Clinical Chemistry 60:4
600–609 (2014)

Review

Vitamin D and Cardiovascular Disease: An Appraisal of the Evidence

Peter F. Schnatz[1,2,3,4] and JoAnn E. Manson[5*]

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, 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)[6] remains the leading cause of mortality in US men and women *(1, 2)*. 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 *(2–5)*. Few Americans achieve optimal control of these risk factors, however, and these traditional risk factors do not explain many CHD events *(6)*. 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 *(7)*. 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 *(8–10)*. Lower serum vitamin D concentrations have also been associated with increased risks of sudden cardiac death *(11)*, peripheral arterial disease *(12)*, and greater carotid intima–medial thickness *(13)*. 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

Departments of [1] Obstetrics and Gynecology and [2] Internal Medicine, The Reading Hospital and Medical Center, Reading, PA; Departments of [3] Obstetrics and Gynecology and [4] Internal Medicine, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA; [5] Department of Medicine, Division of Preventive Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, MA.

* Address correspondence to this author at: Brigham and Women's Hospital, 900 Commonwealth Ave., 3rd Floor, Boston, MA 02215. Fax 617-731-3843; e-mail jmanson@rics.bwh.harvard.edu.

Received June 19, 2013; accepted October 10, 2013.
Previously published online at DOI: 10.1373/clinchem.2013.211037

[6] 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 photoisomerized to vitamin D_3 (cholecalciferol) by sunlight and ultraviolet light. The other major source of vitamin D is intestinal absorption. Vitamin D_3 is then transported to the liver, where it is hydroxylated to 25-hydroxyvitamin D (25OHD), which comprises both $25OHD_2$ and $25OHD_3$. 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 antiinflammatory state via effects on interleukins, C-reactive protein, and antiinflammatory 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 out 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 prespecified 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 D_3 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 D_3 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 D_3 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 D_3 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 D_3 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 D_3 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 D_3 dose ($P = 0.04$). Although the investigators found no statistically significant effect of oral vitamin D_3 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 D_3 on systolic blood pressure, there appeared to be a threshold effect, with individuals receiving 2000 IU/day and 4000 IU/day of vitamin D_3 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 prespecified 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 1. Prospective randomized trials of vitamin D supplementation and CHD events or CVD risk factors.[a]

Design	Primary outcome	Study outcome	Summary of study, sample size, patient age, and route/dose of treatment	Length and follow-up	Outcomes related to CHD or CHD risk factors	Preexisting conditions or disease?	Reference
DB,[b] PC, RCT	Colorectal cancer prevention study	Change in SBP and DBP	Blacks (n = 283; 30–80 years; median, 51 years) randomized for 3 months to oral placebo, 1000, 2000, or 4000 IU/day $VitD_3$, followed by treatment for 3 more months	6 Months	A −1.4-mmHg change in SBP for each additional 1000 IU/day of $VitD_3$ (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)	Generally healthy patients. Patients with preexisting disorders of the parathyroid or calcium metabolism; patients with type I DM, sarcoidosis, malignancy, or thyroid disease excluded	Forman et al. *(60)*
DB, PC, RCT	Changes in brachial artery FMV, carotid–femoral PWV, and aortic augmentation index	Same as primary outcomes	Postmenopausal women [n = 114; mean (SD) age, 63.9 (3) years] with serum 25OHD concentrations >10 and <60 ng/mL; received 2500 IU $VitD_3$ or placebo daily	4 Months	VitD supplementation did not improve endothelial function, arterial stiffness, or inflammation	Generally healthy, community-dwelling, ambulatory women from Madison, WI; patients with CVD excluded	Gepner et al. *(51)*
DB, PC, RCT	Change in small LDL particle number	Other lipid fractions	VitD-insufficient (25OHD ≤20 ng/mL) male and female adults (n = 151); received 50 000 IU $VitD_3$ weekly	8 Weeks	VitD repletion failed to improve lipid profile	Increased risk for CVD (with at least 1 of numerous significant CVD risk factors)	Ponda et al. *(48)*
DB, PC, RCT	BP, FMV of the brachial artery, cholesterol, and other markers of vascular health	Same as primary outcomes	Patients (n = 58; mean age, 67 years) received 100 000 IU oral $VitD_2$ or placebo at baseline	16 Weeks of follow-up	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	History of stroke with baseline 25OHD concentrations <75 nmol/L	Witham et al. *(52)*
DB, PC, RCT	Hip fractures	CAC score	Ca/D trial (1) nested within WHI hormonal trial (2) (estrogen among women who underwent hysterectomy). Women (n = 754; age, 50–59 years) received daily calcium carbonate (1000 mg elemental calcium) and $VitD_3$ (400 IU)	7 Years	Treatment and placebo groups were not different in CAC plaque burden measured at end of trial	Generally healthy postmenopausal women	Manson et al. *(53)*
DB, PC, RCT	Fasting serum lipids, BP, and oral glucose tolerance test	Same as primary outcomes	Obese or overweight patients (n = 438; 21–70 years) received $VitD_2$ (40 000 or 20 000 IU/week) or placebo; all received 500 mg calcium daily; 330 patients completed study	1 Year	No significant effect of VitD on glucose tolerance, BP, or serum lipids	Overweight or obese patients	Jorde et al. *(56)*
PC, RCT	Glycemic control in patients with type 2 DM	Same as primary outcomes	Participants (n = 36) received $VitD_3$ (40 000 IU/week) or placebo	6 Months	No significant effect of VitD on glucose metabolism	Type 2 DM treated with metformin and bedtime insulin	Jorde et al. *(54)*
DB, PC, RCT	Weight loss and traditional + nontraditional CVD risk markers	Same as primary outcomes (PTH, TG, and inflammatory markers)	Participants (n = 200; mean baseline 25OHD, 30 nmol/L) received 3320 IU/day VitD or placebo while participating in weight-reduction program	12 Months	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	Overweight	Zittermann et al. *(55)*

Continued on page 604

Table 1. Prospective randomized trials of vitamin D supplementation and CHD events or CVD risk factors.[a] *(Continued from page 603)*

Design	Primary outcome	Study outcome	Summary of study, sample size, patient age, and route/dose of treatment	Length and follow-up	Outcomes related to CHD or CHD risk factors	Preexisting conditions or disease?	Reference
DB, PC, RCT	Hip fractures	Incident DM	WHI Ca/D trial of postmenopausal women (n = 36 282; age, 50–79 years); received 1000 mg elemental calcium + 400 IU $VitD_3$ daily	7 Years of follow-up	No beneficial effects in reducing DM incidence or metabolic syndrome	Generally healthy postmenopausal women	DeBoer et al. *(47)*
DB, PC, RCT	Hip fractures	Change in BP and development of HTN	WHI Ca/D trial of postmenopausal women (n = 36 282); received 1000 mg elemental calcium + 400 IU $VitD_3$ daily	7 Years of follow-up	No significant beneficial effect on BP or prevention of incident HTN	Generally healthy postmenopausal women	Margolis et al. *(49)*
DB, PC, RCT	Hip fractures	Risk of CHD	WHI Ca/D trial of postmenopausal women (n = 36 282; age, 50–79 years); received 500 mg calcium carbonate + 200 IU $VitD_3$ twice daily	7 Years of follow-up	No beneficial CHD effects attributable to Ca/D	Generally healthy postmenopausal women	Hsia et al. *(50)*
DB, PC, RCT	Survival rate, biochemical variables, and cytokine profile	Same as primary outcomes	Participants (n = 123) received 2000 IU $VitD_3$ and 500 mg Ca/D daily (D+ group), or placebo and 500 mg Ca/D daily (D− group); 93 patients completed study	9-Month intervention, 15 months of follow-up	$VitD_3$ reduced inflammatory milieu in CHF patients; interleukin-10 increased, but significant improvement in PTH and TNF; no difference in survival, however	CHF	Schleithoff et al. *(56)*
DB, PC, RCT	Fracture incidence and total mortality by cause	Same as primary outcome; additional data assessing CVD	2686 Participants (2037 men and 649 women; age, 65–85 years) randomized to receive 100 000 IU supplemental $VitD_3$ every 4 months	5 Years, Britain	No beneficial CVD effects attributable to VitD	Patients recruited from the general community; excluded if history of renal stones, sarcoidosis, or malignancy	Trivedi et al. *(46)*
DB, PC, RCT	Fractures	Coronary mortality	327 Patients (57 men and 270 women >65 years; mean, 79.5 years) received daily all possible combinations of 3 g calcium carbonate, 1000 IU $VitD_3$, 2.5 mg methandienone, and/or placebos	9 Months	Coronary mortality higher among those taking all 3 active substances; significant increase in coronary deaths, most significantly ($P < 0.001$) in patients receiving $VitD_3$ and methandienone		Inkovaara et al. *(58)*

[a] 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.
[b] DB, double-blind; PC, placebo controlled; SBP, systolic blood pressure; DBP, diastolic blood pressure; $VitD_3$, vitamin D_3; 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 D_3 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 D_3 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 D_3 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 D_3 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 $25OHD_2$ and $25OHD_3$ 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 $25OHD_2$ and $25OHD_3$. 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 interlaboratory 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. Manson, 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 D_3 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 D_3 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 $25OHD_3$ concentration remains unclear. A recent study suggested that individuals with the highest plasma $25OHD_3$ 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 $25OHD_3$ concentrations may be detrimental above an upper threshold *(18)*. Data have also suggested a strong link between $25OHD_3$ 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 extraskeletal 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.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: J.E. Manson, National Institutes of Health (CA138962) for VITamin D and OmegA-3 TriaL (VITAL), a randomized trial of vitamin D and omega-3 supplementation in preventing cancer and cardiovascular disease.
Expert Testimony: None declared.
Patents: None declared.

References

1. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. AHA statistical update: heart disease and stroke statistics—2011 update; a report from the American Heart Association. Circulation 2011;123:e18–209.
2. Mosca L, Barrett-Connor E, Kass Wenger N. Sex/gender differences in cardiovascular disease prevention: what a difference a decade makes. Circulation 2011;124:2145–54.
3. Lloyd-Jones DM, Larson MG, Beiser A, Levey D. Lifetime risk of developing coronary heart disease. Lancet 1999;353:89–92.
4. Mosca L, Banka CL, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, et al. Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update. Circulation 2007;115:1481–501.
5. Mosca L, Grundy SM, Judelson D, King K, Limacher M, Oparil S, et al. AHA/ACC scientific statement: consensus panel statement. Guide to preventative cardiology for women. American Heart Association/American College of Cardiology. J Am Coll Cardiol 1999;33:1751–5.
6. Kones R. Primary prevention of coronary heart disease: integration of new data, evolving views, revised goals, and role of rosuvastatin in management. A comprehensive survey. Drug Des Devel Ther 2011;5:325–80.
7. Grimes DS, Hindle E, Dyer T. Sunlight, cholesterol and coronary heart disease. Q J Med 1996;89:579–89.
8. Rostand SG. Ultraviolet light may contribute to geographic and racial blood pressure differences. Hypertension 1997;30:150–6.
9. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-Hydroxyvitamin D and risk of myocardial infarction in men. Arch Intern Med 2008;168:1174–80.
10. Forman JP, Giovannucci E, Homes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension 2007;49:1063–9.
11. Pilz S, Marz W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, et al. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. J Clin Endocrinol Metab 2008;93:3927–35.
12. Melamed ML, Munter P, Michos ED, Uribarri J, Weber C, Sharma J, Raggi P. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease. Atheroscler Thromb Vasc Biol 2008;28:1179–85.
13. Targher G, Bertolini L, Padvoani R, Targher G, Bertolini L, Padovani R, et al. Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. Clin Endocrinol 2006;65:593–7.
14. Tsai KS, Wahner HW, Offord KP, Melton LJ, Kumar R, Riggs BL. Effect of aging vitamin D stores and bone density in women. Calcif Tissue Int 1987;40:241–3.
15. Henry HL, Norman AW. Studies on calciferol metabolism. IX. Renal 25-hydroxy-vitamin D3–1 hydroxylase. Involvement of cytochrome P-450 and other properties. J Biol Chem 1974;249:7529–35.
16. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–81.
17. Bouillon R, Carmeliet G, Verlinden L, Van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008;29:726–76.
18. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008;117:503–11.
19. Manson JE, Bassuk SS. Vitamin D and cardiovascular disease. Menopause Manag 2009;18:28–31.
20. Zella LA, Shevde NK, Hollis BW, Cooke NE, Pike JW. Vitamin D-binding protein influences total

circulating levels of 1,25-dihydroxyvitamin D_3 but does not directly modulate the bioactive levels of the hormone in vivo. Endocrinology 2008;149: 3656–67.
21. Paula B, Ghetu MV, Langan R. Recognition and management of vitamin D deficiency. Am Fam Physician 2009;80:841–6.
22. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690–3.
23. Scragg R. Seasonality of cardiovascular disease mortality and the possible protective effect of ultraviolet radiation. Int J Epidemiol 1981;10: 337–41.
24. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. Am J Med 2002;112:659–62.
25. Merke J, Milde P, Lewicka S, Hugel U, Klaus G, Mangelsdorf DJ, et al. Identification and regulation of 1,25-dihydroxyvitamin D3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D3. Studies in cultured bovine aortic endothelial cells and human dermal capillaries. J Clin Invest 1989; 83:1903–15.
26. Schnatz PF, Nudy M, O'Sullivan DM, Jiang X, Cline JM, Kaplan JR, et al. The quantification of vitamin D receptors in coronary arteries and their association with atherosclerosis. Maturitas 2012; 73:143–7.
27. Schnatz PF, Nudy M, O'Sullivan DM, Jiang X, Cline JM, Kaplan JR, et al. Coronary artery vitamin D receptor expression and plasma concentrations of vitamin D: their association with atherosclerosis. Menopause 2012;19:967–73.
28. Wu-Wong JR, Nakane M, Ma J, Ruan X, Kroeger PE. Effects of vitamin D analogs on gene expression profiling in human coronary artery smooth muscle cells. Atherosclerosis 2006;186:20–8.
29. Rostkowska-Nadolska B, Sliupkas-Dyrda E, Potyka J, Kusmierz D, Fraczek M, Krecicki T, et al. Vitamin D derivatives: calcitriol and tacalcitol inhibits interleukin-6 and interleukin-8 expression in human nasal polyp fibroblast cultures. Adv Med Sci 2010;55:86–92.
30. Schnatz PF, Vila-Wright S, Jiang X, Register TC, Kaplan JR, Clarkson TB, Appt SE. The association between plasma $25OHD_3$ concentrations, C-reactive protein levels, and coronary artery atherosclerosis in postmenopausal monkeys. Menopause 2012;19:1074–80.
31. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. J Nutr 1998;128:68–72.
32. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. J Steroid Biochem Mol Biol 2004;89:387–92.
33. Gedik O, Akalin S. Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man. Diabetologia 1986;29:142–5.
34. Muscogiuri G, Sorice GP, Ajjan R, Mezza T, Pilz S, Prioletta A, et al. Can vitamin D deficiency cause diabetes and cardiovascular diseases? Present evidence and future perspectives. Nutr Metab Cardiovasc Dis 2012;22:81–7.
35. Schnatz PF, Nudy M, O'Sullivan DM, Ethun K, Appt SE, Clarkson TB. Identification of a mechanism for increased cardiovascular risk among individuals with low vitamin D concentrations. Menopause 2011;18:994–1000.
36. Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA. Vitamin D: intake is inversely associated with rheumatoid arthritis. Arthritis Rheum 2004;50:72–7.
37. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child 2008;93:512–7.
38. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 2006;29:650–6.
39. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006;296: 2832–8.
40. Wang L, Song Y, Manson JE, Pilz S, März W, Michaëlsson K, et al. Circulating 25-hydroxyvitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. Circ Cardiovasc Qual Outcomes 2012;5:819–29.
41. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 1985;76:1536–8.
42. Reid D, Toole BJ, Knox S, Talwar D, Harten J, O'Reilly DJS, et al. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. Am J Clin Nutr 2011;93:1006–11.
43. Chung M, Balk EM, Brendel M, Ip S, Lau J, Lee J, et al. Vitamin D and calcium: a systematic review of health outcomes. Evidence report no. 183 (prepared by the Tufts Evidence-based Practice Center under contract no. HHSA 290–2007-10055-I). AHRQ publication no. 09-E015. Rockville, MD: Agency for Healthcare Research and Quality; 2009.
44. IOM. Dietary reference intakes for calcium and vitamin D. Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. Washington (DC): National Academies Press; 2011. http://books.nap.edu/openbook.php?record_id=13050&page=R1 (Accessed February 2014).
45. IOM. Dietary reference intakes for calcium and vitamin D [Report Brief]. 2010 Nov. http://www.iom.edu/~/media/Files/Report%20Files/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/Vitamin%20D%20and%20Calcium%202010%20Report%20Brief.pdf (Accessed February 2014).
46. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D_3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 2003;326:469–75.
47. De Boer IH, Tinker LF, Connelly S, Curb JD, Howard BV, Kestenbaum B, et al. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. Diabetes Care 2008;31:701–7.
48. Ponda MP, Dowd K, Finkielstein D, Holt PR, Breslow JL. The short-term effects of vitamin D repletion on cholesterol: a randomized, placebo-controlled trial. Arterioscler Thromb Vasc Biol 2012;32:2510–5.
49. Margolis KL, Ray RM, Van Horn L, Manson JE, Allison MA, Black HR, et al., for the Women's Health Initiative Investigators. Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. Hypertension 2008;52:847–55.
50. Hsai J, Heiss G, Ren H, Allison M, Dolan NC, Greenland P, et al. Calcium/vitamin D supplementation and cardiovascular events. Circulation 2007;115:846–54.
51. Gepner AD, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH. A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. PLoS One 2012;7:e36617.
52. Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. Nutr Metab Cardiovasc Dis 2012;22:864–70.
53. Manson JE, Allison MA, Carr JJ, Langer RD, Cochrane BB, Hendrix SL, et al. Calcium/vitamin D supplementation and coronary artery calcification in the Women's Health Initiative. Menopause 2010;17:683–91.
54. Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. Eur J Nutr 2009;48:349–54.
55. Zittermann A, Frisch S, Berthold HK, Götting C, Kuhn J, Kleesiek K, et al. Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. Am J Clin Nutr 2009 89:1321–7.
56. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006;83:754–9.
57. Jorde R, Sneve M, Torjesen P, Figenschau Y. No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D3 for 1 year. J Intern Med 2010;267:462–72.
58. Inkovaara J, Gothoni G, Halttula R, Heikinheimo R, Tokola O. Calcium, vitamin D and anabolic steroid in treatment of aged bones: double-blind placebo-controlled long-term clinical trial. Age Ageing 1983;12:124–30.
59. Prentice RL, Pettinger MB, Jackson RD, Wactawski-Wende J, LaCroix AZ, Anderson GL, et al. Health risks and benefits from calcium and vitamin D supplementation: Women's Health Initiative clinical trial and cohort study. Osteoporos Int 2013;24:567–80.
60. Forman JP, Scott JB, Ng K, Drake BF, Suarez EG, Hayden DL, et al. Effect of vitamin D supplementation on blood pressure in blacks. Hypertension 2013;61:779–85.
61. Autier P, Gandini S. Vitamin D supplementation and total mortality. Arch Intern Med 2007;167: 1730–7.
62. Rejnmark L, Avenell A, Masud T, Anderson F, Meyer HE, Sanders KM, et al. Vitamin D with calcium reduces mortality: patient level pooled analysis of 70,528 patients from eight major vitamin D trials. J Clin Endocrinol Metab 2012;97: 2670–81.
63. Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, Almandoz JP, et al. Vitamin

D and cardiovascular outcomes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2011;96:1931–42.

64. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med 2013;10:e1001383.
65. International Osteoporosis Foundation (IOF). IOF position statement: vitamin D recommendations for older adults - position paper. http://www.natap.org/2010/HIV/072310_01.htm (Accessed October 2013).
66. National Osteoporosis Foundation. Calcium and vitamin D: what you need to know. http://www.nof.org/aboutosteoporosis/prevention/vitamind (Accessed October 2013).
67. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
68. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53–8.
69. National Institute of Standards and Technology (NIST). http://www.nist.gov/index.html (Accessed August 2013).
70. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM. Vitamin D status as an international issue: national surveys and the problem of standardization. Scand J Clin Lab Invest Suppl 2012;243:32–40.
71. Graham DC. Accuracy of 25-hydroxyvitamin D assays: confronting the issues. Curr Drug Targets 2011;12:19–28.
72. Lewis JG, Elder PA. Serum 25-OH vitamin D_2 and D_3 are stable under exaggerated conditions. Clin Chem 2008;54:1931–2.
73. Manson JE, Bassuk SS, Li I, Cook NR, Albert MA, Gordon D, et al. The VITamin D and OmegA-3 TriaL (VITAL): rationale and design of a large randomized control trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. Contemp Clin Trials 2012;33:159–71.
74. Pilz S, Rutters F, Dekker JM. Disease prevention: vitamin D trials. Science 2012;338:883.
75. Ginde AA, Liu MC, Camargo CA. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. Arch Intern Med 2009;169:626–32.
76. Hunter D, De Lange M, Snieder H, MacGregor AJ, Swaminathan R, Thakker RV, Spector TD. Genetic contribution to bone metabolism, calcium excretion, and vitamin D and parathyroid hormone regulation. J Bone Miner Res 2001;16:371–8.
77. Karohl C, Su S, Kumari M, Tangpricha V, Veledar E, Vaccarino V, Raggi P. Heritability and seasonal variability of vitamin D concentrations in male twins. Am J Clin Nutr 2010;92:1393–8.
78. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376:180–8.
79. Gutiérrez OM, Farwell WR, Kermah D, Taylor EN. Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. Osteoporos Int 2011;22:1745–53.