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VITAL study: an incomplete picture?

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Two articles published by Manson et al[1,2] in The New England Journal of Medicine have recently gained attention across the scientific community. They showed the results of a large-scale, randomized, double-blind, placebo-controlled trial (VITAL: Vitamin D and Omega-3 Trial) assessing the effects of vitamin D and omega-3 polyunsaturated fatty acids (PUFAs) supplementation on prevention of cancer and cardiovascular (CV) disease. A total of 25,871 participants (mean age: 67.1±7.1 years) recruited throughout the United States underwent randomization, with a median follow-up of 5.3 years. At baseline, mean serum 25-hydroxyvitamin D [25(OH)D] levels were 30.8±10.0 ng/mL (n=15,787) with 12.7% of participants having levels <20 ng/mL and 32.2% with levels between 20-29 ng/mL, indicative of vitamin D deficiency and insufficiency, respectively[3]. Primary endpoints were invasive cancer of any type and major CV events (composite endpoint of myocardial infarction, stroke, and death from CV causes). Secondary cancer endpoints included site-specific cancers (colorectal, breast, and prostate cancers) and death from cancer, whereas secondary CV endpoints consisted of an expanded composite of major CV events plus coronary revascularization and the individual components of major CV events. The authors found that vitamin D supplementation at a dose of 2,000 IU/day did not result in a lower incidence of invasive cancer (p=0.47) or major CV events (p=0.69) compared to placebo[1]. Similarly, supplementation with omega-3 PUFAs at a dose of 1 g/day did not result in a lower incidence of invasive cancer (p=0.56) or major CV events (p=0.24) compared to placebo[2].

Although the primary endpoints of the VITAL trial were not met, other findings from this study are noteworthy. Analyses excluding the first 2 years of follow-up (to account for the latency period of cancer) showed a significant 25% reduction in cancer-related death in the vitamin D group compared to placebo [Hazard ratio (HR), 0.75; 95% CI, 0.59-0.96]. Moreover, subgroup analyses raised the possibility of differential effects of vitamin D supplementation on cancer incidence according to BMI, with the remarkable observation that normal-weight individuals in the vitamin D group showed a lower cancer incidence compared to those in the placebo group (HR, 0.76; 95% CI, 0.63-0.90)[1]. On the other hand, vitamin D supplementation at a dose of 2,000 IU/day may not have led to similar results in overweight and obese participants due to the higher vitamin D requirements of such individuals, because of volumetric dilution or decreased bioactivity of vitamin D associated with obesity[4,5]. Indeed, according to the *Endocrine Society* guidelines, obese adults need at least two to three times higher doses of vitamin D than nonobese adults to treat and prevent recurrent vitamin D deficiency, namely: 6,000-10,000 IU/day for 8 weeks, followed by maintenance therapy of 3,000-6,000 IU/day[3,6].

In addition to these remarks, the VITAL trial may have been negatively affected by some methodology limitations, despite its indisputable strengths (e.g., large sample size; racial, ethnic, and geographic heterogeneity of the participants; high rates of follow-up and adherence to the trial regimen). The authors appropriately state that the study had limitations, including the median duration of follow-up (5.3 years) and the use of only one dose of vitamin D. Nevertheless, additional weaknesses should be taken into account as follows.

It is worth noting that the mean serum 25(OH)D levels at baseline (available only for 15,787 participants, corresponding to approximately 61% of all participants) were 30.8 ng/mL, with only 12.7% of participants being vitamin D deficient. It is unknown what the mean baseline vitamin D status was for all participants, but if the majority were already vitamin D sufficient, this might have had an impact on study outcomes.

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Importantly, all the participants – including the placebo group – were allowed to take up to 800 IU/day of vitamin D from different supplemental sources due to ethical reasons. Given that 800 IU/day of vitamin D amount to 40% of the tested intervention (2,000 IU/day), the study design does not fully meet the criteria of a randomized placebo-controlled trial. Furthermore, at 5 years there was a prevalence of outside use of vitamin D (>800 IU/day) of 6.4% for the vitamin D group and 10.8% for the placebo group, probably due to the initiation/addition of vitamin D supplementation in some participants with low serum levels, as stated by the authors.

This trial undoubtedly presents additional weaknesses, including a lack of information about sun exposure, outdoor physical activity, indoor activity and body-covering habits. Such information could have highlighted potential differences between the placebo and the vitamin D group.

Nevertheless, we believe that the main limitation of this study consists in the lack of assessment of 25(OH)D status during follow-up, except for a subgroup of 1,644 of 25,871 participants (6.35% of all participants) in whom serum 25(OH)D levels were measured at 1 year. In this subgroup, serum 25(OH)D levels increased from 29.8 ng/mL at baseline to 41.8 ng/mL at 1 year (40% increase), with minimal changes observed in the placebo arm (mean: -0.7 ng/mL). However, these findings are not generalizable to the rest of the participants. In fact, serum 25(OH)D response to a given dose of vitamin D is highly variable and dependent upon several factors, including baseline 25(OH)D, body fat percentage, ethnicity, medications, genetics, seasonal variations, and type of vitamin D supplements[7].

Several studies have evaluated different clinical outcomes in relation to a broad range of serum 25(OH)D concentrations, showing interesting results. Importantly, a pooled analysis including a randomized clinical trial and a prospective cohort study (characteristics of the pooled cohort: n=2,304; median age: 64 years; median follow-up time: 3.9 years) found that women with serum 25(OH)D concentrations ≥40 ng/mL (expressed as both baseline concentrations and mean concentrations during the follow-up) had a 67% lower risk of all invasive cancer – excluding skin cancer – compared to women with concentrations <20 ng/ml, adjusting for age, BMI, smoking status, and calcium supplement intake (HR, 0.33; 95% CI, 0.12-0.90; p=0.03)[8]. Similar findings have been observed even for site-specific cancers, such as breast and colorectal cancers. In fact, a recent pooled analysis of two randomized clinical trials and a prospective cohort (characteristics of the pooled cohort: n=5,038; median age: 63 years; median follow-up time: 4.0 years) showed that higher 25(OH)D concentrations were associated with a dose-response reduction in breast cancer risk, with concentrations ≥60 ng/mL being most protective. In particular, multivariate Cox regression revealed that women with serum 25(OH)D levels ≥60 ng/mL had an 80% lower risk of breast cancer compared to women with serum levels <20 ng/mL (HR, 0.20; p=0.03), adjusting for age, BMI, smoking status, calcium supplement intake, and study of origin[9]. Moreover, McCullough et al[10] pooled participant-level data from 17 cohorts (n=5,706 colorectal cancer case participants and 7,107 control participants), and found that serum 25(OH)D levels between 30 and 40 ng/mL were associated with the highest colorectal cancer risk reduction. Notably, circulating 25(OH)D concentrations in the ranges of 30.0-34.9 ng/mL and 35.0-39.9 ng/mL were significantly associated with 19% and 27% lower risks of colorectal cancer, respectively, compared to serum 25(OH)D levels <12 ng/mL. Notwithstanding, colorectal cancer risk did not continue to decline at concentrations ≥40 ng/mL.

Noteworthy, Garland et al[11] published a large meta-analysis of 32 observational studies conducted between 1966 and 2013, which evaluated serum 25(OH)D concentrations in association with age-adjusted all-cause mortality. The authors found that serum 25(OH)D concentrations ≤30 ng/mL were associated with higher all-cause mortality compared to concentrations >30 ng/mL (p <0.01), further mitigating the reliability of the assumed U-shape curve of vitamin D levels and mortality[12]. Of note, the overall age-adjusted HR for all-cause mortality comparing the lowest (0-9 ng/mL) to the highest (>50 ng/mL) ranges of serum 25(OH)D levels was 1.9 (95% CI, 1.6-2.2; p<0.001). A pooled dose-response curve also showed that HR sharply decreased between quantile 0-9 and 30-39 ng/mL, with a further risk reduction in quantile 40-49 ng/mL. Hazard ratios appeared to plateau at serum 25(OH)D levels >50 ng/mL. Moreover, the authors observed a mean drop of 0.1 units in the HR per 10 ng/mL of serum 25(OH)D[11].

Overall, these data suggest that serum 25(OH)D levels ≥40 ng/mL should be the minimum levels to achieve a reduction in malignancy and all-cause mortality, as a result of the extraskeletal benefits of vitamin D[13-15]. This might have relevant clinical implications, given that the aforementioned target serum levels required for extraskeletal benefits of vitamin D are well above the current definition of vitamin D sufficiency based upon the *Institute of Medicine*[16] and *Endocrine Society* guidelines[3], which primarily refer to minimum serum 25(OH)D levels required for bone health.

Another important remark that has to be made on the VITAL trial is that a dose of 2,000 IU/day was considered a "high-dose" of vitamin D supplementation[1]. The *Endocrine Society* guidelines consider 1,500-2,000 IU/day as the vitamin D recommended daily intake for adults, pointing out that up to 10,000 IU/day may be needed to correct vitamin D deficiency (10,000 IU/day is considered as the tolerable upper limit for vitamin D maintenance, not to be exceeded without medical supervision in order to avoid toxicity risk)[3].

Appropriate design, conduct, and analysis of randomized controlled trials (RCTs) are critical for obtaining reliable results on the effects of vitamin D supplementation in different clinical settings. In this regard, Grant et al[17] proposed a hybrid observational approach to vitamin D RCT design based on serum 25(OH) D concentrations and vitamin D supplementation. In fact, vitamin D supplemental intake alone might not be an appropriate index for the influence of vitamin D on health outcomes. We thereby believe that RCT design should include baseline and follow-up 25(OH)D levels, along with vitamin D supplementation doses.

Several studies derived from the VITAL cohort are currently evaluating other clinical outcomes, including hypertension, heart failure, anemia, adiposity, depression, cognitive decline, bone structure and architecture, fractures, mammographic density, and diabetic kidney disease (study protocol details available at https://clinicaltrials.gov; keywords: VITAL, vitamin D, omega-3). Hence, future analyses of VITAL clinical outcomes based on vitamin D serum levels rather than doses may lead to a more accurate interpretation of study results.

Importantly, the analysis of key secondary endpoints showed a significant 28% reduction in risk for total myocardial infarction in the omega-3 group compared to placebo (HR, 0.72; 95% CI, 0.59-0.90)[2]. However, Cox regression models showed a lack of synergistic effects of vitamin D and omega-3 PUFAs when analysing the primary endpoints of cancer and major CV events[2].

We believe that the lack of significant effects of omega-3 PUFAs on primary endpoints could be related to the relatively low-dose of omega-3 PUFAs in the intervention group (1 g/day, administered as a fish-oil capsule containing 840 mg of omega-3 PUFAs distributed as 460 mg of eicosapentaenoic acid [EPA] and 380 mg of docosahexaenoic acid [DHA]). In this regard, the large, randomized, double-blind, placebo-controlled trial REDUCE-IT investigated the effects of the supplementation with icosapent ethyl – a highly purified and stable EPA ethyl ester – at a higher dose (4 g/day) on CV risk among patients with hypertriglyceridemia and established CV disease or diabetes and other risk factors (n=8,179; median follow-up: 4.9 years), who had been receiving statin therapy[18]. The primary endpoint was a composite of CV death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina, while the secondary endpoint was a composite of CV death, nonfatal myocardial infarction, or nonfatal stroke. Importantly, icosapent ethyl supplementation led to a significant reduction in the rates of primary (HR, 0.75; 95% CI, 0.68 to 0.83; p <0.001) and secondary endpoints (HR, 0.74; 95% CI, 0.65 to 0.83; p <0.001) compared to the placebo group[18]. Also, a study addressing the effect of EPA on CV events in 18,645 Japanese patients with hypercholesterolemia (JELIS study) randomized participants assigned to receive either statin therapy plus EPA at a dose of 1.8 g/day or statin therapy alone during a 5-year follow-up period[19]. The group that received statin therapy plus EPA had a 19% reduction in major coronary events compared to the statin therapy alone group (HR, 0.81; 95% CI, 0.69 to 0.95; p=0.011)[19].

Omega-3 PUFAs exert anti-inflammatory properties acting as the precursor of a series of mediators known as resolvins, which are involved in the resolution of inflammation[20]. On the other hand, the omega-6 PUFAs (especially arachidonic acid, AA) exert opposing properties, functioning as the precursor of the pro-inflammatory eicosanoids[21]. Increased plasma levels of omega-6/omega-3 ratio and AA/EPA ratio have been proposed as biomarkers of systemic inflammation[22-24]. Inflammation has been shown to play a pivotal role in CV disease[25,26]. Hence, the beneficial CV effects derived from the use of omega-3 PUFAs may rely on their anti-inflammatory role[27]. In fact, JELIS study showed significant CV benefits when the AA/EPA ratio was lowered to approximately 0.8[19]. In addition, higher doses of omega-3 PUFAs appear to be required to achieve a significant reduction in AA/EPA ratio[28-33], which in turn may lead to a more potent anti-inflammatory effect. Therefore, use of higher doses of omega-3 PUFAs and assessment of omega-6/omega-3 ratio and AA/EPA ratio during the follow-up may add further information on future studies evaluating the therapeutic effects of omega-3 PUFAs in different clinical settings[34-39]. Figure 1 illustrates all the aforementioned potential limitations of the VITAL study, which may have negatively affected its final results. In conclusion, the relative contribution of high-dose vitamin D and high-dose omega-3 PUFA supplementation to reduce inflammation may be answered in the ongoing POSEIDON trial (Pilot Study of Omega-3 and Vitamin D in High-Dose in Type 1 Diabetic Patients, ClinicalTrials.gov Identifier: NCT03406897)[39].

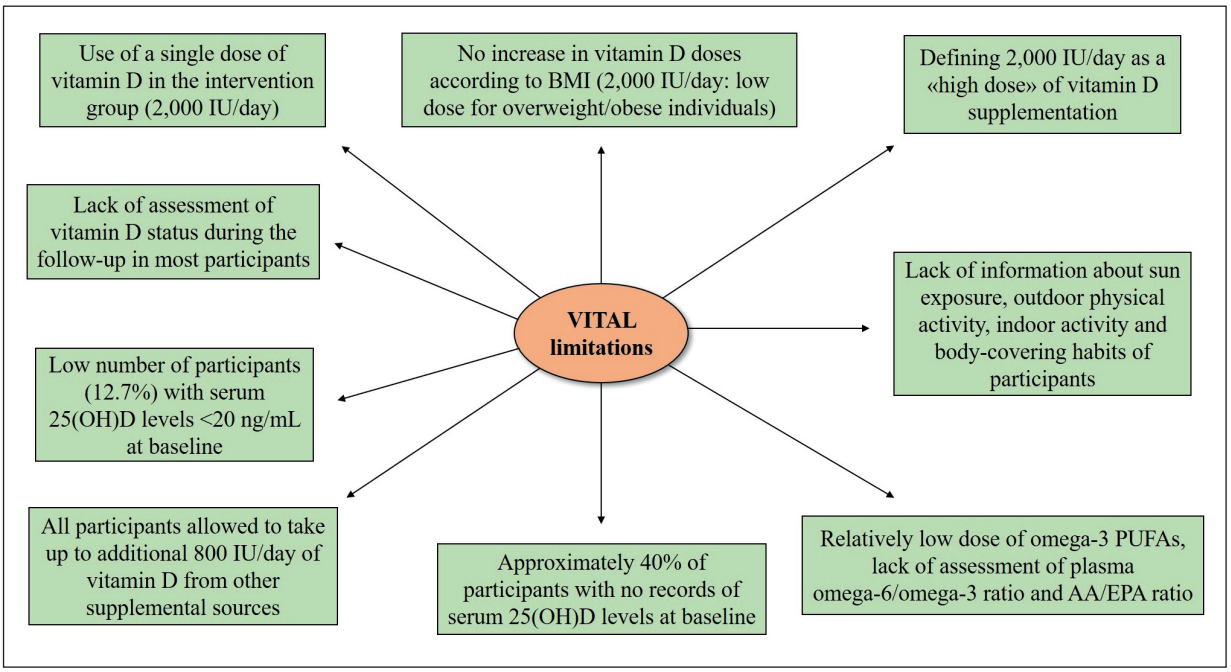

Figure 1. Limitations of the VITAL study. Abbreviations: 25(OH)D: 25-hydroxyvitamin D; omega-3 PUFAs: omega-3 polyunsaturated fatty acids.

Disclosure Statement

Barry Sears is the President of Zone Labs, a medical food company that produces supplements and dietary food products, including purified omega-3 fatty acid concentrates. The other authors declare that they have no conflict of interest to disclose.

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