly reduce CVD endpoints or all-cause mortality in any subgroup. Intriguingly, individuals with normal BMI (<25 kg/m^2) experienced a significant treatment-associated reduction in cancer risk (HR=0.76 [0.63–0.90]), but overweight or obese individuals did not (p, interaction=0.002). African Americans assigned to vitamin D also had a suggestive reduction in cancer risk (HR=0.77 [0.59–1.01]), although the p-value for interaction by race/ethnicity was not significant (p, interaction=0.21).

Discussion and Context of Other Research

VITAL is the first large (N≥10,000) trial of moderate- or high-dose vitamin D for CVD and cancer prevention. Although VITAL was designed to overcome methodologic limitations of

earlier randomized trials, the observed absence of cardiovascular benefit in VITAL is consistent with results of other trials. Among the largest of these trials are the Women's Health Initiative (WHI) calcium-vitamin D trial,[84, 85] the Randomized Evaluation of Calcium or vitamin D (RECORD) trial,[86, 87] and a UK trial by Trivedi et al.[88] The WHI randomized >36,000 US postmenopausal women to 7 years of daily calcium (1000 mg) plus vitamin D_3 (400 IU) or to placebo; the intervention did not affect the incidence of CHD[84] or stroke[84] or mortality from these outcomes,[85] but the below-RDA dose is a limitation. In RECORD, 5,292 UK adults aged ≥70 were randomized to daily vitamin D_3 (800 IU), calcium (1000 mg), both, or placebo for 2–5.2 years for secondary fracture prevention and then followed observationally for 3 years; vitamin D did not reduce the incidence of MI (HR=0.97 [0.75–1.26]),[86] stroke (HR=1.06 [0.85–1.32]),[86] or vascular disease mortality (HR=0.91 [0.79–1.05]).[87] Trivedi et al.[88] randomized 2,686 older adults to vitamin D_3 (100,000 IU every 4 months [~833 IU/d]) or placebo for up to 5 years and found nonsignificant reductions in CHD incidence (0.94 [0.77–1.15]), CHD mortality (0.84 [0.56–1.27]), CVD incidence (0.90 [0.77–1.06]), and CVD mortality (0.84 [0.65–1.10]); however, the trial's modest size and intermittent bolus dosing, which has been associated with nonphysiological fluctuations in vitamin D blood levels,[89] are limitations. Given such results, it is not surprising that meta-analyses of these (and smaller) vitamin D trials, even those restricted to trials of RDA-level (800 IU/d) or higher doses,[90] have not found cardiovascular benefit.[86, 90–93] More recently, the 3.3-year Vitamin D Assessment Study (ViDA), which tested high-dose vitamin D (100,000 IU/month [~3300/d]) vs. placebo for CVD prevention in 5,110 New Zealanders, also reported null results (MI: RR=0.90 [0.54–1.50]; stroke: RR=0.95 [0.55–1.62]),[94] although the short duration and intermittent bolus dosing limit definitive conclusions.[89] A 2019 meta-analysis of vitamin D trials that included VITAL and ViDA found that, compared with placebo, vitamin D supplementation did not reduce major adverse cardiovascular events (10 trials, 6243 events, 79,111 participants; RR=1.00 [0.95–1.06]), MI (18 trials, 2550 events, 82,576 participants; RR=1.00 [0.93–1.08]), stroke (15 trials, 2354 events, 82,239 participants; RR=1.06 [0.98–1.15]), or CVD mortality (10 trials, 2202 events, 76,783 participants; RR=0.98 [0.90–1.07]).[95] Results did not significantly vary by baseline 25(OH)D level, vitamin D dose or administration frequency, or presence or absence of calcium co-administration. Supplemental calcium, often administered concurrently with vitamin D, raises blood calcium levels more rapidly than dietary calcium and could theoretically raise cardiovascular risks. However, results of calcium or calcium-plus-vitamin D trials do not clearly support this hypothesis.[91, 96–98] In the WHI, for example, a 22% increase in MI risk occurred in participants who first started calcium supplements as part of the trial but not in those already taking them at baseline.[99] As noted earlier, there was no treatment-associated elevation in MI or stroke risk in the total study population. In addition, the intervention did not increase coronary artery calcification at trial's end.[100] Large trials of calcium plus high-dose vitamin D supplementation are lacking, however.

As did VITAL, ViDA found that vitamin D failed to reduce all-cause mortality.[94] Lower-dose vitamin D trials have also shown neutral effects or at most modest reductions in this endpoint.[91–93] A 2019 meta-analysis of 20 vitamin D trials (6,502 deaths among 83,059

participants) that included VITAL and ViDA reported a RR of 0.97 (0.93–1.02) for all-cause mortality.[95] However, longer follow-up may be needed to detect a benefit.

The lack of benefit of vitamin D for cancer incidence and the suggestive benefit for cancer mortality in the overall VITAL cohort is also consistent with findings from previous vitamin D trials. In a 2019 meta-analysis of vitamin D trials including VITAL and ViDA, treatment-associated RRs for cancer mortality (5 trials, 6547 cancer deaths) and cancer incidence (10 trials, 6,547 incident cancers) were 0.87 (0.79–0.96) and 0.98 (0.93–1.03), respectively.[101] The VITAL findings for cancer mortality are also supported by laboratory research suggesting that vitamin D decreases tumor invasiveness and metastatic propensity[102] and by observational studies showing that higher 25(OH)D levels at diagnosis predict longer survival in cancer patients.[103–105]

Although the reasons for the significant treatment-associated cancer reductions among normal-weight participants and suggestive reductions among African Americans require further exploration, these benefits sharply contrast with the null cardiovascular findings in these groups. It is possible that the divergence may be explained by differing vitamin D requirements for CVD and cancer prevention. In observational studies, the 25(OH)D levels associated with lowest risks tend to be between 20–25 ng/mL for CVD[106] but above 30 ng/mL for cancer (at least colorectal cancer).[107] Thus, it is possible that many participants entered VITAL (and other contemporary clinical trials) with their vitamin D requirements for cardiovascular health already met. Although neither VITAL nor ViDA found significant cardiovascular benefit for vitamin D among participants with low 25(OH)D at baseline, a trial among individuals with vitamin D levels well below the 20 ng/mL recommended for bone health[3] might show stronger risk reductions. However, it would be neither ethical nor feasible to target patients with vitamin D deficiency and maintain them in this state for 5 or more years (as would be the case for the 50% assigned to placebo).

There are several plausible mechanisms by which vitamin D may prevent CVD (Figure 5). Vitamin D, obtained from diet, supplements, or conversion of 7-dehydrocholesterol in the skin by ultraviolet-B radiation, is hydroxylated in the liver to 25(OH)D, the major circulating vitamin D metabolite, which is then further hydroxylated to the active metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), primarily in the kidneys.[108, 109] Vascular smooth muscle cells, endothelial cells, cardiomyocytes, and macrophages also express the vitamin D receptor and/or produce 1α-hydroxylase, allowing for extra-renal production of $1,25(OH)_2D$. Laboratory and animal study data suggest that $1,25(OH)_2D$ inhibits vascular smooth muscle cell proliferation and vascular calcification, controls volume homeostasis and blood pressure via regulation of the renin-angiotensin-aldosterone system, exerts anti-inflammatory effects, and improves insulin sensitivity and secretion.[9, 110–112] In addition, observational studies have found inverse associations between 25(OH)D levels and cardiovascular risk factors and/or incident CVD.[106, 113, 114] However, meta-analyses of mostly small, short-term trials in humans have found null or mixed results for effects of supplemental vitamin D on intermediate cardiovascular endpoints,[115] including blood pressure,[116–119] glucose or insulin homeostasis and type 2 diabetes,[119–122] inflammation,[123–125] and markers of vascular function,[118, 126–129] as well as lipid profiles.[90, 130] Of interest, a hypotensive effect of supplemental vitamin D in normal-weight individuals but a

hypertensive effect in those who are overweight has been noted.[117] Most recently, the Vitamin D and Type 2 Diabetes Study, in which 2,423 adults with mean baseline 25(OH)D of 28.0 ng/mL were randomized to 4000 IU/d of vitamin D vs. placebo for a median of 2.5 years, also found differences in the effect of supplemental vitamin D by BMI, with a significant treatment-associated reduction in risk of diabetes in those without, but not with, obesity.[131]

Future Directions

Alternative vitamin D biomarkers.—Although total 25(OH)D has traditionally been viewed as the optimal marker of clinical vitamin D status, most 25(OH)D circulates bound to vitamin D binding protein (DBP, ~85%) or albumin (~15%). Some studies have suggested that alternative biomarkers of vitamin D status, including DBP; bioavailable 25(OH)D, defined as 25(OH)D not bound to DBP; free 25(OH)D, defined as 25(OH)D not bound to either DBP or albumin; and parathyroid hormone may be potentially useful adjuncts to—or more biologically relevant indicators than—total 25(OH)D in characterizing vitamin D status[132, 133] in relation to various clinical outcomes, including CHD[134–136] and cancer.[137–139] However, the data are not entirely consistent.[133, 140] Whether baseline levels or changes in novel vitamin D markers influence the likelihood of deriving CVD benefit or harm from supplemental vitamin D requires further examination.

Obesity.—Obesity is associated with lower levels of both free and total 25(OH)D,[141] may disproportionately lower the former,[142] and may affect the correlation between the two markers.[143] In a recent mouse study, diet-induced obesity decreased free 25(OH)D but not total 25(OH)D, and increased expression of *CYP2R1*, a key vitamin D-related gene.[142] Among patients with CHD, higher levels of free 25(OH)D predicted reduced cardiovascular and all-cause mortality in those with normal BMI but not in those who were overweight/obese.[136] These data suggest that vitamin D bioactivity is altered in obesity and lend credence to the aforementioned more favorable treatment effects on blood pressure,[117] diabetes,[131] and cancer incidence[19] in normal-weight than in overweight/obese participants observed in some vitamin D trials.

Vitamin K.—Vitamin K may optimize the benefit-risk ratio of vitamin D supplementation. Vitamin K-vitamin D interactions appear to promote bone and cardiovascular health and may also protect against possible adverse effects of high-dose vitamin D such as kidney stones and vascular calcification.[144] Vitamin D stimulates the transcription and translation of osteocalcin (OC), matrix Gla protein (MGP), and other proteins in bone, the vasculature, and other tissues. Vitamin K then helps to carboxylate and activate these proteins. Carboxylated OC is involved in calcium binding in bone, and carboxylated MGP inhibits calcium deposition in the vasculature, kidney, and soft tissues. High-dose vitamin D supplementation may increase synthesis of OC and MGP, resulting in the need for higher levels of vitamin K to fully activate these proteins and achieve maximal bone and cardiovascular benefits. Whether vitamin K status modifies effects of supplemental vitamin D on CVD warrants further study in a large trial.

Magnesium.—Magnesium plays a key role in vitamin D synthesis and metabolism,[145, 146] suggesting that adequate magnesium is required for optimal response to vitamin D supplementation. Among US National Health and Nutrition Examination Survey participants, magnesium intake significantly interacted with vitamin D intake to affect vitamin D status, and also interacted with circulating 25(OH)D to influence risk of CVD mortality.[147] An inverse association between 25(OH)D and mortality was primarily seen in individuals with above-median magnesium intakes. In a trial in people without overt magnesium deficiency, supplemental magnesium raised vitamin D levels in those with low levels and lowered vitamin D levels in those with high levels—a pattern suggestive of this mineral's importance in optimizing vitamin D status.[148] In mice with induced chronic kidney disease, co-administration of magnesium blunted the adverse impact of vitamin D on vascular calcification.[149] Whether magnesium status affects the relation between vitamin D supplementation and cardiovascular endpoints has yet to be tested in a large trial.

Dose, administration frequency.—VITAL examined only one vitamin D dose and administration frequency and therefore could not address dose-response issues or the relative efficacy of daily vs. less frequent dosing. Ongoing vitamin D trials[150] may help clarify these uncertainties. D-Health[151]—the only large (N≥10,000) trial of high-dose vitamin D other than VITAL—is testing a bolus vitamin D dose of 60,000 IU/month for 5 years in 21,315 Australians aged 60 and older; the primary endpoints are cancer and all-cause mortality, but CVD will also be examined. Results are expected in 2021.

Heart failure.—In VITAL, results of ancillary studies of supplemental vitamin D on incident heart failure, cardiac structure and function, and other CVD-related endpoints will soon be available. Vitamin D deficiency may lead to heart failure through deleterious effects on the renin-angiotensin-aldosterone system and cardiac morphology. In the RECORD trial, supplemental vitamin D (800 IU/d) was associated with a significant reduction in incident heart failure (HR=0.75 [0.58–0.97]).[86] A 2014 meta-analysis of seven vitamin D trials, including RECORD and six smaller trials, also reported a significant reduction in this endpoint (HR=0.79 [0.59–0.99]).[86] However, ViDA did not find a benefit of monthly high-dose vitamin D on incident heart failure (HR=1.19 [0.84–1.68]).[94] Small trials in heart failure patients suggest that daily high-dose vitamin D (4000 IU) for 1–3 years may improve left ventricular structure and/or function,[152, 153] although a reduction in all-cause mortality has not been found.[154]

Impaired kidney function.—Individuals with chronic kidney disease, even at early stages, have lower vitamin D levels (in part because of reduced conversion to its active metabolite) and higher CVD rates than members of the general population. Adequately powered randomized trials testing the effect of vitamin D supplementation on cardiovascular outcomes in such patients are lacking[155] but challenging to conduct because such supplementation is routinely prescribed for bone health maintenance in this population. Thus, investigation of potential modification of the effect of supplemental vitamin D on cardiovascular outcomes according to baseline markers of kidney function in a general population is of interest; such analyses are underway and will be soon be reported in VITAL.

Benefit-risk balance.—Forthcoming results from VITAL ancillary studies of noncardiovascular outcomes (e.g., fractures, falls, cognition, depression, and infections) will also inform the overall benefit-risk balance of vitamin D supplementation. As noted earlier for n-3 FAs, post-intervention follow-up of the VITAL cohort to capture potential latent and long-term treatment effects and to increase statistical power, especially for assessment of secondary endpoints and subgroup effects, is in progress.

CONCLUSION

In VITAL, n-3 FA supplementation among initially healthy adults led to a small but statistically nonsignificant reduction in a composite endpoint of major CVD events, a statistically significant 28% reduction in total MI, reductions in other coronary outcomes, but no reduction in stroke or cardiovascular deaths not related to heart disease. The reduction in total MI supports a possible cardioprotective role for n-3 FAs in a usual-risk setting, especially in people with low dietary fish intake or with cardiovascular risk factors, and in African Americans. Daily high-dose vitamin D supplementation did not reduce the incidence of major CVD events or secondary CVD endpoints in initially healthy adults but showed a promising signal for reducing cancer death. VITAL and other recent trials have contributed to updated meta-analyses of these interventions and suggest that the benefit-risk pattern may vary by subgroup. Additional research is needed to determine which individuals may be most likely to derive a net benefit from these supplements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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VITAL has been approved by the Institutional Review Board of Partners Healthcare/Brigham and Women's Hospital. The study agents have received Investigational New Drug Approval from the U.S. Food and Drug Administration.

Non-standard abbreviations and acronyms:

ASCEND	A Study of Cardiovascular Events in Diabetes
CABG	coronary artery bypass grafting
CHD	coronary heart disease
CVD	cardiovascular disease
CTSC	Clinical and Translational Science Center
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
HR	hazard ratio
MI	myocardial infarction
PCI	percutaneous coronary intervention
n-3 FA	n-3 fatty acid
RDA	recommended dietary allowance
REDUCE-IT	Reduction of Cardiovascular Events with Icosapent Ethyl—Intervention Trial
RR	relative risk
SD	standard deviation
ViDA	Vitamin D Assessment Study
VITAL	*VIT*amin D and Omeg*A*-3 Tria*L*
25(OH)D	25-hydroxyvitamin D

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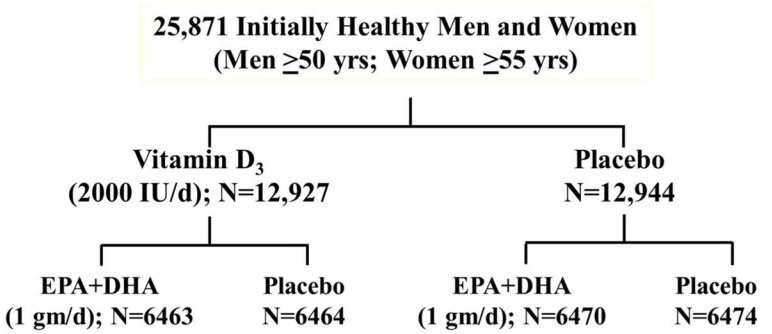
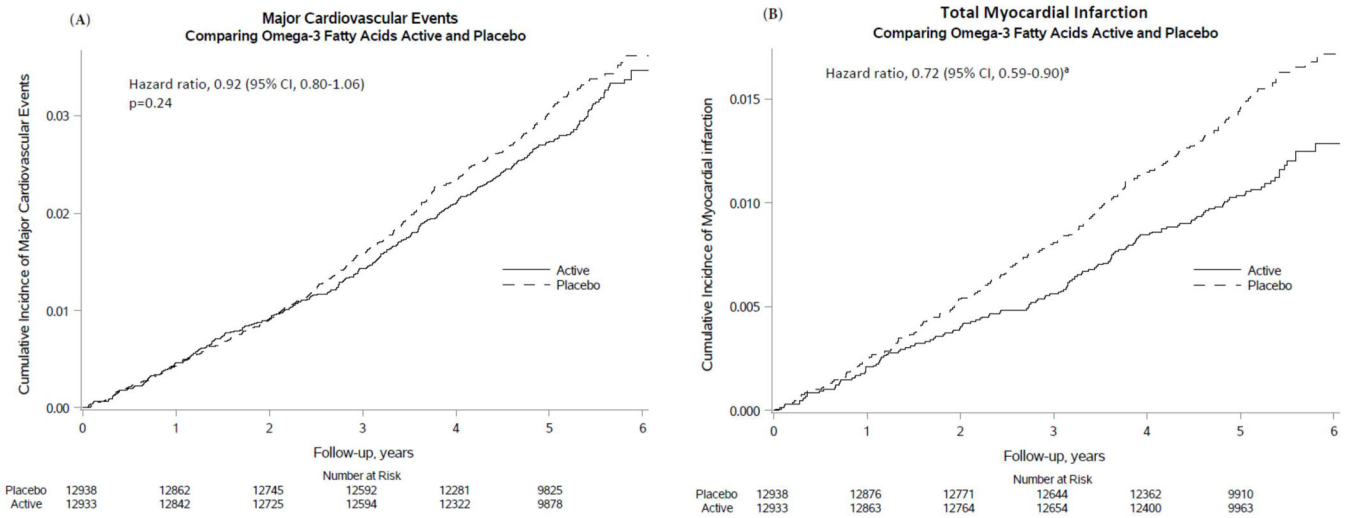


Adapted from Bassuk SS et al., *Contemp Clin Trials* 2016; 47:235-243

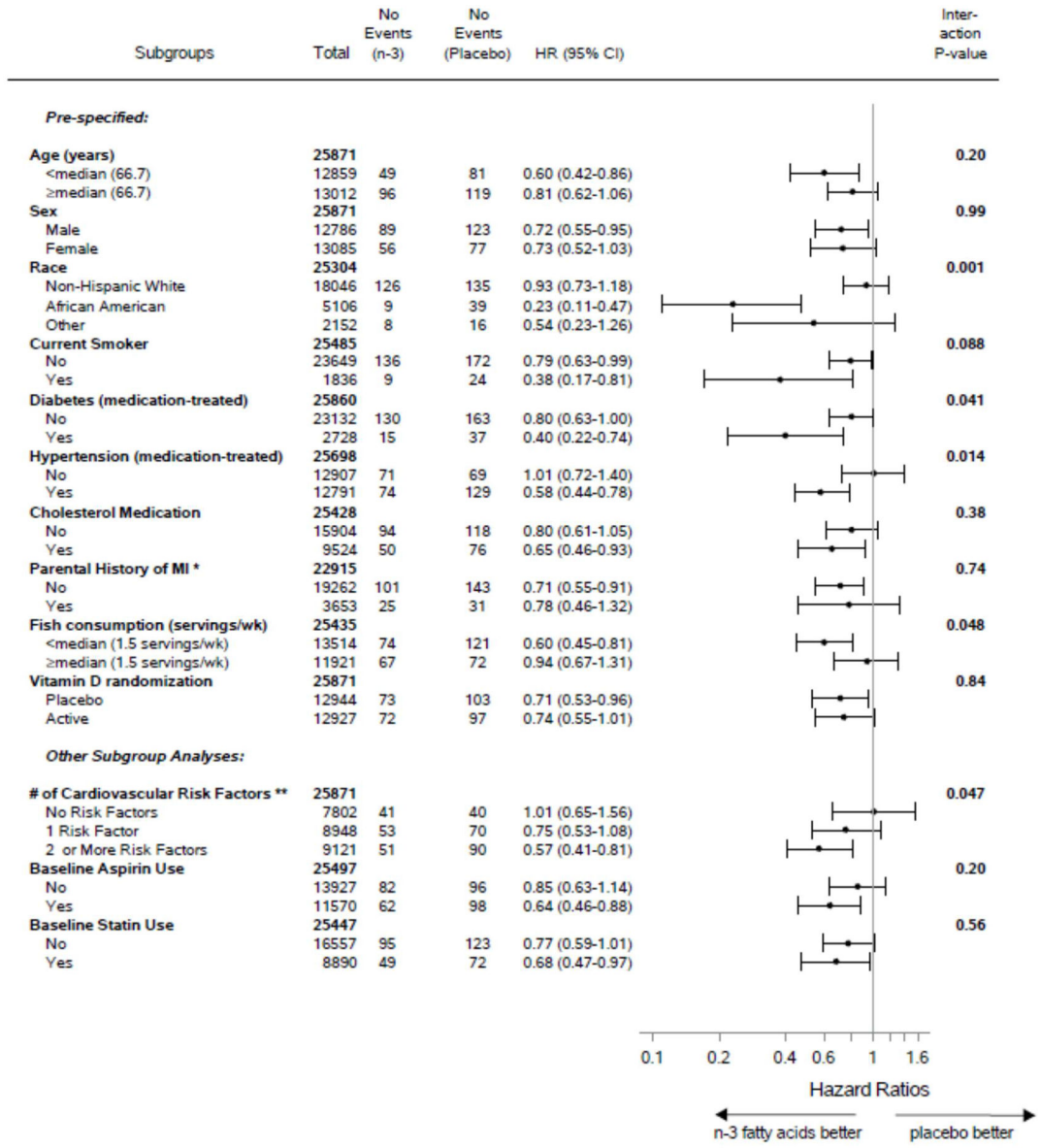
Figure 1:
VITAL Factorial Design.



Adapted from Manson JE et al., *N Eng J Med* 2019; 380:23-32

Figure 2.

Cumulative incidence rates of major CVD events and total MI by year of follow-up, in the n-3 fatty acid group and the placebo group.



HR = hazard ratio; CI = confidence interval; MI = myocardial infarction

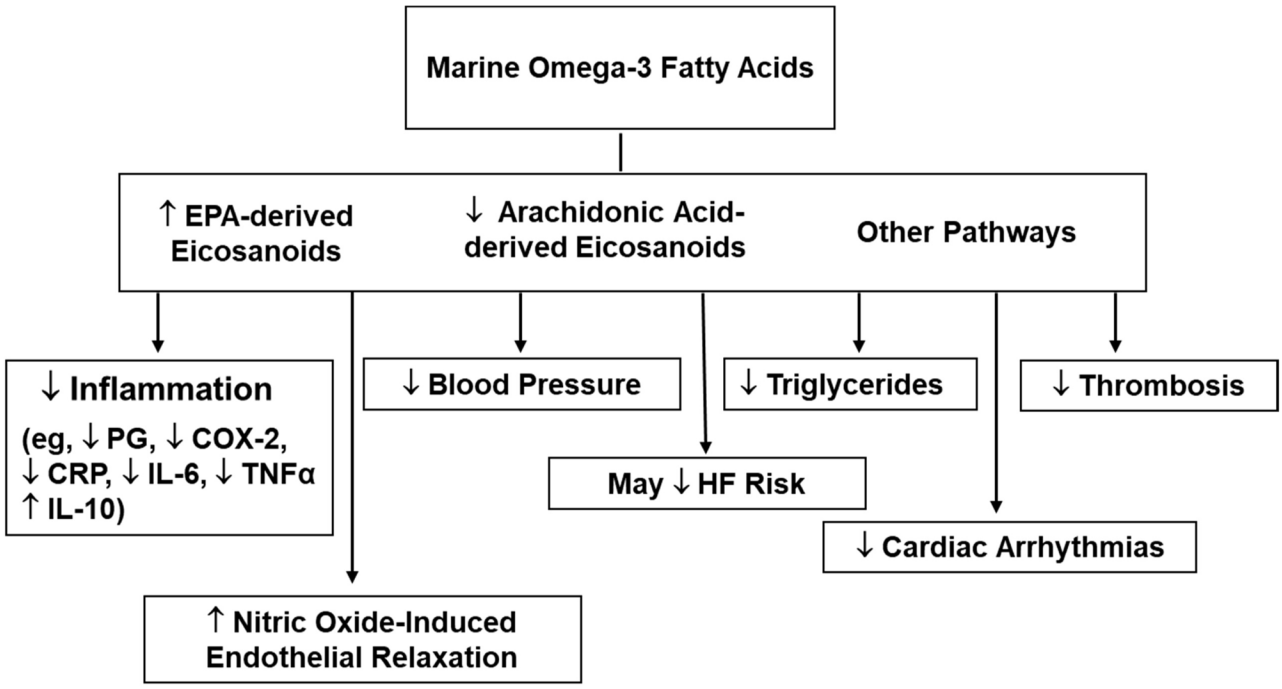
* Premature MI in a parent (before age 60 in father and before 65 in mother)

** Number of traditional cardiovascular disease risk factors (smoking, diabetes, hypertension high cholesterol, parental history of premature MI)

From: Manson JE et al., *N Engl J Med* 2019; 380:23-32.

Figure 3.

Hazard ratios (HR) and 95% confidence intervals (CI) of total myocardial infarction according to subgroups, comparing n-3 fatty acid and placebo groups. (From Cox regression models controlling for age, sex, and vitamin D randomization group.)

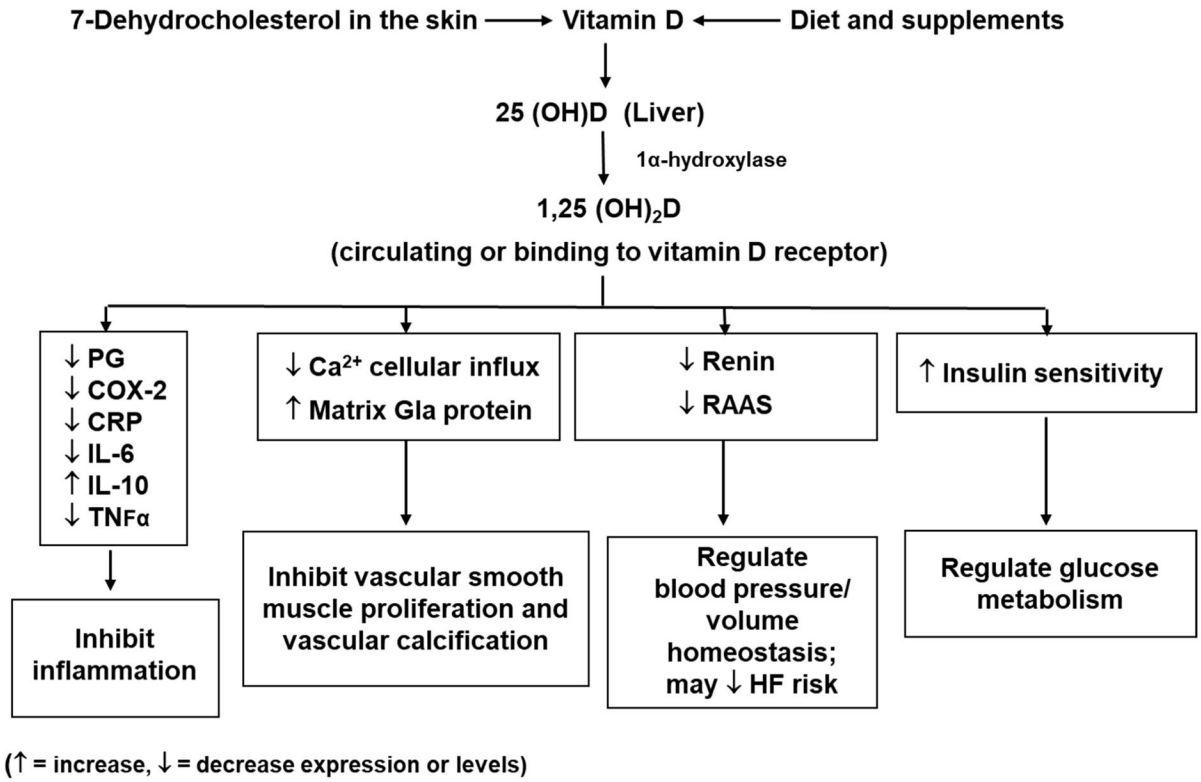


COX-2, cyclooxygenase-2; CRP, C-reactive protein; EPA, eicosapentaenoic acid; HF, heart failure; IL-6, interleukin-6; IL-10, interleukin-10; PG, prostaglandin; TNFα, tumor necrosis factor-α

Adapted from: Manson JE et al., *Contemp Clin Trials* 2012; 33:159-171.

Figure 4. Mechanisms by which marine omega-3 fatty acids may lower cardiovascular disease risk.

COX-2, cyclooxygenase-2; CRP, C-reactive protein; EPA, eicosapentaenoic acid; HF, heart failure; IL-6, interleukin-6; IL-10, interleukin-10; PG, prostaglandin; TNFα, tumor necrosis factor-α



COX-2, cyclooxygenase-2; CRP, C-reactive protein; HF, heart failure; IL-6, interleukin-6; IL-10, interleukin-10; PG, prostaglandin; RAAS, renin-angiotensin-aldosterone system; TNFα, tumor necrosis factor-α

Adapted from: Manson JE et al., *Contemp Clin Trials* 2012; 33:159-171.

Figure 5. Mechanisms by which vitamin D may lower cardiovascular disease risk.

COX-2, cyclooxygenase-2; CRP, C-reactive protein; HF, heart failure; IL-6, interleukin-6; IL-10, interleukin-10; PG, prostaglandin; RAAS, renin-angiotensin-aldosterone system; TNFα, tumor necrosis factor-α

Table 1.

Baseline characteristics of the 25,871 VITAL participants, according to randomized treatment assignment

Baseline characteristic	All participants		n-3 fatty acids			Vitamin D	
			Active	Placebo		Active	Placebo
Total number	25,871		12,933	12,938		12,927	12,944
Female sex—number (%)	13,085/25,871 (50.6)		6547 (50.6)	6538 (50.5)		6547 (50.6)	6538 (50.5)
Age, years, mean ± SD	67.1 ± 7.1		67.2 ± 7.1	67.1 ± 7.1		67.1 ± 7.0	67.1 ± 7.1
Race/ethnicity—number (%)[a]							
Non-Hispanic White	18,046/25,304 (71.3)		9044 (71.5)	9002 (71.2)		9013 (71.3)	9033 (71.4)
African American	5106/25,304 (20.2)		2549 (20.1)	2557 (20.2)		2553 (20.2)	2553 (20.2)
Hispanic (not African American)	1013/25,304 (4.0)		491 (3.9)	522 (4.1)		516 (4.1)	497 (3.9)
Asian/Pacific Islander	388/25,304 (1.5)		200 (1.6)	188 (1.5)		188 (1.5)	200 (1.6)
Native American	228/25,304 (0.9)		120 (0.9)	108 (0.9)		118 (0.9)	110 (0.9)
Other or unknown	523/25,304 (2.1)		249 (2.0)	274 (2.2)		259 (2.0)	264 (2.1)
Body mass index, kg/m^2, mean ± SD[b]	28.1 (5.7)		28.1 ± 5.7	28.1 ± 5.8		28.1 ± 5.7	28.1 ± 5.8
Current smoking—number (%)	1836/25,485 (7.2)		920 (7.2)	916 (7.2)		921 (7.2)	915 (7.2)
Hypertension treated with medication—number (%)	12,791/25,698 (49.8)		6338 (49.3)	6453 (50.2)		6352 (49.5)	6439 (50.1)
Cholesterol-lowering medication, current use—number (%)	9524/25,428 (37.5)		4788 (37.7)	4736 (37.2)		4822 (38.0)	4702 (36.9)
Diabetes—number (%)	3549/25,828 (13.7)		1799 (13.9)	1750 (13.5)		1812 (14.0)	1737 (13.4)
Current regular aspirin use—number (%)[c]	11,570/25,497 (45.4)		5771 (45.3)	5799 (45.5)		5756 (45.2)	5,814 (45.6)

Abbreviations: SD = standard deviation. There were no significant differences in the baseline characteristics between the groups.

[a] Race and ethnic group were reported by participants.

[b] For body mass index, data were missing for 2.4% of participants.

[c] At least monthly.

Table 2.

Hazard ratios (HR) and 95% confidence intervals (CI) of primary, secondary, and other outcomes by randomized assignment to n-3 fatty acids (n-3 FAs)[a]

Endpoint	n-3 FAs (N =12,933)	Placebo (N =12,938)	HR	95% CI
	# of participants w/event			
Cardiovascular disease (CVD), primary and secondary outcomes				
Major CVD event[b,c]	386	419	0.92	0.80–1.06
Expanded CVD event[d]	527	567	0.93	0.82–1.04
Total myocardial infarction (MI)	145	200	0.72	0.59–0.90
Total stroke	148	142	1.04	0.83–1.31
Cardiovascular mortality	142	148	0.96	0.76–1.21
Other vascular outcomes[e]				
Percutaneous coronary intervention (PCI)	162	208	0.78	0.63–0.95
Coronary artery bypass graft (CABG)	85	86	0.99	0.73–1.33
Fatal MI	13	26	0.50	0.26–0.97
Coronary heart disease (CHD) mortality	37	49	0.76	0.49–1.16
Total CHD[f]	308	370	0.83	0.71–0.97
Ischemic stroke	111	116	0.96	0.74–1.24
Hemorrhagic stroke	25	19	1.32	0.72–2.39
Fatal stroke	22	20	1.10	0.60–2.01
Total invasive cancer[b]	820	797	1.03	0.93–1.13
Cancer mortality	168	173	0.97	0.79–1.20
All-cause mortality	493	485	1.02	0.90–1.15
Excluding the first two years of follow-up:				
Cardiovascular outcomes				
Major CVD event	269	301	0.89	0.76–1.05
Total myocardial infarction	94	131	0.72	0.55–0.93
Total stroke	103	112	0.92	0.70–1.20
Total invasive cancer	536	476	1.13	1.00–1.28
Cancer mortality	126	135	0.93	0.73–1.19
All-cause mortality	371	381	0.97	0.84–1.12

[a] Analyses were from Cox regression models that were controlled for age, sex, and randomization group in the vitamin D portion of the trial. Analyses were not adjusted for multiple comparisons.

[b] Primary outcomes

[c] A composite of MI, stroke, and cardiovascular mortality

[d] A composite of MI, stroke, cardiovascular mortality, and coronary revascularization (CABG, PCI)

[e] Not prespecified as primary or secondary outcomes.

[f] A composite of MI, coronary revascularization (CABG, PCI), and CHD death

Table 3.

Hazard ratios (HR) and 95% confidence intervals (CI) of primary, secondary, and other outcomes by randomized assignment to vitamin D[a]

Endpoint	Vitamin D (N = 12,927)	Placebo (N = 12,944)	HR	95% CI
	no. of participants w/event			
Cardiovascular disease (CVD), primary and secondary outcomes				
Major CVD event[b,c]	396	409	0.97	0.85–1.12
Expanded CVD event[d]	536	558	0.96	0.86–1.08
Total myocardial infarction	169	176	0.96	0.78–1.19
Total stroke	141	149	0.95	0.76–1.20
Cardiovascular mortality	152	138	1.11	0.88–1.40
Other vascular outcomes[e]				
Percutaneous coronary intervention (PCI)	182	188	0.97	0.79–1.19
Coronary artery bypass graft (CABG)	73	98	0.75	0.55–1.01
Fatal MI	24	15	1.60	0.84–3.06
Fatal stroke	19	23	0.84	0.46–1.54
Total invasive cancer[b]	793	824	0.96	0.88–1.06
Cancer mortality	154	187	0.83	0.67–1.02
All-cause mortality	485	493	0.99	0.87–1.12
Excluding the first two years of follow-up:				
Major CVD event	274	296	0.93	0.79–1.09
Total invasive cancer	490	522	0.94	0.83–1.06
Cancer mortality	112	149	0.75	0.59–0.96
All-cause mortality	368	384	0.96	0.84–1.11

[a]Analyses were from Cox regression models controlling for age, sex, and n-3 fatty acid randomization group. Analyses were not adjusted for multiple comparisons.

[b]Primary outcome.

[c]A composite of myocardial infarction, stroke, and cardiovascular mortality.

[d]A composite of major cardiovascular events plus coronary revascularization (CABG + PCI).

[e]Not prespecified as primary or secondary outcomes.