Published in final edited form as:
Arch Intern Med. 2008 June 9; 168(11): 1174–1180. doi:10.1001/archinte.168.11.1174.

A Prospective Study of 25-Hydroxy-Vitamin D and Risk of Myocardial Infarction in Men

Edward Giovannucci, MD, ScD[1,2,3], **Yan Liu, MS**[1], **Bruce W. Hollis, MD, PhD**[4], and **Eric B. Rimm, ScD**[2,3]

[1]Department of Nutrition, Harvard School of Public Health, Boston MA 02115 USA

[2]Department of Epidemiology, Harvard School of Public Health, Boston MA 02115 USA

[3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston MA 02115 USA

[4]Department of Pediatrics, Medical University of South Carolina, Charleston, SC 29425 USA

Abstract

Background—Vitamin D deficiency may be involved in the development of atherosclerosis and coronary heart disease (CHD) in humans. We assessed prospectively whether plasma 25(OH)vitamin D (25(OH)D) concentrations are associated with risk of CHD.

Methods—A nested case-control study was conducted among 18,225 male participants of the Health Professionals Follow-up Study aged 40 to 75 years who were free of diagnosed cardiovascular disease at the time of blood draw (1993–1995). During 10 years of follow-up through January 31, 2004, 454 men developed MI (nonfatal myocardial infarction) or fatal CHD. Using risk set sampling, controls were selected in a 2:1 ratio matched for age, date of blood draw, and smoking status (n=900).

Results—After adjustment for matched variables, men deficient (≤15 ng/mL) in 25(OH)D were at increased risk of MI compared to those considered to be sufficient (≥30 ng/mL) in 25(OH)D (relative risk (RR)=2.42; 95% confidence interval (CI), 1.53–3.84; *P* for trend <.001). After additional adjustment for family history of MI, body mass index, alcohol consumption, physical activity, history of diabetes and hypertension, ethnicity, region, marine omega-3 intake, low- and high-density lipoprotein cholesterol and triglyceride levels, this relationship remained significant (RR=2.09; 95% CI, 1.24–3.54; *P* for trend=0.02). Even men with intermediate values for 25(OH)D were at elevated risk relative to those with sufficient 25(OH)D (22.6–29.9 ng/mL: RR=1.60; 95% CI, 1.10–2.32; 15.0–22.5 ng/mL: RR=1.43; 95% CI, 0.96–2.13).

Conclusions—This study provides evidence that optimal levels of 25(OH)D should be at least 30 ng/mL to lower risk of MI.

Correspondence / Reprints to: Edward Giovannucci, MD, ScD, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, Tel: 617-432-4648; Fax: 617-432-2435; egiovann@hsph.harvard.edu.

Author Contributions: Dr. Giovannucci had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Giovannucci, Rimm. *Acquisition of data:* Giovannucci, Liu, Rimm. *Analysis and interpretation of data:* Giovannucci, Liu, Hollis, Rimm. *Drafting of the manuscript:* Giovannucci. *Critical revision of the manuscript for important intellectual content:* Giovannucci, Liu, Hollis, Rimm. *Statistical analysis:* Giovannucci, Liu. *Obtained funding:* Giovannucci, Rimm. *Administrative, technical, and material support:* Giovannucci. *Study supervision:* Giovannucci.

Financial Disclosures: Dr. Hollis is a consultant for Diasorin Corp. No other disclosures are reported.

INTRODUCTION

A number of observations are not easily explained by known cardiovascular disease (CVD) risk factors. In most populations studied, the rate of CVD death is elevated at higher latitudes, increases during the winter months, and is lower at high altitudes. As has been noted previously by others, this pattern is consistent with an adverse effect of hypovitaminosis D, which is more prevalent in higher latitudes, during the winter, and at lower altitudes.[1] Alternative explanations for these observations are possible, but a number of plausible biologic mechanisms support a role of vitamin D. The vitamin D axis influences vascular smooth cell proliferation, inflammation, vascular calcification, and the renin-angiotensin system (RAS) and blood pressure, [1]all which influence risk of CVD and of myocardial infarction (MI).

Despite these suggestive ecologic data and plausible mechanisms, data directly linking vitamin D levels to risk of MI are sparse. A Danish study[2] examined 25(OH)D levels measured in 128 patients admitted with ischemic heart disease (75 with angina pectoris and 53 with acute MI) and 409 controls and found that 25(OH)D levels were significantly lower in those with angina (23.5 ng/ml) or MI (24.0 ng/ml) than in the controls (28.8 ng/ml). In a New Zealand case-control study of 179 MI cases,[3] cases had a lower mean 25(OH)D level (P=0.017), which was more pronounced in the winter-spring (P=0.029) than in the summer-autumn (P=0.21). The relative risk (RR) of MI decreased across increasing quartiles of 25(OH)D: <10 ng/mL: RR =1 (reference); 10–13 ng/mL: RR=0.56 (95% CI, 0.32–1.03); 13.1–16.8 ng/mL: RR=0.33 (95% CI, 0.17–0.64); >16.8 ng/mL: RR=0.30 (95% CI, 0.15–0.61). Multivariate analyses of major CVD risk factors did not appreciably alter the results. A small nested-case control study of MI based in the Tromso Heart Study (northern Norway) based on only 30 cases and 60 matched controls found a slightly non-significant lower 25(OH)D level in cases (23.6 ng/mL) compared to controls (25.4 ng/mL).[4]

Because hypovitaminosis D is highly prevalent and easily correctable,[5] establishing the relationship between vitamin D and risk of MI is important. Thus, we examined prospectively 25(OH)D concentrations in relation to risk of MI in the Health Professionals Follow-Up Study (HPFS), a large cohort of U.S. men.

METHODS

Study Population

The HPFS is a prospective cohort investigation among 51 529 US male health care professionals aged 40 to 75 years at baseline in 1986. This cohort was designed primarily to evaluate associations between diet and chronic disease incidence.[6] We assessed information about health and disease every 2 years by a self-administered questionnaire, and diet every 4 years by a self-administered food frequency questionnaire.[7] Between 1993 and 1995, a blood sample was requested from all surviving cohort participants, of which 18 225 provided samples. The respondents were somewhat younger but were otherwise similar to non-participants. Based on the cohort who provided samples, and after exclusion of participants with a history of CVD prior to 1994, we identified 454 participants with incident nonfatal MI or fatal coronary heart disease (CHD) between date of blood draw and January 31, 2004. We were able to assess disease status through January 31, 2004 for 97.3% of the men. Controls were randomly selected from participants with a blood sample and who were alive and who did not have history of CVD at the time of case ascertainment. For the controls, we used a 2:1 ratio and matched for age, month and year of blood draw, and smoking status (risk set sampling).[8] Our analysis includes 8 participants who were selected as a control and subsequently had an event later during follow-up, leaving 900 controls available for analysis. No control was selected twice during the random selection process.

All participants gave written informed consent, and the Harvard School of Public Health Human Subjects Committee Review Board approved the study protocol.

Assessment of Nonfatal MI and Fatal CHD

Study physicians reviewed the medical records of all participants for whom nonfatal MI or fatal CHD was reported during follow-up. The reviewers were blinded to participants' exposure status. Each questionnaire that is mailed biennially to HPFS participants contains a question on whether the man has had “professionally diagnosed … myocardial infarction (heart attack)” in the preceding 2 years. Myocardial infarction (MI) was confirmed if it met the World Health Organization's criteria, which include symptoms plus either diagnostic electrocardiographic changes or elevated levels of cardiac enzymes.[9] For about 70% of the men who self-reported MI, we confirmed the diagnosis using these methods. Reasons for nonconfirmation of MI were either that no further information was available, typically because the participant did not consent or the hospital did not send the hospital records, or that a reported case was rejected based on the medical record information received. We excluded nonconfirmed participants from the control selection process. Deaths were identified from state vital statistics records and the National Death Index or reported by next of kin or by the postal system. Fatal CHD was considered to have occurred if there was fatal MI confirmed by hospital records or on autopsy or if CHD was listed as the cause of death on the death certificate, if it was the underlying and most plausible cause, and if evidence of previous CHD was available. In our analysis, 352 participants had nonfatal MI and 102 had fatal CHD as the qualifying event.

Assessment of Medical History, Anthropometric Data, and Diet and Lifestyle Factors

For the analysis, anthropometric data, lifestyle factors, and diet were based on the 1994 questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Nutrient intake was computed based on a validated semiquantitative food frequency questionnaire, which inquires about average food intake during the past year, using composition values from the US Department of Agriculture sources,[10] supplemented with other data. Physical activity was expressed as metabolic equivalent task (MET)–hours based on self-reported types and durations of activities over the previous year.[11] One MET-hour is equivalent to energy expenditure while sitting quietly for 1 hour. We derived medical history information from the questionnaires completed between 1986 and 1994. We have previously reported on the validity and reproducibility of the collected data and measurements.[7, 12–16]

Measurement of Biochemical Variables

We collected blood samples in three 10-mL liquid EDTA blood tubes, placed on ice packs, stored in Styrofoam containers, and returned to our laboratory via overnight courier. More than 95% arrived within 24 hours of blood draw. The blood samples were then centrifuged and aliquoted for storage in the vapor phase of liquid nitrogen freezers (−130°C or colder). Fewer than 15% of the samples were slightly hemolyzed and very few were moderately hemolyzed (<3%), lipemic (<1%), or not cooled on arrival (<0.5%).

Plasma 25(OH)D was determined by radioimmunoassay (RIA) in the laboratory of Dr. Bruce Hollis as previously described.[17] The coefficient of variation for 25(OH)D was 11.5%. In a sub-cohort of the 144 men who provided baseline blood samples in 1993–94 and again in 1997 (mean of 3.03 0.46 years apart), the Pearson correlation coefficient for 25(OH)D was 0.70 (P<0.0001).[18] LDL cholesterol was measured by a homogeneous direct method from Genzyme Corp, Cambridge, MA,[19] HDL cholesterol using a direct enzymatic colorimetric assay,[20] and triglycerides enzymatically with correction for endogenous

glycerol.[21] The assays used for lipoprotein and lipid analysis are approved by the US Food and Drug Administration for clinical use, and coefficients of variation were less than 6%.

Statistical Analyses

Plasma 25(OH)D levels were categorized into four categories based on common definitions of "deficient" (≤15 ng/mL), "insufficient" (15.1–29.9 ng/mL) and "sufficient" 25(OH)D (≥30 ng/mL) levels.[22] We further dichotomized the "insufficient" range at its midpoint into 15.1–22.5 ng/mL and 22.6 to 29.9 ng/mL. For our main analysis to investigate the association between 25(OH)D concentrations and incidence of nonfatal MI or fatal CHD (events), we used conditional logistic regression. For tests for trend, we modeled the median value of the four categories as continuous variables in the regression model. In our multivariable model, we further adjusted for family history of MI before age 60 years (yes/no), alcohol intake (nondrinker; 0.1–4.9, 5.0–14.9, 15.0–29.9, or 30.0 g/d; or missing), body mass index (continuous), physical activity (quintiles), and history of diabetes (yes/no) and hypertension (yes/no), ethnicity (Caucasian/other), region (Northeast/mid-Atlantic, Midwest, South) marine omega-3 intake (quintiles), low- and high-density lipoprotein cholesterol and triglyceride levels (quintiles) at baseline. Because of the design of our study, the odds ratio derived from logistic regression directly estimates the incidence rate (hazard) ratio and, therefore, the relative risk.[8, 23] We assessed the goodness of fit of the models using the method described by Hosmer and Lemeshow,[24] which did not show any significant lack of fit.

For stratified analysis, we used unconditional logistic regression adjusted for matched variables (age <50, 50–54, 55–59, 60–64, or 65 years; smoking status never, past, or current; and month of blood draw in 5 categories). For the main analysis, unconditional logistic regression with adjustment for matched variables yielded similar results as conditional logistic regression. Tests for interaction were based on the Wald test for the interaction term (variable times 25(OH)D) with both the variable and vitamin D in the model as continuous variables. All P values presented are 2-tailed; $P<.05$ was considered statistically significant. All analyses were performed using SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Selected characteristics and biomarker levels of cases and controls are presented in Table 1. As expected, cases had a higher BMI, lower level of physical activity and less alcohol intake, and were more likely to have a family history of MI before age 60 years and history of diabetes and hypertension. In addition, cases had higher levels of total and LDL cholesterol and of triglycerides, but lower levels of HDL cholesterol. Plasma 25(OH)D levels were significantly lower ($P=0.002$) in cases (23.0 ng/mL) compared to controls (24.5 ng/mL).

We next measured levels or percentages of selected risk factors across 25(OH)D concentrations in controls, shown in Table 2. Men with lower 25(OH)D levels were more likely to be current smokers, less physically active and heavier, and less likely to be white and to have a parental history of MI. They were also less likely to take a multivitamin supplement, drank less alcohol, and were more likely to live in northern states. Men with lower 25(OH)D levels also had a suggestive but not statistically significant higher prevalence of diabetes and hypertension, and significantly higher levels of triglycerides and lower levels of HDL cholesterol.

Table 3 presents RRs of MI (incident nonfatal MI plus fatal CHD) over 10 years of follow-up across the categories of plasma 25(OH)D. After adjustment for matching factors, men with deficient levels of 25(OH)D had a significantly elevated risk of MI (RR=2.42; 95% CI,

1.53–3.84; *P* for trend=0.0006). In the multivariable model without plasma lipids, the RR was somewhat attenuated but remain statistically significant (RR=2.01; 95% CI, 1.22–3.30; *P* for trend=0.02). After further adjustment for plasma lipids, the RR=2.09 (95% CI, 1.24–3.54; *P* for trend=0.02). The results did not change appreciably after excluding the first two years of follow-up (RR=1.97; 95% CI, 1.10–3.51; *P* for trend=0.05 across levels of 25(OH)D).

In alternative analyses, we examined season-specific quintiles of 25(OH)D in relation to MI risk. For those in the bottom versus top quintiles, the multivariable RR=1.94 (95% CI, 1.23–3.06). Compared with men with adequate 25(OH)D, those with insufficient values of 25(OH)D were associated with approximately a 50% increase in risk. In the full multivariable model, the β coefficient for a 1 ng/mL increment in 25(OH)D was −0.0214 (SE=0.00987), indicating the risk of MI decreased by 2.1% per a 1 ng/mL increment in 25(OH)D. We did not have adequate power to test for differences between non-fatal (n=352) and fatal (n=102) cases, but the β coefficient for MI risk per 1 ng/mL increase of 25(OH)D suggested a stronger association for fatal (4.3% decrease in risk) compared to non-fatal MI (1.8% decrease in risk). Further, the multivariable RR for deficient vs. sufficient 25(OH)D did not change appreciably when we included C-reactive protein (RR=2.04; 95% CI, 1.20–3.46) or included estimated glomerular filtration rate based on measured plasma creatinine, age, sex, and race (RR=2.09; 95% CI, 1.23–3.54) in the full multivariable model.

We tested for interaction across various factors to examine whether the association between 25(OH)D level and MI risk varied. We did not find statistical evidence of interaction by age (*P* for interaction=0.85), high blood pressure (*P*=0.84), BMI (*P*=0.87), aspirin use (*P*=0.92), physical activity (*P*=0.21), alcohol (*P*=0.53), LDL cholesterol (*P*=0.33), or triglycerides (*P*=0.73). Suggestive evidence was observed for an interaction by HDL cholesterol level, with a suggestive stronger inverse association with 25(OH)D among men with a higher HDL cholesterol level (*P*=0.06). Excluding men taking cholesterol-lowering drugs at baseline, the multivariable RR for deficient versus sufficient 25(OH)D was 2.30; 95% CI, 1.33–3.97. Because our subset included only a limited number of participants with diabetes at baseline, we were not able to calculate effect estimates within this subgroup; however, exclusion of these participants did not substantively alter the results as presented in Table 3. Similarly, exclusion of participants with a parental history of MI before age 60 years or current smokers did not affect the results.

DISCUSSION

In this cohort study, men with circulating 25(OH)D levels of at least 30 ng/mL had about half the risk of MI, independent of other CVD factors. The association was suggestively stronger for fatal CHD, but the number of cases was too small to make definitive conclusions. Although traditional CVD risk factors (e.g., lipids, hypertension, diabetes, smoking) remained strong risk factors in this population, vitamin D deficiency appeared to be an independent risk factor. Only 23% of the men in the HPFS had levels of 25(OH)D of at least 30 ng/mL. This percentage is typical of many populations, and the prevalence of deficiency is even higher in sub-populations, such as dark-skinned individuals and the elderly. Of note, individuals in sun-rich environments, where clothing or cultural practices do not appreciably limit vitamin D production, 25(OH)D levels of 54–90 ng/mL are attained,[25] but from our study we cannot evaluate whether levels above 35 ng/mL would be associated with an even greater MI risk reduction.

The prospective design, high follow-up rate, and use of a plasma marker largely precluded major sources of bias, such as recall bias or selection bias. We excluded men with diagnosed CVD at baseline, and furthermore, results were similar after excluding the first two years of

follow-up, arguing against reverse causation bias, which might occur if men predisposed to a MI stayed indoors and thereby avoided sun exposure. We controlled for and stratified by major covariates that could influence MI risk and influence 25(OH)D concentrations, including exercise, which increases sun exposure, BMI, region, race, multivitamin use and marine n-3s, as fatty fish is the only significant natural source of dietary vitamin D. We controlled for the major lipid risk factors of MI. In addition, we considered estimated glomerular filtration rate because circulating 25(OH)D levels could be lower in those with chronic kidney disease, which is a risk factor for CVD.[26] Controlling for estimated glomerular filtration rate did not change our results. Given the strength of the association that we observed between 25(OH)D level and MI risk, and the fact that controlling for these factors did not appreciably influence the magnitude of the association, substantial residual confounding by these factors is not likely, but cannot be ruled out.

Because 25(OH)D levels are largely influenced by sun exposure, it is plausible that some other consequence of sun exposure other than vitamin D production is responsible for the observed association with MI. Nonetheless, a large body of evidence supports mechanisms whereby vitamin D could influence CVD risk. Among the potentially relevant mechanisms, vitamin D influences vascular smooth cell proliferation, inflammation, vascular calcification and blood pressure through the renin-angiotensin system.[1]

The renin-angiotensin system (RAS) helps regulate blood pressure, electrolyte and volume homeostasis, and excessive RAS stimulation is associated with hypertension. Animal studies show that vitamin D is an important regulator of the RAS system, and that $1,25(OH)_2D$, the activated form of vitamin D, suppresses renin gene expression.[27] Disruption of the vitamin D receptor gene leads to elevated renin production, cardiac hypertrophy, and elevated blood pressure in mice.[28] In an RCT of either UV-B or UV-A administered through tanning booths, UV-B, which increased 25(OH)D by 162%, was effective in reducing 24-h ambulatory blood pressure (by −6/−6 mmHg; $P < 0.001$) whereas UV-A radiation did not influence 25(OH)D levels or blood pressure.[29] In another RCT of subjects with low vitamin D status (<20 ng/mL; mean approximately 10 ng/mL),[30] supplementation with 800 IU vitamin D, resulted in an increase in serum 25(OH)D of 72% ($P < 0.01$), a decrease in systolic blood pressure (SBP) of 9.3% ($P=0.02$) and a suggestive decrease of diastolic blood pressure of 8.5% ($P=0.10$). In the HPFS and Nurses' Health Study, during 4 years of follow-up, men and women who had plasma 25(OH)D levels <15 ng/mL were three times as likely to have a new diagnosis of hypertension within the next 4 years compared to those with 25(OH)D levels >30 ng/mL.[31] An inverse association between 25(OH)D levels and blood pressure was also found using NHANES III data.[32]

Calcification is a common feature of atherosclerosis, and nearly all angiographically significant lesions are calcified.[33] Calcification of coronary arteries has been associated with increased risk of MI[34] and poorer 5-year survival.[35] Atherosclerotic calcification is a process regulated in ways similar to skeletal osteogenesis.[36] A significant association exists between osteoporosis and vascular calcification, suggesting that osteoregulatory mechanisms related to bone development may also influence calcification in the vasculature. $1,25(OH)_2D$ levels have been shown to be inversely associated with vascular calcification,[36] suggesting that vitamin D may influence MI risk through its effects on vascular calcification.

Other mechanisms could account for or contribute to the association between 25(OH)D and MI risk. Vitamin D deficiency, possibly in combination with low calcium intake, has been associated with impaired fasting glucose and possibly risk of type 2 diabetes,[37–40] risk factors for CVD. Vitamin D deficiency has also been associated with a cytokine profile that favors greater inflammation (e.g. higher CRP, IL-6 and lower IL-10),[41–46] which could

predispose to heightened MI risk. Finally, seasonal respiratory infections, particularly influenza, have been proposed to account for the winter rise in mortality due to CVD,[47] and hypovitaminosis D could contribute to these infections.[48, 49]

Two case-control studies[2, 3] and a small prospective study[4] found that individuals with low 25(OH)D levels were at higher risk of ischemic heart disease. The strongest test of the hypothesis that vitamin D lowers MI risk would be from an RCT. Two RCTs reported on CVD. In a UK study of 2,686 men and women, the subjects were randomized to 830 IU vitamin D per day (administered as 100,000 IU oral vitamin D3 every 4 months) or placebo for 5 years. The in-study 25(OH)D levels were 29.7 ng/mL in the vitamin D group and 21.4 ng/mL in the placebo group. There was a non-significant decrease in CVD incidence (RR=0.90, 95%CI 0.77–1.06) and CVD mortality (RR=0.84, 95% CI 0.65–1.10) in the intervention group. Based on our study, a difference of 8.3 ng/mL of 25(OH)D would be associated with a RR of 0.92, which is compatible with their results. A recent meta-analysis of total mortality as a secondary endpoint of RCTs with varying levels of vitamin D versus placebo controls found a statistically significant 8% reduction in risk of total mortality in subjects that had received vitamin D.[50] Although the authors could not evaluate cause-specific mortality, the relatively immediate effect of a large enough magnitude to influence total mortality would suggest a benefit on CVD risk.

The largest RCT of vitamin D (and calcium) supplementation and CVD risk was from the Women's Health Initiative, in which 36, 282 postmenopausal women received either calcium (1000 mg daily) and vitamin D3 (400 IU daily) or placebo.[51] No reduction was observed in MI or CHD death (hazard ratio, 1.04; 95% CI, 0.92–1.18]. These results appear to be in contrast to our findings, suggesting two possible explanations. First, despite our efforts to exclude confounding, it is possible that uncontrolled or residual confounding explained our results. Alternatively, the range of vitamin D studied was much wider in the HPFS, which allowed us to detect an association. The difference between the medians of the top and bottom category, for which we observed a RR of about 2, was 23.5 ng/mL (35.5 – 12.0 ng/mL), and the calculated reduction in MI risk per increment of 1 ng/mL of 25(OH)D was 2%. In the WHI study, the influence of the treatment on the 25(OH)D levels was not reported, but based on the dose and compliance, is estimated to be only 2 ng/mL.[52] Based on our data, such an increment would be expected to have only a 4% reduction in risk. Of note, to increase 25(OH)D levels from 12 to 35.5 ng/mL would require approximately 3000 IU daily of vitamin D.[53] Although such intakes may seem high by current standards, an increasing body of evidence demonstrates no toxicity at intakes below 10,000 IU/day.[54] Because current sources of vitamin D provide much less (e.g. a glass of milk has about 100 IU), those who achieve such high levels naturally do so largely through sun exposure.

Vitamin D deficiency has been related to an increasing number of conditions[5] and to total mortality.[50] These results provide further support of an important role of vitamin D on MI risk. If this association is causal, which remains to be established, the amount of vitamin D required for optimal benefit may be much higher than would be provided by current recommendations (200–600 IU per day), especially in those with minimal sun exposure. Thus, our findings add further support that the current dietary requirements of vitamin D need to be increased to have an effect on circulating 25(OH)D substantially large enough for potential health benefits.[25]

Acknowledgments

Funding/Support: The project described was supported by Grants P01CA055075 from the National Cancer Institute and R01 HL035464 from the National Heart, Lung and Blood Institute.

Role of the Sponsors: The sponsors had no role in the design and conduct of the study; collection, management, analyses, and interpretation of the data; or preparation, review, or approval of the manuscript. The content is solely the responsibility of the authors and should not be constituted to represent the official views of the National Cancer Institute or the National Institutes of Health.

REFERENCES

1. Zittermann A, Schleithoff SS, Koerfer R. Putting cardiovascular disease and vitamin D insufficiency into perspective. Br J Nutr. 2005; 94:483–492. [PubMed: 16197570]
2. Lund B, Badskjaer J, Lund B, Soerensen OH. Vitamin D and ischaemic heart disease. Horm Metab Res. 1978; 10:553–556. [PubMed: 744575]
3. Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. Int J Epidemiol. 1990; 19:559–563. [PubMed: 2262248]
4. Vik B, Try K, Thelle DS, Forde OH. Tromsø Heart Study: vitamin D metabolism and myocardial infarction. Br Med J. 1979; 2:176. [PubMed: 466339]
5. Holick MF. Vitamin D deficiency. N Engl J Med. 2007; 357:266–281. [PubMed: 17634462]
6. Rimm EB, Giovannucci EL, Willett WC, et al. Prospective study of alcohol consumption and risk of coronary disease in men. Lancet. 1991; 338:464–468. [PubMed: 1678444]
7. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol. 1992; 135:1114–1126. [PubMed: 1632423]
8. Prentice RL, Breslow NE. Retrospective studies and failure time models. Biometrika. 1978; 65:153–158.
9. Rose, GA.; Blackburn, H. WHO Monograph Series No. 58. Geneva: World Health Organization; 1982. Cardiovascular Survey Methods.
10. Department of Agriculture. Composition of foods: raw, processed, prepared, 1963–1991, Agricultural handbook. Washington, D. C: Government Printing Office; 1992.
11. Ainsworth BE, Haskell WL, Leon AS, et al. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc. 1993; 25:71–80. [PubMed: 8292105]
12. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. Epidemiology. 1990; 1:466–73. [PubMed: 2090285]
13. Giovannucci E, Colditz G, Stampfer MJ, et al. The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol. 1991; 133:810–817. [PubMed: 2021148]
14. Chasan-Taber S, Rimm EB, Stampfer MJ, et al. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. Epidemiology. 1996; 7:81–86. [PubMed: 8664406]
15. Ascherio A, Rimm EB, Giovannucci EL, et al. A prospective study of nutritional factors and hypertension among US men. Circulation. 1992; 86:1475–1484. [PubMed: 1330360]
16. Hu FB, Leitzmann MF, Stampfer MJ, Colditz GA, Willett WC, Rimm EB. Physical activity and television watching in relation to risk for type 2 diabetes mellitus in men. Arch Intern Med. 2001; 161:1542–1548. [PubMed: 11427103]
17. Hollis BW, Kamerud JW, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay using I^{125}-labelled tracer. Clin Chem. 1993; 39:529–533. [PubMed: 8448871]
18. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control. 2004; 15:255–265. [PubMed: 15090720]
19. Rifai N, Iannotti E, DeAngelis K, Law T. Analytical and clinical performance of a homogenous enzymatic LDL-cholesterol assay compared with the ultracentrifugation-dextran sulfate-Mg2+ method. Clin Chem. 1998; 44:1242–1250. [PubMed: 9625048]

20. Sugiuchi H, Uji Y, Okabe H, et al. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated alpha-cyclodextrin. Clin Chem. 1995; 41:717–723. [PubMed: 7729051]
21. Stinshoff K, Weisshaar D, Staehler F, Hesse D, Gruber W, Steier E. Relation between concentrations of free glycerol and triglycerides in human sera. Clin Chem. 1977; 23:1029–1032. [PubMed: 870252]
22. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998; 338:777–783. [PubMed: 9504937]
23. Rothman, KJ.; Greenland, S. Case-control studies. In: Rothman, KJ.; Greenland, S., editors. Modern Epidemiology. 2nd ed.. Philadelphia, PA: Lippincott-Raven Publishers; 1998. p. 93-114.
24. Hosmer, DW.; Lemeshow, S. Applied Logistic Regression. 2nd Edition. New York, NY: John Wiley & Sons, Inc; 2000.
25. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr. 2005; 135:317–322. [PubMed: 15671234]
26. Chonchol M, Scragg R. 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. Kidney Int. 2007; 71:134–139. [PubMed: 17082756]
27. 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; 89–90:387–392.
28. Xiang W, Kong J, Chen S, et al. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. Am J Physiol Endocrinol Metab. 2005; 288:E125–E132. [PubMed: 15367398]
29. Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. Lancet. 1998; 352:709–710. [PubMed: 9728997]
30. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab. 2001; 86:1633–1637. [PubMed: 11297596]
31. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007; 49:1063–1069. [PubMed: 17372031]
32. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. Am J Hypertens. 2007; 20:713–719. [PubMed: 17586404]
33. Honye J, Mahon DJ, Jain A, et al. Morphological effects of coronary balloon angioplasty in vivo assessed by intravascular ultrasound imaging. Circulation. 1992; 85:1012–1025. [PubMed: 1537099]
34. Beadenkopf WG, Daoud AS, Love BM. Calcification in the coronary arteries and its relationship to arteriosclerosis and myocardial infarction. Am J Roentgenol Radium Ther Nucl Med. 1964; 92:865–871.
35. Margolis JR, Chen JT, Kong Y, Peter RH, Behar VS, Kisslo JA. The diagnostic and prognostic significance of coronary artery calcification. A report of 800 cases. Radiology. 1980; 137:609–616. [PubMed: 7444045]
36. Watson KE, Abrolat ML, Malone LL, et al. Active serum vitamin D levels are inversely correlated with coronary calcification. Circulation. 1997; 96:1755–1760. [PubMed: 9323058]
37. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care. 2007; 30:980–986. [PubMed: 17277040]
38. Pittas AG, Dawson-Hughes B, Li T, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care. 2006; 29:650–656. [PubMed: 16505521]
39. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab. 2007:92.

40. Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D(3) deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev. 2007 Jul 2. Epub ahead of print.
41. Zittermann A, Dembinski J, Stehle P. Low vitamin D status is associated with low cord blood levels of the immunosuppressive cytokine interleukin-10. Pediatr Allergy Immunol. 2004; 15:242–246. [PubMed: 15209957]
42. 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; 83:754–759. [PubMed: 16600924]
43. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol. 2003; 134:128–132. [PubMed: 12507780]
44. Timms PM, Mannan N, Hitman GA, et al. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? QJM. 2002; 95:787–796. [PubMed: 12454321]
45. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab. 2003; 88:4623–4632. [PubMed: 14557432]
46. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. Trends Mol Med. 2002; 8:174–179. [PubMed: 11927275]
47. Reichert TA, Simonsen L, Sharma A, Pardo SA, Fedson DS, Miller MA. Influenza and the winter increase in mortality in the United States, 1959–1999. Am J Epidemiol. 2004; 160:492–502. [PubMed: 15321847]
48. Cannell JJ, Vieth R, Umhau JC, et al. Epidemic influenza and vitamin D. Epidemiol Infect. 2006; 134:1129–1140. [PubMed: 16959053]
49. Aloia JF, Li-Ng M. Correspondence: epidemic influenza and vitamin D. Epidemiol Infect. 2007 Mar 12.135:1095–1096. Epub ahead of print. [PubMed: 17352842]
50. Autier P, Gandini S. Vitamin D supplements and total mortality: a meta-analysis of randomised controlled trials. Arch Intern Med. 2007; 167:1730–1737. [PubMed: 17846391]
51. Hsia J, Heiss G, Ren H, et al. Calcium/vitamin D supplementation cardiovascular events. Women's Health Initiative. Circulation. 2007; 115:846–854. [PubMed: 17309935]
52. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007; 85:1586–1591. [PubMed: 17556697]
53. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr. 2003; 77:204–210. [PubMed: 12499343]
54. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. Am J Clin Nutr. 2007; 85:6–18. [PubMed: 17209171]

Table 1

Baseline Characteristics of Men with Incident MI and Matched Controls During 10 Years of Follow-up[≠]

Characteristics	Cases (n = 454)		Controls (n = 900)		P Value
Age, mean (SD), y	63.8	(8.6)	63.8	(8.6)	
Current smoker	42	(9.3)	80	(8.9)	
Body mass index, mean (SD)[†]	26.1	(3.3)	25.6	(3.4)	0.01
Major ancestry					
White	427	(94.1)	851	(94.6)	0.70
African American	0		2	(0.22)	0.55
Asian	3	(0.7)	5	(0.6)	1.0
Other	24	(5.3)	42	4.7)	0.62
Region of residence					
South	197	(43.5)	426	(47.3)	0.17
Northeast	146	(32.2)	313	(34.8)	0.34
Midwest	111	(24.4)	161	(17.9)	0.004
Family history of MI before age 60 y	72	(15.9)	100	(11.1)	0.01
Current aspirin use, ≥2/wk	177	(39.0)	310	(34.4)	0.10
History of diabetes	42	(9.3)	33	(3.7)	<0.0001
History of hypertension	169	(37.2)	265	(29.4)	0.004
Fat intake, mean (SD), % energy					
Total	30.6	(6.7)	30.2	(6.9)	0.40
Saturated fat	10.2	(2.7)	10.0	(2.9)	0.22
Marine omega-3	0.12	(0.16)	0.13	(0.20)	0.85
Alcohol consumption, median (IQR), g/d	4.2	(0–14.6)	6.9	(0.88–17.8)	0.002
Multivitamin use	211	(46.5)	436	(48.44)	0.49
Physical activity, median (IQR), MET-h/wk	22.8	(9.5–46.5)	26.1	(11.9–48.8)	0.05
Cholesterol level, mean (SD), mg/dL					
Total	210.7	(39.0)	202.4	(36.3)	0.0001
HDL	42.2	(11.1)	46.0	(12.6)	<0.0001

Characteristics	Cases (n = 454)		Controls (n = 900)		P Value
LDL	133.9	(34.5)	126.0	(31.2)	<0.0001
Triglycerides level, mean (SD), mg/dL	164.4	(100.4)	142.6	(101.4)	0.0002
25(OH)vitamin D, mean (SD), ng/mL	23.0	(7.6)	24.5	(8.3)	0.002

Abbreviations: HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; MET-h, metabolic equivalent task-hours; MI, myocardial infarction.

SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259. To convert triglycerides to mmpl/L, multiply by 0.0113.

[≠]Data are expressed as No. (%) unless otherwise indicated. Age, smoking status, and month of blood draw were matched variables.

[†]Body mass index is expressed as weight in kilograms divided by the square of height in meters.

Arch Intern Med. Author manuscript; available in PMC 2013 July 23.

Table 2

Baseline Characteristics of Selected Covariates by Plasma 25(OH)Vitamin D Level Among Control Subjects

	25(OH)D (ng/mL)				
Characteristics	**≤15 (n=87)**	**15.1–22.5 (n=307)**	**22.6–29.9 (n=299)**	**≥30 (n=207)**	**P value (trend)**
25(OH)vitamin D, mean	12.0	19.2	25.9	35.5	
Age, mean, y	63.8	63.0	64.9	63.4	
Current smoker, %	20.7	7.0	8.0	8.1	0.01
Body mass index[†]	26.4	25.9	25.5	24.9	<0.0001
White Ancestry, %	93.1	95.2	92.9	96.7	0.31
Region of residence, %					
South	43.7	42.8	50.3	51.3	0.15
Northeast	36.8	40.0	31.4	31.3	0.10
Midwest	19.5	16.9	18.3	17.4	0.97
Family history of MI before age 60 y, %	6.9	8.7	14.2	12.0	0.02
Current aspirin use, ≥2/wk, %	36.8	34.6	31.3	37.9	0.80
History of diabetes, %	5.7	3.5	4.4	2.1	0.12
History of hypertension, %	31.0	31.6	28.2	27.4	0.19
Fat intake, % energy					
Total	30.7	30.2	30.2	30.0	0.23
Saturated fat	10.1	10.0	10.0	9.8	0.21
Marine omega-3	0.09	0.13	0.13	0.14	0.59
Alcohol consumption, g/d	11.5	12.0	13.1	15.5	0.009
Multivitamin use, %	39.1	48.3	49.0	51.8	0.01
Physical activity, MET-h/wk	27.1	35.6	39.7	42.3	0.002
Cholesterol level, mg/dL					
Total	200.1	202.9	200.7	205.3	0.20
HDL	42.0	43.9	47.0	49.4	<0.0001
LDL	122.8	126.0	125.0	128.6	0.21
Triglycerides level, mean (SD), mg/dL	155.3	156.8	133.9	128.8	0.001

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET-h, metabolic equivalent task-hours; MI, myocardial infarction.

All vartiables other than age and 25()H)vitamin D are age-standardized.

SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259. To convert triglycerides to mmpl/L, multiply by 0.0113.

* Data are expressed as No. unless otherwise indicated.

† Body mass index is expressed as weight in kilograms divided by the square of height in meters.

Table 3

Estimated Relative Risks of MI By Level of 25(OH)Vitamin D at Baseline During 10 Years of Follow-up

	Plasma 25(OH)D (ng/mL)				P value (trend)
	≤15	15.1–22.5	22.6–29.9	≥30	
N (cases, controls)	63, 87	156, 307	165, 299	70, 207	
RR – matching variables	2.42 (1.53–3.84)	1.65 (1.15–2.37)	1.72 (1.22–2.42)	1.0	0.0006
RR – MV1	2.01 (1.22–3.30)	1.45 (0.99–2.12)	1.56 (1.09–2.22)	1.0	0.02
RR – MV2	2.09 (1.24–3.54)	1.43 (0.96–2.13)	1.60 (1.10–2.32)	1.0	0.02

Abbreviations: CI, confidence interval; MI, myocardial infarction; MV, multivariable; RR, relative risk

MV1: matching variables (age, month and year of blood draw, smoking status) and family history of myocardial infarction before age 60, history of diabetes, history of hypertension, alcohol intake, BMI, physical activity, region, race, multivitamin use, marine omega-3 intake, and fasting status.

MV2: All the variables in MV1, and HDL and LDL cholesterol and triglycerides.