Vitamin D and Cardiovascular Disease: An Appraisal of the Evidence

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BACKGROUND: Supplementation with vitamin D has received attention as a potential cardioprotective strategy. Biologically plausible mechanisms have been proposed to link vitamin D to coronary heart disease (CHD) prevention, and observational studies suggest an inverse association between serum 25-hydroxyvitamin D (25OHD) concentrations and CHD. Few randomized clinical trials of vitamin D supplementation and CHD have been conducted, however, and no trial with CHD as the primary prespecified outcome has been completed.

CONTENT: A search was conducted in PubMed to find prospective studies of the use of vitamin D supplementation and its relationship to cardiovascular risk factors (RFs) and/or cardiovascular disease (CVD). The exact search query was: “(vitamin D supplement*[Title/Abstract]) AND cardiovascular [Title/Abstract]” AND prospective [Title/Abstract]. This query yielded 42 results. “Randomized Controlled Trial” (article type) was used as a filter in a subsequent query with the same search terms. We review the evidence that vitamin D supplementation modifies coronary RFs, such as blood pressure, lipids, and glucose tolerance, and/or affects the development of clinical CHD events. We address potential sources of confounding in observational epidemiologic studies of the relationship between serum 25OHD and CHD. We also address laboratory assay issues relevant to the reliable measurement of 25OHD.

SUMMARY: Most vitamin D supplementation trials have not demonstrated improvement in CVD, but they have tested relatively low vitamin D doses. Thus, the evidence remains inconclusive, highlighting the need for rigorous randomized trials of higher vitamin D doses with cardiovascular events as prespecified outcomes.

While we await the results of ongoing trials, the recommended dietary allowances from the Institute of Medicine remain the best guidepost for nutritional requirements.

Coronary heart disease (CHD) remains the leading cause of mortality in US men and women. The primary risk factors for CHD in both sexes include older age, smoking, diabetes mellitus, dyslipidemia, hypertension, physical inactivity, obesity, the metabolic syndrome, a family history of premature CHD (males and females <55 and <65 years of age, respectively), and a personal history of peripheral arterial disease. Few Americans achieve optimal control of these risk factors, however, and these traditional risk factors do not explain many CHD events. Thus, novel approaches to reducing CHD risk remain of great interest.

Vitamin D has garnered recent attention for its potential cardioprotective properties and has become a topic of considerable interest in both the clinical and research communities. An increased incidence of CHD and hyperlipidemia in higher latitudes has been ecologically correlated with less sunlight. Other studies have reported that individuals with less exposure to ultraviolet light have lower vitamin D concentrations and a higher risk of CHD, myocardial infarction, and hypertension. Lower serum vitamin D concentrations have also been associated with increased risks of sudden cardiac death, peripheral arterial disease, and greater carotid intima–medial thickness. Randomized trials of these relationships have been sparse, however, and data related to these outcomes have been inconsistent. Postmenopausal women, as well as older men, may be at particularly high risk for vitamin D deficiency because of age-associated decreases in skin photoisomerization of 25-hydroxyvitamin D, 1,25(OH)2D, 1,25-dihydroxyvitamin D; VDR, vitamin D receptor; IOM, Institute of Medicine; AHRQ, Agency for Healthcare Research and Quality; IOF, International Osteoporosis Foundation; NOF, National Osteoporosis Foundation; LC-MS/MS, liquid chromatography–tandem mass spectrometry; CVD, cardiovascular disease; VITAL, Vitamin D and Omega-3 Trial.

Nonstandard abbreviations: CHD, coronary heart disease; 25OHD, 25-hydroxyvitamin D; 1,25(OH)2D, 1,25-dihydroxyvitamin D; VDR, vitamin D receptor; IOM, Institute of Medicine; AHRQ, Agency for Healthcare Research and Quality; IOF, International Osteoporosis Foundation; NOF, National Osteoporosis Foundation; LC-MS/MS, liquid chromatography–tandem mass spectrometry; CVD, cardiovascular disease; VITAL, Vitamin D and Omega-3 Trial.
7-dehydrocholesterol (14) and lower dietary intakes of oral vitamin D.

**Vitamin D Production and Homeostasis**

Vitamin D (calciferol) is a term that refers to a group of lipid-soluble compounds with a 4-ringed cholesterol backbone. In the skin, pro–vitamin D is photo-isomerized to vitamin D₃ (cholecalciferol) by sunlight and ultraviolet light. The other major source of vitamin D is intestinal absorption. Vitamin D₃ is then transported to the liver, where it is hydroxylated to 25-hydroxyvitamin D (25OHD), which comprises both 25OHD₂ and 25OHD₃. 25OHD then travels to the kidney, where it is further hydroxylated to 1,25-dihydroxyvitamin D [1,25(OH)₂D or calcitriol] (15), the physiologically active form of vitamin D (16, 17). The most representative measure of vitamin D status is the serum 25OHD concentration (18, 19). Serum 25OHD is an excellent marker of vitamin D sufficiency, because it reflects the total stored quantity from both endogenous and exogenous sources (18). As serum 25OHD concentrations decrease, parathyroid hormone concentrations increase and positively influence the conversion of 25OHD to 1,25(OH)₂D, which subsequently maintains normal intestinal absorption of calcium. Therefore, 1,25(OH)₂D is not representative of the total body storage of vitamin D, because serum calcium and 1,25(OH)₂D concentrations will be normal or slightly increased during vitamin D deficiency, owing to secondary hyperparathyroidism (18, 20).

**Risk Factors for Vitamin D Deficiency**

Risk factors for developing vitamin D deficiency, or lower serum vitamin D concentrations, include an age > 65 years (21), dark skin pigmentation, obesity (from storage in adipose tissue (22)), kidney and/or liver disease (23), disorders affecting fat absorption (e.g., celiac disease, Crohn disease, ulcerative colitis, some types of bariatric surgery), and end-organ insensitivity to 1,25(OH)₂D. In addition, 25OHD deficiency is also known to be related to environmental variables that lead to decreased exposure to ultraviolet light, such as institutionalization, decreased outdoor physical activity, and frailty (24).

**Potential Mechanisms for an Association between Vitamin D Deficiency and CHD**

Vitamin D receptors (VDRs) have been identified in many tissues, including vascular smooth muscle cells (25), cardiomyocytes, and coronary arteries (26, 27). Given the presence of VDRs in the vascular system, including the coronary arteries, there are several biologically plausible pathways through which vitamin D could lead to improved cardiovascular health. Activation of the VDRs, for instance, has been shown to inhibit vascular smooth muscle cell proliferation, which is believed to be cardioprotective (28). Some studies have associated higher 25OHD concentrations and/or vitamin D supplementation with a systemic anti-inflammatory state via effects on interleukins, C-reactive protein, and anti-inflammatory cytokines—a milieu which, again, is believed to foster cardioprotection (29–31). Vitamin D may control blood pressure through its regulatory effects on the renin–angiotensin–aldosterone system (32). Limited research has suggested that vitamin D supplementation can decrease the incidence of impaired glucose tolerance and diabetes mellitus (33, 34), along with improving values for lipid parameters (35). Furthermore, the results of various studies have suggested a link between vitamin D and a lower likelihood of autoimmune conditions such as rheumatoid arthritis (36), diabetes (both type 1 and type 2) (37, 38), and multiple sclerosis (39).

**Observational Data**

Much of the excitement concerning a correlation between vitamin D deficiency and CHD stems from observational data. For example, Giovannucci et al. (9) followed 18 000 healthy male participants for 10 years. Individuals with vitamin D deficiency, defined as serum 25OHD concentrations ≤ 15 ng/mL (≈ 38 nmol/L), had a greater risk of a myocardial infarction than men with 25OHD concentrations ≥ 30 ng/mL (≥ 75 nmol/L) (9), with a relative risk of 2.42 (95% CI, 1.53–3.84; P < 0.001). The Framingham Offspring Study, a prospective analysis of 1739 individuals with 25OHD concentrations ≤ 15 ng/mL (< 38 nmol/L), obtained an adjusted hazard ratio for a first-incident cardiovascular event of 1.6 (95% CI, 1.11–2.36; P = 0.01). Participants who had hypertension along with 25OHD deficiency had a hazard ratio of 2.1 (95% CI, 1.3–3.5; P = 0.003) for a first cardiovascular event (18). A meta-analysis of prospective but observational studies of 25OHD and cardiovascular events demonstrated a generally linear, inverse association up to 24 ng/mL (60 nmol/L) but revealed no further reductions (i.e., a threshold effect) at higher 25OHD concentrations (40).

**Limitations of the Observational Data**

Despite the considerable data demonstrating an association between vitamin D deficiency and poor cardiovascular outcomes, caution is advisable in interpreting the data from observational studies. Confounding by other lifestyle factors and a “healthy user” bias in non-
randomized studies may play a role in the current evidence suggesting an association. For example, age must be carefully controlled in analyses, because older age (21) increases both the risk of vitamin D deficiency and the risk of myocardial infarction (41). Decreased dietary intake and poor nutritional status can each lead to vitamin D nutritional deficiency. The lower intake could be due to other disease(s) or general malnutrition, either of which could increase the likelihood of CHD. Decreased exposure to ultraviolet light can be due to less outdoor activity/exercise and hence lead to an increased risk of CHD and low 25OHD. An additional confounding risk factor is obesity, which both increases the risk of CHD and lowers 25OHD concentrations because 25OHD can be sequestered in adipose tissues (22).

Few observational studies are able to adjust fully for these confounding factors. Therefore, all of these risk factors, which are more likely to be associated with low 25OHD, may confound the relationship between 25OHD and CHD in nonrandomized studies. As we pointed earlier, vitamin D has been associated with a systemic antiinflammatory milieu. Although relevant pathways may include a beneficial interaction between vitamin D and C-reactive protein, interleukins, and/or cytokines (29–31), vitamin D deficiency has also been suggested to be a direct consequence of an inflammatory condition or state (42).

Randomized Controlled Trials

Few prospective randomized clinical trials evaluating the effects of vitamin D supplementation on CHD have been conducted, and currently none of the prospective trials have presupposed CHD as the primary outcome (43–45). Among the sparse randomized trials that have assessed CHD—or CHD risk factors—as a secondary or tertiary outcome, no correlation has been identified for CHD, and few have been found for CHD risk factors (Table 1) (46–58). In a study of 327 men and women older than 65 years, individuals who received vitamin D3 actually had an increased risk of coronary death (P < 0.001) (58). In a double-blind, placebo-controlled, randomized clinical trial in the UK conducted among 2686 men and women 65–85 years of age, participants received 100,000 IU of supplemental vitamin D3 every 4 months (equivalent to approximately 833 IU daily) for 5 years (46). No beneficial CHD effect could be attributable to vitamin D (46).

Additional results from the Women’s Health Initiative suggested that postmenopausal women who received 400 IU/day of oral vitamin D3 combined with 1000 mg/day of calcium had no reduction in their risk of CHD events or stroke (50). In additional prospective trials (from subanalyses of the Women’s Health Initiative), calcium and vitamin D3 supplementation was not found to improve blood pressure (49) or coronary artery calcium scores (53). Furthermore, there was no decrease in incident hypertension (59) or any prevention of or improvement in the metabolic syndrome or diabetes (47). An 8-week prospective trial of 151 male and female vitamin D–deficient adults randomized to receive 50,000 IU of vitamin D3 or placebo found no improvement in lipid parameters (48). Two small prospective trials that evaluated endothelial function produced mixed results, with one showing no effect (51) and the other showing a short-term improvement in stroke patients with well-controlled hypertension; however, the effect was not sustained by the completion of the 16-week study (52).

Several small prospective studies have shown some improvement in CHD risk factors (55) and inflammation (30, 56). Additional data have shown no effect on glycemic control, however (54, 57). A recent prospective randomized, double-blind, placebo-controlled clinical trial assessed the change in systolic and diastolic blood pressures in a healthy black population randomized to oral placebo or to 1000, 2000, or 4000 IU/day vitamin D3 for 3 months (60). The results of this study revealed a 1.4-mmHg decrease in systolic blood pressure for each additional 1000 IU/day increase in the vitamin D3 dose (P = 0.04). Although the investigators found no statistically significant effect of oral vitamin D3 on diastolic blood pressure, their study did reveal a 0.2-mmHg decrease in systolic blood pressure for each 1-ng/mL (2.5-nmol/L) increase in 25OHD (P = 0.02). Despite this significant effect of oral vitamin D3 on systolic blood pressure, there appeared to be a threshold effect, with individuals receiving 2000 IU/day and 4000 IU/day of vitamin D3 having similar results. Furthermore, those with baseline 25OHD concentrations ≥20 ng/mL (≥50 nmol/L) experienced little benefit from supplementation, compared with participants with baseline 25OHD concentrations <20 ng/mL (<50 nmol/L), who had a 2.2-mmHg decrease in systolic blood pressure (P = 0.03). In addition, adjustment for baseline differences in blood pressure attenuated the study’s findings. Whereas this trial was designed to assess change in blood pressure, it is noteworthy that most other trials were designed to assess bone health. Cardiovascular outcomes were not presupposed primary end points for most previous studies.

Metaanalyses of Randomized Trials

Several metaanalyses have evaluated both mortality and CHD risk with respect to their relationship to vitamin D supplementation. A metaanalysis of 18 randomized clinical trials including 57,311 individuals was published in 2008 (61). This analysis revealed a statis-
<table>
<thead>
<tr>
<th>Design</th>
<th>Primary outcome</th>
<th>Study outcome</th>
<th>Summary of study, sample size, patient age, and route/dose of treatment</th>
<th>Length and follow-up</th>
<th>Outcomes related to CHD or CHD risk factors</th>
<th>Preexisting conditions or disease?</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>DB, PC, RCT</td>
<td>Colorectal cancer prevention study</td>
<td>Change in SBP and DBP</td>
<td>Blacks (n = 283; 30–80 years; median, 51 years) randomized for 3 months to oral placebo, 1000, 2000, or 4000 IU/day VitD3, followed by treatment for 3 more months</td>
<td>6 Months</td>
<td>A ~1.4-mmHg change in SBP for each additional 1000 IU/day of VitD3 (P = 0.04). No significant effect on DBP. Each 1-ng/mL increase in 25OHD accompanied by a ~0.2-mmHg change in SBP (P = 0.02)</td>
<td>Generally healthy patients. Patients with preexisting disorders of the parathyroid or calcium metabolism; patients with type I DM, sarcoidosis, malignancy, or thyroid disease excluded</td>
<td>Forman et al. (60)</td>
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<tr>
<td>DB, PC, RCT</td>
<td>Changes in brachial artery FMV, carotid–femoral PWV, and aortic augmentation index</td>
<td>Same as primary outcomes</td>
<td>Postmenopausal women [n = 116; mean (SD) age, 63.9 (3) years] with serum 25OHD concentrations &gt;20 and &lt;60 ng/mL, received 2500 IU VitD3 or placebo daily</td>
<td>4 Months</td>
<td>VitD supplementation did not improve endothelial function, arterial stiffness, or inflammation</td>
<td>Generally healthy, community-dwelling, ambulatory women from Madison, WI; patients with CVD excluded</td>
<td>Gepner et al. (51)</td>
</tr>
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<td>DB, PC, RCT</td>
<td>Change in small LDL particle number</td>
<td>Other lipid fractions</td>
<td>VitD-insufficient (25OHD &lt;20 ng/mL) male and female adults (n = 151); received 50 000 IU VitD3 weekly</td>
<td>8 Weeks</td>
<td>VitD repletion failed to improve lipid profile</td>
<td>Increased risk for CVD (with at least 1 of numerous significant CVD risk factors)</td>
<td>Ponda et al. (48)</td>
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<tr>
<td>DB, PC, RCT</td>
<td>BP, FMV of the brachial artery, cholesterol, and other markers of vascular health</td>
<td>Same as primary outcomes</td>
<td>Patients (n = 58; mean age, 67 years) received 100 000 IU oral VitD3 or placebo at baseline</td>
<td>16 Weeks of follow-up</td>
<td>High-dose oral VitD supplementation did not improve BP but produced short-term improvement in endothelial function in stroke patients with well-controlled baseline BP, which was not sustained by end of study</td>
<td>History of stroke with baseline 25OHD concentrations &lt;75 nmol/L</td>
<td>Witham et al. (52)</td>
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<tr>
<td>DB, PC, RCT</td>
<td>Hip fractures</td>
<td>CAC score</td>
<td>CaD trial (1) nested within WHI hormonal trial (2) (estrogen among women who underwent hysterectomy). Women (n = 75; age, 50–59 years) received daily calcium carbonate (1000 mg elemental calcium) and VitD3 (400 IU)</td>
<td>7 Years</td>
<td>Treatment and placebo groups were not different in CAC plaque burden measured at end of trial</td>
<td>Generally healthy postmenopausal women</td>
<td>Manson et al. (53)</td>
</tr>
<tr>
<td>DB, PC, RCT</td>
<td>Fasting serum lipids, BP, and oral glucose tolerance test</td>
<td>Same as primary outcomes</td>
<td>Obese or overweight patients (n = 438; 21–70 years) received VitD3 (40 000 or 20 000 IU/week) or placebo; all received 500 mg calcium daily. 330 patients completed study</td>
<td>1 Year</td>
<td>No significant effect of VitD on glucose tolerance, BP, or serum lipids</td>
<td>Overweight or obese patients</td>
<td>Jorde et al. (56)</td>
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<tr>
<td>PC, RCT</td>
<td>Glycemic control in patients with type 2 DM</td>
<td>Same as primary outcomes</td>
<td>Participants (n = 36) received VitD3 (40 000 IU/week) or placebo</td>
<td>6 Months</td>
<td>No significant effect of VitD on glucose metabolism</td>
<td>Type 2 DM treated with metformin and bedtime insulin</td>
<td>Jorde et al. (54)</td>
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<tr>
<td>DB, PC, RCT</td>
<td>Weight loss and traditional + nontraditional CVD risk markers</td>
<td>Same as primary outcomes (PTH, TG, and inflammatory markers)</td>
<td>Participants (n = 200; mean baseline 25OHD, 30 nmol/L) received 3320 IU/day VitD3 or placebo while participating in weight-reduction program</td>
<td>12 Months</td>
<td>No adverse effect of VitD on weight loss but significantly decreased PTH, TG, TNF (although LDL increased significantly) in overweight individuals with inadequate VitD status while participating in weight-reduction program</td>
<td>Overweight</td>
<td>Zittermann et al. (55)</td>
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Table 1. Prospective randomized trials of vitamin D supplementation and CHD events or CVD risk factors. (Continued from page XX)

<table>
<thead>
<tr>
<th>Design</th>
<th>Primary outcome</th>
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<th>Summary of study, sample size, patient age, and route/dose of treatment</th>
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<tbody>
<tr>
<td>DB, PC, RCT</td>
<td>Hip fractures</td>
<td>Incident DM</td>
<td>WHI Ca/D trial of postmenopausal women (n = 36,282; age, 50–79 years); received 1000 mg elemental calcium + 400 IU VitD&lt;sub&gt;3&lt;/sub&gt; daily</td>
<td>7 Years of follow-up</td>
<td>No beneficial effects in reducing DM incidence or metabolic syndrome</td>
<td>Generally healthy postmenopausal women</td>
<td>DeBoer et al. (47)</td>
</tr>
<tr>
<td>DB, PC, RCT</td>
<td>Hip fractures</td>
<td>Change in BP and development of HTN</td>
<td>WHI Ca/D trial of postmenopausal women (n = 36,282); received 1000 mg elemental calcium + 400 IU VitD&lt;sub&gt;3&lt;/sub&gt; daily</td>
<td>7 Years of follow-up</td>
<td>No significant beneficial effect on BP or prevention of incident HTN</td>
<td>Generally healthy postmenopausal women</td>
<td>Margolis et al. (49)</td>
</tr>
<tr>
<td>DB, PC, RCT</td>
<td>Hip fractures</td>
<td>Risk of CHD</td>
<td>WHI Ca/D trial of postmenopausal women (n = 36,282; age, 50–79 years); received 500 mg calcium carbonate + 200 IU VitD&lt;sub&gt;3&lt;/sub&gt; twice daily</td>
<td>7 Years of follow-up</td>
<td>No beneficial CHD effects attributable to Ca/D</td>
<td>Generally healthy postmenopausal women</td>
<td>Hsia et al. (50)</td>
</tr>
<tr>
<td>DB, PC, RCT</td>
<td>Survival rate, biochemical variables, and cytokine profile</td>
<td>Same as primary outcomes</td>
<td>Participants (n = 123) received 2000 IU VitD&lt;sub&gt;3&lt;/sub&gt; and 500 mg Ca/D daily (D&lt;sub&gt;1&lt;/sub&gt; group), or placebo and 500 mg Ca/D daily (D&lt;sub&gt;2&lt;/sub&gt; group); 93 patients completed study</td>
<td>9-Month intervention, 15 months of follow-up</td>
<td>VitD&lt;sub&gt;3&lt;/sub&gt; reduced inflammatory milieu in CHF patients; interleukin-10 increased, but significant improvement in PTH and TNF; no difference in survival, however</td>
<td>CHF</td>
<td>Schleithoff et al. (56)</td>
</tr>
<tr>
<td>DB, PC, RCT</td>
<td>Fracture incidence and total mortality by cause</td>
<td>Same as primary outcomes; additional data assessing CVD</td>
<td>2686 Participants (2037 men and 649 women; age, 65–85 years) randomized to receive 100,000 IU supplemental VitD&lt;sub&gt;3&lt;/sub&gt; every 4 months</td>
<td>5 Years, Britain</td>
<td>No beneficial CVD effects attributable to VitD&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Patients recruited from the general community; excluded if history of renal stones, sarcoidosis, or malignancy</td>
<td>Trivedi et al. (46)</td>
</tr>
<tr>
<td>DB, PC, RCT</td>
<td>Fractures</td>
<td>Coronary mortality</td>
<td>327 Patients (57 men and 270 women &gt;65 years; mean, 79.5 years) received daily all possible combinations of 3 g calcium carbonate, 1000 IU VitD&lt;sub&gt;3&lt;/sub&gt;, 2.5 mg methandienone, and/or placebos</td>
<td>9 Months</td>
<td>Coronary mortality higher among those taking all 3 active substances; significant increase in coronary deaths, most significantly (P &lt; 0.001) in patients receiving VitD&lt;sub&gt;3&lt;/sub&gt; and methandienone</td>
<td></td>
<td>Inkovaara et al. (58)</td>
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*Note that because this article is not an all-inclusive systematic review, this table may not list all randomized controlled trials (RCTs) reporting on vitamin D supplementation and CHD risk factors as well as CHD events.

DB, double-blind; PC, placebo controlled; SBP, systolic blood pressure; DBP, diastolic blood pressure; VitD<sub>3</sub>, vitamin D<sub>3</sub>; DM, diabetes mellitus; FMV, flow-mediated vasodilation; PWV, pulse wave velocity; BP, blood pressure; CAC, coronary artery calcium; WHI, Women's Health Initiative; Ca/D, calcium and vitamin D; PTH, parathyroid hormone; TG, triglycerides; TNF, tumor necrosis factor; HTN, hypertension; CHF, congestive heart failure.
tically significant 7% decrease in all-cause mortality in participants who received vitamin D supplementation (61); however, a subsequent metaanalysis by Rejnmark et al. (62), which included 24 randomized trials of patients receiving vitamin D supplementation, with or without oral calcium, showed similar results, but with the following important variation. Although the patients who received supplements of vitamin D and calcium had a similar 7% reduction in all-cause mortality, as was seen in the earlier study (61), the patients who received vitamin D alone did not have a significant decrease in mortality. These results raise a number of questions, including the role calcium may have in any potential beneficial effect related to vitamin D supplementation. In considering a role of supplemental vitamin D, a recent systematic review and metaanalysis identified randomized trials published through August 2010 in which patients were randomized to vitamin D supplementation or to no treatment (63). The outcome measures of interest included mortality, cardiovascular events, and CHD risk factors. A total of 51 studies were eligible. Of note is that the analysis was unable to identify statistically significant differences in any of the outcomes, including myocardial infarction, stroke, all-cause mortality, or such CHD risk factors as lipid fractions, glucose, and systolic and diastolic blood pressures. Most of the trials tested relatively low vitamin D doses, however.

Although the association between 25(OH)D deficiency and obesity is not new (22), the results of a recent large metaanalysis suggest that a higher body mass index leads to lower plasma 25(OH)D concentrations, implying a causative relationship. In contrast, lower 25(OH)D concentrations did not appear to lead to a high body mass index (64). If these findings are confirmed, strategies to decrease obesity could also lower the prevalence of 25(OH)D deficiency.

Current Recommendations

The Institute of Medicine (IOM) (44) and the Agency for Healthcare Research and Quality (AHRQ) (43) reviewed the literature related to vitamin D and health-related outcomes. Both concluded that although sufficient evidence existed to support a role for calcium and vitamin D related to skeletal health, evidence supporting effects on non–bone-related health outcomes was lacking (44, 45). The IOM recommended a dietary allowance of 600 IU/day for individuals 1–70 years of age, with 800 IU/day recommended for individuals older than 70 years (18, 61). The IOM and AHRQ reports have generated some controversy, and some investigators have stated that higher dietary allowances should be encouraged. The International Osteoporosis Foundation (IOF), for instance, recommends 800–1000 IU/day as the mean supplemental dose to achieve an appropriate plasma 25OHD concentration (65). The IOF adds that individuals at higher risk may require doses of up to 2000 IU/day to reach an appropriate concentration (65). The National Osteoporosis Foundation (NOF) recommends 400–800 IU/day of oral vitamin D₃ for adults ≤50 years of age and 800–1000 IU/day for those ≥50 years (66). In line with the IOF guidelines, the NOF has clarified that some people may need higher oral vitamin D₃ doses, with 4000 IU/day noted as an upper limit of safety (66). Similarly, the Endocrine Society’s clinical guideline recommends at least 600 IU/day for adults 19–50 years of age and 600–800 IU/day for those older than 50 years. They also point out that doses of 1500–2000 IU/day may be required for all adults to consistently raise plasma 25OHD concentrations to >30 ng/mL (>75 nmol/L) (67). Although controversy surrounds the 25OHD concentration to use as a cutpoint for vitamin D deficiency or insufficiency, the IOM suggests that a serum 25OHD concentration of at least 20 ng/mL (50 nmol/L) will meet the vitamin D requirements for ≥97.5% of the US and Canadian populations (68). As we discussed above, results of the recent study assessing oral vitamin D₃ and blood pressure (60) likewise support a 25OHD concentration ≥20 ng/mL as being adequate; only individuals with 25OHD concentrations <20 ng/mL at baseline experienced a significant improvement in systolic blood pressure with vitamin D₃ supplementation (60).

Laboratory Testing

The current assays available for 25OHD testing include antibody-based methods and liquid chromatography. The methodologies for plasma 25OHD analyses, however, have changed greatly over the years. The early testing methods used competitive protein-binding assays, which were difficult to perform and lacked consistency. The introduction of early liquid chromatography techniques in the 1970s allowed, for the first time, the ability to detect 25OHD₂ and 25OHD₃ separately. As liquid chromatography assays were being refined in the 1980s, antibody-based assays were introduced. More recently, antibody assays have been modified to accommodate the automated multiwell plate format, which has made these assays quite popular. A notable drawback is the inability to distinguish between 25OHD₂ and 25OHD₃. It is also noteworthy that much of the past research related to plasma vitamin D concentrations were performed with antibody-based techniques. The variety of testing methods and questions about the reliability of testing also add to the complexity of interpreting previous research. Most recently, the liquid chromatography method has ad-
vanced substantially with the incorporation of a tandem mass spectrometer—the liquid chromatography–tandem mass spectrometry (LC-MS/MS) technique. This improvement produces data with very high specificity and sensitivity, along with outstanding reproducibility (44).

Because a majority of the previous data were produced with the antibody-based assays, it is important to point out the concern about inconsistencies between testing methods. Research that has incorporated inter-laboratory comparisons suggests a high and concerning degree of variation (44). These results have led to external quality-assurance programs, including NIST reference standards (69, 70), which use a “validated” LC-MS/MS technique for calibration (44). A Standard Reference Material and a calibration solution are now available through the NIST to help assure the accuracy and reliability of 25OHD measurements (44).

A study by Lia et al. compared DiaSorin LIAISON results with those for the LC-MS/MS assay (selected as the nominal gold standard) obtained with same participant samples and found a vitamin D deficiency rate that was 16% to 29% higher, respectively, based just on the laboratory test used. Furthermore, the DiaSorin RIA has been shown to produce lower serum 25OHD values than the LC-MS/MS method (71). The LC-MS/MS assay, which is considered inherently more accurate (71) with its high sensitivity, high specificity, and better reproducibility, tends to produce values that are slightly higher than obtained with the RIA techniques (71). Some have argued that LC-MS/MS results may need to be adjusted downward according to a mathematical formula, whereas others have suggested that DiaSorin RIA results may need to be corrected upward (71). Regardless, the value of using consistent and reliable laboratory testing is therefore of paramount importance, and LC-MS/MS is currently the preferred laboratory assay.

For quality assurance, it is critically important that test measurements be performed on standardized samples, with the inclusion of NIST samples and split-replicate samples for laboratory assessment. Such protocols allow laboratories to compute means, SDs, and CVs. Samples should be protected from direct sunlight to ensure the accuracy and precision of assays (30, 44, 72). The new NIST reference standards offer hope that 25OHD measurements can achieve improved accuracy and reliability and thus diminish the variation between tests and laboratory centers seen in the past (44).

Future Research Directions

Although our understanding of vitamin D deficiency and its ramifications is rapidly expanding, there is still much to learn. Several large-scale randomized trials of moderate to high dosages of vitamin D supplementation in cardiovascular disease (CVD) prevention are being conducted in the US and throughout the world. As one example, the Vitamin D and Omega-3 Trial (VITAL) (J.E.M., principal investigator) is a randomized, double-blind, placebo-controlled clinical trial of 20,000 US men and women older than 50 years. It tests 2000 IU/day of oral vitamin D3 and ω-3 fatty acid supplements in a 2 × 2 factorial design, with CVD and cancer as prespecified primary outcomes (73). Results are expected in 2017. While we await the results of VITAL and other ongoing randomized trials of vitamin D, it is also important to point out that many of the trials will analyze the effects of vitamin D3 supplementation in a general population of patients with and patients without 25OHD deficiency. There may be value, therefore, in stratifying by baseline concentrations or subsequently analyzing results in vitamin D–deficient patients (74).

Although data have suggested that the VDR concentration may be inversely correlated with the degree of coronary artery atherosclerosis (26), the link to or relationship with the plasma 25OHD3 concentration remains unclear. A recent study suggested that individuals with the highest plasma 25OHD3 concentration and the lowest VDR abundance had the greatest degree of coronary artery atherosclerosis (27), but these findings need corroboration. If confirmed, they suggest a therapeutic-window phenomenon, that high 25OHD3 concentrations may be detrimental above an upper threshold (18). Data have also suggested a strong link between 25OHD3 deficiency and race/heritability (75–79). A recent study (76) indicated that individual differences in 25OHD concentrations have both genetic and environmental associations and that the relative contributions to CHD outcomes remains unclear. In this particular study, the variation attributable to genetics was predominantly demonstrated in the winter when ultraviolet exposure was minimal but was not apparent in the summer months, implying that environmental factors (mostly sun exposure) may compensate for vitamin D deficiency related to genetics (77).

As the review above indicates, we are clearly in need of well-designed and adequately powered prospective randomized trials with vitamin D supplementation and CHD or CHD biomarkers as primary outcomes. It will be important to determine whether supplementation makes a clinically meaningful difference for CHD and whether the baseline 25OHD concentration modifies the response. Such trials will help to elucidate whether low vitamin D concentrations represent a marker for other processes, are indicators of genetic predisposition to disease, or are causally re-
lated to risk. On the assumption that vitamin D supplementation truly is of value for preventing CVD, what is the optimal dose, and what role does calcium supplementation play in the equation? Furthermore, does a therapeutic-window phenomenon exist such that not only lower but also higher serum concentrations can be detrimental? In contrast, an examination of the completed randomized, prospective placebo-controlled trials (Table 1) shows that a major weakness of many of these trials is that they tested lower vitamin D doses than currently postulated to be of benefit for extraskelatal outcomes. What role does the VDR play, would a VDR agonist be of benefit, or are there ways to prevent VDR loss and hence delay the onset of coronary atherosclerosis? As we await the results of ongoing research, we eagerly await answers to these questions.

Conclusions

The IOM review suggested that higher plasma concentrations of vitamin D have not been shown to reduce chronic disease beyond the established bone health benefits, but it recommended that more targeted research continue to explore the role of vitamin D supplementation in preventing CVD and other chronic illnesses. Despite plausible biological mechanisms for a role of vitamin D in cardioprotection, a cause-and-effect relationship has not yet been established. Although observational studies point to a potential association, such data are hampered by potential confounding and selection factors. A correlation found in an observational study does not prove causality, and so the available randomized-trial data do not yet demonstrate a clear benefit. Therefore and in line with the recommendations from the IOM and the AHRQ, additional research is needed to advance our knowledge of this subject. While clinicians await the results of ongoing randomized trials, including VITAL and several other trials worldwide, they should be cautious to avoid not only overtreatment with high-dose vitamin D supplementation but also undertreatment until we know the true risks and benefits.

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