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Vitamin D, Calcium, and Atherosclerotic Risk: Evidence from serum levels and supplementation studies

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Abstract

Vitamin D and calcium have been traditionally been viewed in relation to bone health. However, recent research has suggested relations between these nutrients and cardiovascular disease (CVD). Specifically, evidence from both observational studies and clinical trials suggests that vitamin D may be related to lower risk of CVD. The picture for calcium is more complex. Dietary intake of calcium may be associated with lower CVD risk, while calcium supplementation may elevate CVD risk. In this review we summarize evidence of these relations, and comment on the recent Institute of Medicine (IOM) recommendations regarding use of vitamin D and calcium supplements.

Keywords

calcium; vitamin D; supplements; cardiovascular disease; review

Introduction

Atherosclerotic cardiovascular disease (CVD) is the number one cause of morbidity and mortality in developed countries. Therefore, there remains a great interest in identifying novel risk factors to identify those at risk for CVD, particularly markers that are modifiable/treatable.

Vitamin D is a fat-soluble vitamin obtained through cutaneous synthesis resulting from sun exposure, and through oral intake from food and supplement sources. Insufficient vitamin D, as assessed by low circulating 25-hydroxyvitamin D [25(OH)D], has recently drawn attention as a potential CVD risk factor. This possibility is of immense public health and clinical interest, given the vast potential for intervention as globally it is estimated that more than 50% of the world population has inadequate vitamin D levels¹. Although intriguing, much remains unknown about the relation of vitamin D to CVD events.

Calcium is vital to the functionality of all cells, though more than 99% of the calcium in the body is used as a structural component of bones and teeth². The biology of calcium and vitamin D is intricately intertwined. As such, these nutrients are often prescribed in combination for the promotion of skeletal health, particularly among postmenopausal women. Relations of calcium supplementation to reduced fracture risk are, however, perhaps less pronounced than commonly assumed^{3, 4}. As such, it is important to be cognizant of

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other ways in which calcium supplementation may impact health. In particular, recent studies have highlighted concerns regarding increased cardiovascular risk associated with calcium supplementation.

Vitamin D Metabolism

The major source of vitamin D is endogenous production via the action of the sun's ultraviolet b (UVB) light on 7-dehydrocholesterol precursors in the skin, converting them to vitamin D₃ (cholecalciferol)^{2, 5}. Exogenous D₃ can also be obtained from the diet from oily fish, fortified foods, or supplements. Vitamin D can also be found in the form of D₂ (ergocalciferol) which is produced in small amounts by plants after UVB radiation of ergosterol, and can also be consumed in the diet as a supplement or from fortified food. Fortified foods provide the majority of the vitamin D in the American diet⁶; however most people meet their vitamin D needs predominantly through sun exposure⁶.

Vitamin D undergoes 25-hydroxylation in the liver, to form 25(OH)D, the primary circulating form of vitamin D and the metabolite that best reflects stores of vitamin D (i.e. sufficiency or deficiency states). The active metabolite, 1,25(OH)₂D, (also called calcitriol), is formed after 1-hydroxylation in the kidneys, and both 1,25(OH)₂D₂ and 1,25(OH)₂D₃ bind to the vitamin D receptor. Although the main source of 1,25(OH)₂D in the circulation is from renal 1-hydroxylation, it has been recently appreciated that many other tissues, including breast, colon and vascular smooth muscle cells also express 1-hydroxylase. In these tissues, the locally formed 1,25(OH)₂D serves as a cell-differentiating factor. Extra-renal production of 1,25(OH)₂D is thought to have a paracrine/autocrine function including an important role in innate (macrophage) immunity. Levels of activated 1,25(OH)₂D levels do not correlate well with 25(OH)D levels, and given tight regulation by parathyroid hormone (PTH), 1,25(OH)₂D levels may remain in the normal range even in the face of 25(OH)D deficiency⁵⁻⁷.

Vitamin D and CVD Risk Factors

Suboptimal vitamin D is thought to influence CVD risk predominantly by acting on established CVD risk factors, namely hypertension, diabetes, and inflammation.

Vitamin D is involved in regulation of the renin-angiotensin system, and low 25(OH)D levels are associated with hypertension and hypertrophy of the left ventricle and vascular smooth muscle cells⁸⁻¹⁰. The majority of observational prospective cohort studies^{11, 12}, though not all¹³, have reported inverse associations between 25(OH)D and risk of incident hypertension. Experimentally, several small clinical trials have found that supplementing vitamin D (either by dietary supplements or with UVB radiation) successfully reduced blood pressure¹⁴⁻¹⁸. However, a recent small clinical trial enrolling patients with chronic kidney disease and left ventricular hypertrophy did not find the activated vitamin D analog paricalcitol compared to placebo reduced left ventricular mass or measures of diastolic dysfunction after 48 weeks of treatment¹⁹.

Suboptimal vitamin D is also believed to elevate diabetes risk. The mechanism of action is thought to be mediated through regulation of plasma calcium levels, which in turn regulate insulin synthesis and secretion, and also through a direct action on pancreatic beta-cell function²⁰⁻²³. Most²⁴⁻²⁸, but not all²⁹, prospective epidemiological studies have shown an association between low serum 25(OH)D and diabetes incidence. Intervention studies evaluating the relation between vitamin D supplementation and markers of glucose metabolism have yielded mixed results, with some finding vitamin D supplementation to be protective, while others failed to show an association^{16, 23, 30-32}. These interventions were,

however, limited in that they were often short in duration, included very few subjects, and/or had varying supplement formulations. Clearly additional research is needed.

Proinflammatory effects for low 25(OH)D levels and increased PTH have also been posited, as they appear to stimulate cytokine release from vascular smooth muscle cells and lymphocytes^{33–36}. In several small clinical trials, most in populations with non-cardiac inflammatory diseases, vitamin D supplementation has been shown to lower inflammatory marker levels^{37, 38}.

Additional mechanisms through which low vitamin D may influence CVD risk include its association with elevated triglyceride levels^{39–41}, promotion of atherogenic foam cells⁴², and direct cardiac effects, such as cardiomyocyte hypertrophy, apoptosis, and fibrosis of the left ventricle and vascular medial smooth muscle tissue^{33, 43–45}.

Observational Studies of Vitamin D and CVD Outcomes

The vast majority of the literature on vitamin D and CVD is based upon observational epidemiologic data. Low 25(OH)D levels have been associated with greater risk of combined CVD endpoints^{46, 47}, and CVD mortality^{47–53, 54, 55}, as well as specific CVD outcomes.

Relations between low 25(OH)D levels and myocardial infarction (MI) have been observed in three prospective cohort studies^{47, 56, 57}, several small case-control studies^{58–60}, and cross-sectional data from the third National Health and Nutrition Examination Survey (NHANES)⁶¹. In a prospective nested case-control study of 18,225 men from the Health Professional's Follow-up Study, the risk of MI was 50% higher among men with serum 25(OH)D levels <15 ng/mL as compared to men with levels >30 ng/mL⁵⁶.

In the Cardiovascular Health Study of older adults, low levels of 25(OH)D <20 ng/ml were also associated with an increased risk of sudden cardiac death,⁶² as well as a composite outcome that included hip fractures, MIs, cancer, and death⁶³.

A few studies have explored the associations of low vitamin D with risk of cerebrovascular disease. In the Mini-Finland Health Survey, which included 6,219 initially healthy individuals who were followed for over 27 years, participants in the highest quintile of 25(OH)D levels were at lower risk of incident stroke than were those in the lowest quintile [HR:0.48 (95% CI: 0.31–0.75)]⁴⁸. Inverse associations between 25(OH)D levels and fatal stroke were also observed among members of the Intermountain Healthcare system⁴⁷, among white but not black participants in an analysis of NHANES-III data⁶⁴, in a sample of patients referred for coronary angiography⁶⁵, and in two small case-control studies^{66, 67}. Low dietary vitamin D intake predicts 34-year stroke incidence among Japanese-American men⁶⁸.

Low vitamin D levels have also been associated with heart failure. Members of the Intermountain Healthcare system who had low 25(OH)D (<15 ng/mL) were at double the risk of incident heart failure relative to those with levels >30 ng/mL⁴⁷. In a population of Germans referred for coronary angiography low vitamin D levels were also associated with increased risk of heart failure mortality⁶⁹. Additionally, low 25(OH)D has also been linked to congestive heart failure in cross-sectional data from NHANES⁶¹ and in a number of small clinical studies^{70–73}. In the one study to date, no association was observed between serum vitamin D levels and risk of atrial fibrillation⁷⁴.

In summary, the epidemiological evidence suggests that lower 25(OH)D levels are associated with greater risk of CVD events. However, caution should be used in interpreting

these findings as residual or uncontrolled confounding may have contributed to these associations. Of additional potential concern is reverse causality bias, as individuals who are less healthy may participate in fewer outdoor activities as compared to healthy adults, which may in turn lead to less sunlight exposure and a greater propensity for suboptimal vitamin D levels.

Clinical Trials of Vitamin D and CVD Outcomes

The key question regarding the role of vitamin D in CVD risk is whether a causal relationship exists. To date, no large-scale randomized trials have been completed with clinical CVD events as the primary prespecified outcome⁷⁵.

Two randomized trials which tested moderate to high doses of vitamin D supplements (100,000 IU/4 months⁷⁶ and 1000 IU/d⁷⁷) without co-administration of calcium found a slight but nonsignificant reduction in CVD risk among those randomized to vitamin D versus placebo [meta-analysis HR:0.90 (95% CI: 0.77–1.05)]⁷⁸. Two other trials tested combined vitamin D and calcium supplementation versus placebo [including the large Women's Health Initiative (WHI)], and both trials showed no differences in CVD risk between the treatment groups^{79, 80}. These studies were, however, limited. It is widely believed that in WHI the vitamin D supplement dose (400 IU/d) was insufficient to alter rates between the treatment groups^{37, 79}. Every 100 IU vitamin D ingested daily increases the 25(OH)D level by about 1 ng/mL^{81–83}. The other study was very small (n = 192), and likely underpowered for CVD events⁸⁰.

Two meta-analyses of randomized vitamin D supplementation trials on any health condition found a reduced mortality from any cause for those randomized to supplements as compared to placebo. The first meta-analysis showed a statistically significant reduction in mortality, albeit modest⁸⁴. The more recent one from the Cochrane Collaboration was a meta-analysis of 50 randomized vitamin D supplementation trials, with a total of nearly 100,000 participants, which found a trend in reduced mortality from any cause for those randomized to vitamin D supplements as compared to placebo of borderline statistical significance [HR: 0.97 (95% CI: 0.94–1.00)]. Notably, most trials included only elderly women (older than 70 years)⁸⁵. Thus, it is uncertain how generalizable this finding is to men and younger adults. Also, the meta-analyses are limited by combining heterogeneous studies involving different types of vitamin D, differing dosing, and differing patient populations. Despite being unclear which conditions contributed to the lower mortality, this finding is intriguing.

Calcium Metabolism

Dairy products, such as milk, yogurt, and cheese are the most common sources of dietary calcium. As noted in the introduction, calcium is involved in the function of all cells. In addition to its major roles in forming and maintaining bones, calcium is also involved in cell metabolism, muscle contraction, blood coagulation, and transmitting nerve impulses.

The physiology of calcium and vitamin D are intricately intertwined. Calcitriol, the active hormone form of vitamin D (1,25(OH)₂D), enhances intestinal absorption of calcium². Low concentrations of 1,25(OH)₂D are associated with impaired calcium absorption, a negative calcium balance, and a compensatory rise in PTH. Elevated PTH stimulates the osteoclast activity, thereby resulting in increased release of calcium from the bones and higher serum calcium levels. Levels of serum calcium are highly regulated in the body, thus bone mass will be lost in order to maintain serum calcium levels. Given the complex interrelations between calcium and vitamin D, these nutrients are often prescribed in combination for the promotion of skeletal health, particularly among postmenopausal women who are at high risk of osteoporosis and fracture.

Observational Studies of Calcium and CVD Risk

Dietary calcium has been reported to be associated with lower risk of obesity, hypertension, and the metabolic syndrome,⁸⁶ with generally favorable relations reported between calcium markers of glucose/insulin homeostasis or diabetes^{87–89}, and lipid levels⁹⁰.

As has recently been reviewed elsewhere⁹¹, results of studies of dietary calcium intake and CVD risk have been mixed, with a few studies showing an inverse (protective) association, and many others yielding null results. Interestingly, a recent epidemiologic paper from the EPIC-Heidelberg cohort (n=23, 980 participants aged 35–64 years free of CVD at baseline) found a benefit for moderate intake of total dietary calcium with the third quartile (mean=820 mg/day) compared to lowest quartile having about a 30% lower risk of MI (HR 0.69, 95% CI 0.50–0.94)⁹¹. However, users of calcium supplements compared to non-users had increased risk of MI (HR 2.39, 95% CI 1.12–5.12). This data suggests that large boluses of calcium taken once or twice daily by supplements do not confer the same metabolic effect as calcium ingested throughout the day in small doses via a balanced diet.

Clinical Trials of Calcium Supplements and Cardiovascular Risk

Calcium supplements modestly increase bone mineral density and reduce fracture risk^{3, 4}. A relatively strong body of evidence suggests that calcium supplementation is associated with lower blood pressure, compared to placebo. In a 2006 meta-analysis of 40 randomized clinical trials reported that calcium supplementation (mean daily dose = 1200 mg) reduced systolic BP by 1.86 (95% CI: 0.81 to 2.91) mmHg and diastolic BP by 0.99 (95% CI: 0.37 to 1.61) mmHg⁹². However recent studies have suggested possible CVD harm associated with calcium supplementation, although results are inconclusive.

A clinical trial of calcium supplements (without vitamin D) vs. placebo conducted in New Zealand in 2008 suggested an increased risk of MI associated with calcium supplements which was not statistically significant when additional adjudicated events were included⁹³. This was followed by a 2010 meta-analysis from the same group including 15 clinical trials (5 with patient level data) which again found an increased risk of MI for those assigned to calcium therapy (without vitamin D) [HR:1.31 (95% CI 1.02–1.67)] but no statistically increased risk for mortality or the composite CVD outcome⁹⁴. This was in contrast to another 2010 meta-analysis of clinical trials which found no statistically significant increased CVD risk for calcium supplementation alone or in combination with vitamin D⁷⁸.

Next, there was a recent re-analysis of the data from WHI. While the overall WHI results showed no evidence for CVD benefit or harm with assignment to calcium plus vitamin D therapy vs. placebo⁷⁹, Bolland et al found an interaction that suggested women who were not taking personal calcium supplements before randomization had a higher risk for CVD events when assigned to calcium/vitamin D therapy during the trial compared to placebo but no alteration in CVD risk for women already taking supplements before entry into the study⁹⁵. However, post-hoc subgroup analyses should always be viewed with caution. Furthermore, there was also no dose response seen which leads to the question if calcium is so harmful, why did the group taking the most not fare the worst? It is possible that a threshold exists, whereby calcium supplementation beyond a certain level does not result in further increased risk. An updated 2011 meta-analysis of placebo controlled trials by these authors found that calcium supplements with or without vitamin D modestly increases the risk of MI [RR:1.24 (95% CI: 1.07–1.45)].⁹⁵

The Calcium and Heart Disease Quandary

Counter to studies of dietary calcium intake and CVD risk, most^{96–98}, though not all⁹⁹, studies of serum calcium levels have shown a positive relation with CVD risk. These higher serum calcium levels are thought to promote CVD and atherogenesis through vascular calcifications and increased coagulability^{3, 4}. It has been suggested that the potential harm of calcium supplements is that large boluses can transiently increase serum calcium levels, even above the normal range, for several hours after ingestion. This may potentially cause similar vascular effects in these normocalcemic patients, as described in patients with higher serum calcium levels. However the current data is insufficient to confirm or refute this concern. More studies are clearly needed to clarify this potential risk.

Food sources, which have not been linked to increased CVD risk, may be the best source of calcium, perhaps because calcium ingested from food sources is absorbed more slowly in smaller amounts throughout the day, thus not substantially changing serum calcium levels. Pending future studies, given the only modest reduction in fractures and the mildly increased CVD risk, there may be no net benefit for calcium supplementation in post-menopausal women and possibly even potential harm.

Current IOM Recommendations Regarding Supplement Use

As of the 2010 Institute of Medicine (IOM) report, the recommended daily allowance of vitamin D for adults is 600 IU/day and 800 IU/day for those aged <70 and ≥70 years, respectively.¹⁰⁰ Notably, the IOM report did not recommend vitamin D supplementation for CVD prevention and suggested serum levels >20 ng/ml may be sufficient for health (rather than the higher targets >30 or >40 ng/ml recommended by many experts in the field). The calcium recommended daily allowances from those same guidelines range from 1000–1200 mg/day for adults.

The IOM authors emphasized cautious interpretation of observational epidemiological studies, as correlation does not equal causation, and stressed the need for large-scale clinical trials. Historically, several micronutrients have appeared to be associated with lower CVD risk in observational epidemiologic studies, but have failed to show an effect when tested in supplementation trials.

Several randomized clinical trials are currently underway, which will hopefully provide insight as to whether suboptimal vitamin D is causally related to CVD risk. The largest is the VITamin D and Omega-3 Trial (VITAL). It is a 2×2 factorial, double-blind, placebo-controlled clinical trial which will randomize 20,000 participants to 2000 IU/day of vitamin D (without calcium) and omega-3 dietary supplements¹⁰¹. VITAL began recruitment in 2010 and will follow participants for 5 years for cancer and CVD outcomes. Hopefully trials such as VITAL will provide important insight into whether associations between vitamin D and CVD are causal. Concerns about VITAL trial do, however exist. For instance, 25(OH)D deficiency was not a criteria for enrollment. Likely vitamin D replacement would most benefit those with 25(OH)D deficiency and may have little benefit in those who already have adequate 25(OH)D levels. Additionally, if vitamin D impacts CVD risk through diabetes, hypertension, and inflammation, given the age of participants (i.e. men ≥50 years, women ≥55 years), it is possible that the intervention is taking place too late in the natural history of the disease.

Conclusions

In summary, vitamin D status remains a marker of interest given the encouraging findings from observational data and the limited clinical trials to date. Although biologically

plausible mechanisms have been proposed and the epidemiologic evidence is intriguing, only clinical trials are capable of establishing causality. Thus, pending the results of ongoing clinical trials, we agree with the IOM that presently there is insufficient evidence to recommend vitamin D supplements for the sole purpose of CVD prevention. At this time, the evidence for calcium supplements and increased CVD risk is also inconclusive but raises some concern. Moderate intake of calcium from dietary foods may be the best way to achieve target daily calcium goals, although calcium supplements may be needed for individuals not meeting the recommended daily allowances as outlined by IOM. Given remaining uncertainty about how to best advise patients, large designated randomized clinical trials with CVD events as the primary clinical endpoint are warranted for both calcium and vitamin D supplements.

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References

Papers of particular interest, published recently, have been highlighted as:

* Of importance

** Of outstanding importance

1. Lips P. Worldwide status of vitamin d nutrition. *The Journal of Steroid Biochemistry and Molecular Biology*. 2010; 121:297–300. [PubMed: 20197091]
2. Wardlaw, GKM. *Perspectives in nutrition*. Boston: McGraw Hill; 2002.
3. Reid I, Bolland M, Avenell A, Grey A. Cardiovascular effects of calcium supplementation. *Osteoporosis International*. 2011; 22:1649–1658. [PubMed: 21409434]
4. Reid IR, Bolland MJ. Calcium supplements: Bad for the heart? *Heart*. 2012; 98:895–896. [PubMed: 22626897]
5. Holick MF. Vitamin d deficiency. *N Engl J Med*. 2007; 357:266–281. [PubMed: 17634462]
6. Office of Dietary Supplements. *Dietary supplement fact sheet: Vitamin d*. 2008. 2009
7. Willett, WC. *Nutritional epidemiology*. New York: Oxford University Press; 1998.
8. Li YC. Vitamin d regulation of the renin-angiotensin system. *J Cell Biochem*. 2003; 88:327–331. [PubMed: 12520534]
9. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-dihydroxyvitamin d(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest*. 2002; 110:229–238. [PubMed: 12122115]
10. Xiang W, Kong J, Chen S, Cao LP, Qiao G, Zheng W, Liu W, Li X, Gardner DG, Li YC. 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]
11. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC. Plasma 25-hydroxyvitamin d levels and risk of incident hypertension. *Hypertension*. 2007; 49:1063–1069. [PubMed: 17372031]
12. Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin d levels and risk of incident hypertension among young women. *Hypertension*. 2008; 52:828–832. [PubMed: 18838623]
13. Jorde R, Figenschau Y, Emaus N, Hutchinson M, Grimnes G. Serum 25-hydroxyvitamin d levels are strongly related to systolic blood pressure but do not predict future hypertension. *Hypertension*. 2010; 55:792–798. [PubMed: 20065152]
14. Krause R, Bühring M, Hopfenmüller W, Holick MF, Sharma AM. Ultraviolet b and blood pressure. *Lancet*. 1998; 352:709–710. [PubMed: 9728997]

15. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin d3 and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab.* 2001; 86:1633–1637. [PubMed: 11297596]
16. Witham M, Dove F, Dryburgh M, Sugden J, Morris A, Struthers A. The effect of different doses of vitamin d₃ on markers of vascular health in patients with type 2 diabetes: A randomised controlled trial. *Diabetologia.* 2010; 53:2112–2119. [PubMed: 20596692]
17. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin d improves endothelial function in patients with type 2 diabetes mellitus and low vitamin d levels. *Diabet Med.* 2008; 25:320–325. [PubMed: 18279409]
18. Judd SE, Raiser SN, Kumari M, Tangpricha V. 1,25-dihydroxyvitamin d3 reduces systolic blood pressure in hypertensive adults: A pilot feasibility study. *The Journal of Steroid Biochemistry and Molecular Biology.* 2010; 121:445–447. [PubMed: 20420907]
19. Thadhani R, Appelbaum E, Pritchett Y, et al. Vitamin d therapy and cardiac structure and function in patients with chronic kidney disease: The primo randomized controlled trial. *JAMA: The Journal of the American Medical Association.* 2012; 307:674–684. [PubMed: 22337679]
20. Boucher BJ. Inadequate vitamin d status: Does it contribute to the disorders comprising syndrome x? *Br J Nut.* 1998; 79:315–327.
21. Palomer X, González-Clemente J, Blanco-Vaca F, Mauricio D. Role of vitamin d in the pathogenesis of type 2 diabetes mellitus. *Diabetes Obes Metab.* 2008; 10:185–197. [PubMed: 18269634]
22. Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin d3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. *Int J Clin Pract.* 2003; 57:258–261. [PubMed: 12800453]
23. 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; 92:2017–2029. [PubMed: 17389701]
24. Knekt P, Laaksonen M, Mattila C, Harkanen T, Marniemi J, Heliovaara M, Rissanen H, Montonen J, Reunanen A. Serum vitamin d and subsequent occurrence of type 2 diabetes. *Epidemiology.* 2008; 19:666–671. 610.1097/EDE.1090b1013e318176b318178ad. [PubMed: 18496468]
25. Mattila C, Knekt P, Männistö S, Rissanen H, Laaksonen MA, Montonen J, Reunanen A. Serum 25-hydroxyvitamin d concentration and subsequent risk of type 2 diabetes. *Diabetes Care.* 2007; 30:2569–2570. [PubMed: 17626891]
26. Grimnes G, Emaus N, Joakimsen RM, Figenschau Y, Jenssen T, Njølstad I, Schirmer H, Jorde R. Baseline serum 25-hydroxyvitamin d concentrations in the tromsø study 1994–95 and risk of developing type 2 diabetes mellitus during 11 years of follow-up. *Diabetic Medicine.* 2010; 27:1107–1115. [PubMed: 20854377]
27. Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Shaw JE, Zimmet PZ, Sikaris K, Grantham N, Ebeling PR, Daly RM. Serum 25-hydroxyvitamin d, calcium intake, and risk of type 2 diabetes after 5 years: Results from a national, population-based prospective study (the australian diabetes, obesity and lifestyle study). *Diabetes Care.* 2011; 34:1133–1138. [PubMed: 21430082]
28. Pittas AG, Sun Q, Manson JE, Dawson-Hughes B, Hu FB. Plasma 25-hydroxyvitamin d concentration and risk of incident type 2 diabetes in women. *Diabetes Care.* 2010; 33:2021–2023. [PubMed: 20805275]
29. Robinson JG, Manson JE, Larson J, Liu S, Song Y, Howard BV, Phillips L, Shikany JM, Allison M, Curb JD, Johnson KC, Watts N. Lack of association between 25(oh)d levels and incident type 2 diabetes in older women. *Diabetes Care.* 2011; 34:628–634. [PubMed: 21289227]
30. 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. *Journal of Internal Medicine.* 2010; 267:462–472. [PubMed: 20141565]
31. von Hurst PR, Stonehouse W, Coad J. Vitamin d supplementation reduces insulin resistance in south asian women living in new zealand who are insulin resistant and vitamin d deficient? A randomised, placebo-controlled trial. *British Journal of Nutrition.* 2010; 103:549–555. [PubMed: 19781131]

32. Mitri J, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin d and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: The calcium and vitamin d for diabetes mellitus (caddm) randomized controlled trial. *The American Journal of Clinical Nutrition*. 2011; 94:486–494. [PubMed: 21715514]
33. Zittermann A. Vitamin d and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol*. 2006; 92:39–48. [PubMed: 16600341]
34. Martin-Ventura JL, Ortego M, Esbrit P, Hernandez-Presa MA, Ortega L, Egido J. Possible role of parathyroid hormone-related protein as a proinflammatory cytokine in atherosclerosis. *Stroke*. 2003; 34:1783–1789. [PubMed: 12805493]
35. Somjen D, Weisman Y, Kohen F, Gayer B, Limor R, Sharon O, Jaccard N, Knoll E, Stern N. 25-hydroxyvitamin d3-1 α -hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation*. 2005; 111:1666–1671. [PubMed: 15795327]
36. Rigby WF, Denome S, Fanger MW. Regulation of lymphokine production and human t lymphocyte activation by 1,25-dihydroxyvitamin d3. Specific inhibition at the level of messenger rna. *J Clin Invest*. 1987; 79:1659–1664. [PubMed: 2884234]
37. Michos ED, Blumenthal RS. Vitamin d supplementation and cardiovascular disease risk. *Circulation*. 2007; 115:827–828. [PubMed: 17309931]
38. 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]
39. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin d in the united states: Data from the third national health and nutrition examination survey. *Archives of internal Medicine*. 2007; 167:1159–1165. [PubMed: 17563024]
40. Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin d and the metabolic syndrome among u.s. Adults. *Diabetes Care*. 2005; 28:1228–1230. [PubMed: 15855599]
41. Zittermann A, Frisch S, Berthold HK, Götting C, Kuhn J, Kleesiek K, Stehle P, Koertke H, Koerfer R. Vitamin d supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. *The American Journal of Clinical Nutrition*. 2009; 89:1321–1327. [PubMed: 19321573]
42. Oh J, Weng S, Felton SK, Bhandare S, Riek A, Butler B, Proctor BM, Petty M, Chen Z, Schechtman KB, Bernal-Mizrachi L, Bernal-Mizrachi C. 1,25(OH)₂ vitamin d inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. *Circulation*. 2009; 120:687–698. [PubMed: 19667238]
43. Rostand SG, Drueke TB. Parathyroid hormone, vitamin d, and cardiovascular disease in chronic renal failure. *Kidney Int*. 1999; 56:383–392. [PubMed: 10432376]
44. Perkovic V, Hewitson TD, Kelynack KJ, Martic M, Tait MG, Becker GJ. Parathyroid hormone has a prosclerotic effect on vascular smooth muscle cells. *Kidney Blood Press Res*. 2003; 26:27–33. [PubMed: 12697974]
45. Amann K, Tornig J, Flechtenmacher C, Nabokov A, Mall G, Ritz E. Blood-pressure-independent wall thickening of intramyocardial arterioles in experimental uraemia: Evidence for a permissive action of pth. *Nephrol Dial Transplant*. 1995; 10:2043–2048. [PubMed: 8643165]
46. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin d deficiency and risk of cardiovascular disease. *Circulation*. 2008; 117:503–511. [PubMed: 18180395]
47. Anderson JL, May HT, Horne BD, Bair TL, Hall NL, Carlquist JF, Lappe DL, Muhlestein JB. Relation of vitamin d deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol*. 2010; 106:963–968. [PubMed: 20854958]
48. Kilkkinen A, Knekt P, Aro A, Rissanen H, Marniemi J, Heliovaara M, Impivaara O, Reunanen A. Vitamin d status and the risk of cardiovascular disease death. *Am J Epidemiol*. 2009; 170:1032–1039. [PubMed: 19762371]

49. Virtanen JK, Nurmi T, Voutilainen S, Mursu J, Tuomainen TP. Association of serum 25-hydroxyvitamin d with the risk of death in a general older population in finland. *European journal of nutrition*.
50. Pilz S, Dobnig H, Nijpels G, Heine RJ, Stehouwer CD, Snijder MB, van Dam RM, Dekker JM. Vitamin d and mortality in older men and women. *Clinical endocrinology*. 2009; 71:666–672. [PubMed: 19226272]
51. Semba RD, Houston DK, Bandinelli S, Sun K, Cherubini A, Cappola AR, Guralnik JM, Ferrucci L. Relationship of 25-hydroxyvitamin d with all-cause and cardiovascular disease mortality in older community-dwelling adults. *European journal of clinical nutrition*. 64:203–209. [PubMed: 19953106]
52. Fiscella K, Franks P. Vitamin d, race, and cardiovascular mortality: Findings from a national us sample. *Ann Fam Med*. 2010; 8:11–18. [PubMed: 20065273]
53. Ginde AA, Scragg R, Schwartz RS, Camargo CA Jr. Prospective study of serum 25-hydroxyvitamin d level, cardiovascular disease mortality, and all-cause mortality in older u.S. Adults. *Journal of the American Geriatrics Society*. 2009; 57:1595–1603. [PubMed: 19549021]
54. Wang AY, Lam CW, Sanderson JE, Wang M, Chan IH, Lui SF, Sea MM, Woo J. Serum 25-hydroxyvitamin d status and cardiovascular outcomes in chronic peritoneal dialysis patients: A 3-y prospective cohort study. *The American journal of clinical nutrition*. 2008; 87:1631–1638. [PubMed: 18541550]
55. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med*. 2008; 168:1340–1349. [PubMed: 18574092]
56. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin d and risk of myocardial infarction in men: A prospective study. *Arch Intern Med*. 2008; 168:1174–1180. [PubMed: 18541825]
57. Kestenbaum B, Katz R, de Boer I, Hoofnagle A, Sarnak MJ, Shlipak MG, Jenny NS, Siscovick DS. Vitamin d, parathyroid hormone, and cardiovascular events among older adults. *J Am Coll Cardiol*. 2011; 58:1433–1441. [PubMed: 21939825]
58. Lund B, Badskaer J, Lund B, Soerensen OH. Vitamin d and ischaemic heart disease. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme*. 1978; 10:553–556. [PubMed: 744575]
59. 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. *International journal of epidemiology*. 1990; 19:559–563. [PubMed: 2262248]
60. Vik B, Try K, Thelle DS, Forde OH. Tromso heart study: Vitamin d metabolism and myocardial infarction. *British medical journal*. 1979; 2:176. [PubMed: 466339]
61. Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis d in cardiovascular diseases (from the national health and nutrition examination survey 2001 to 2004). *The American journal of cardiology*. 2008; 102:1540–1544. [PubMed: 19026311]
62. Deo R, Katz R, Shlipak MG, Sotoodehnia N, Psaty BM, Sarnak MJ, Fried LF, Chonchol M, de Boer IH, Enquobahrie D, Siscovick D, Kestenbaum B. Vitamin d, parathyroid hormone, and sudden cardiac death. *Hypertension*. 2011; 58:1021–1028. [PubMed: 22068871]
63. de Boer IH, Levin G, Robinson-Cohen C, Biggs ML, Hoofnagle AN, Siscovick DS, Kestenbaum B. Serum 25-hydroxyvitamin d concentration and risk for major clinical disease events in a community-based population of older adults: a cohort study. *Annals of Internal Medicine*. 2012; 156:627–634. [PubMed: 22547472] **This recent observational study adds to the epidemiology literature by suggesting (1) season specific levels may be most appropriate for targeting 25(OH)D concentrations and (2) the season-specific threshold of increased risk centers close to levels below 20 ng/ml (rather than 30 ng/ml), reaffirming the optimal serum level of 25(OH)D 20 ng/ml set by the IOM guidelines.
64. Michos ED, Reis JP, Post WS, Lutsey PL, Gottesman RF, Mosley TH, Sharrett AR, Melamed ML. 25-hydroxyvitamin d deficiency is associated with fatal stroke among whites but not blacks: The nhanes-iii linked mortality files. *Nutrition*.

65. Pilz S, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, Marz W. Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke*. 2008; 39:2611–2613. [PubMed: 18635847]
66. Marniemi J, Alanen E, Impivaara O, Seppanen R, Hakala P, Rajala T, Ronnemaa T. Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. *Nutr Metab Cardiovasc Dis*. 2005; 15:188–197. [PubMed: 15955467]
67. Poole KE, Loveridge N, Barker PJ, Halsall DJ, Rose C, Reeve J, Warburton EA. Reduced vitamin d in acute stroke. *Stroke; a journal of cerebral Circulation*. 2006; 37:243–245.
68. Kojima G, Bell C, Abbott RD, Launer L, Chen R, Motonaga H, Ross GW, Curb JD, Masaki K. Low dietary vitamin d predicts 34-year incident stroke. *Stroke*. 2012
69. Pilz S, Marz W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, Boehm BO, Dobnig H. Association of vitamin d deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. *The Journal of clinical endocrinology and metabolism*. 2008; 93:3927–3935. [PubMed: 18682515]
70. Arroyo M, Laguardia SP, Bhattacharya SK, Nelson MD, Johnson PL, Carbone LD, Newman KP, Weber KT. Micronutrients in african-americans with decompensated and compensated heart failure. *Transl Res*. 2006; 148:301–308. [PubMed: 17162251]
71. Laguardia SP, Dockery BK, Bhattacharya SK, Nelson MD, Carbone LD, Weber KT. Secondary hyperparathyroidism and hypovitaminosis d in african-americans with decompensated heart failure. *Am J Med Sci*. 2006; 332:112–118. [PubMed: 16969139]
72. Shane E, Mancini D, Aaronson K, Silverberg SJ, Seibel MJ, Adesso V, McMahon DJ. Bone mass, vitamin d deficiency, and hyperparathyroidism in congestive heart failure. *The American journal of Medicine*. 1997; 103:197–207. [PubMed: 9316552]
73. Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Korfer R, Stehle P. Low vitamin d status: A contributing factor in the pathogenesis of congestive heart failure? *Journal of the American College of Cardiology*. 2003; 41:105–112. [PubMed: 12570952]
74. Rienstra M, Cheng S, Larson MG, McCabe EL, Booth SL, Jacques PF, Lubitz SA, Yin X, Levy D, Magnani JW, Ellinor PT, Benjamin EJ, Wang TJ. Vitamin d status is not related to development of atrial fibrillation in the community. *American Heart Journal*. 2011; 162:538–541. [PubMed: 21884873]
75. Shapses SA, Manson JE. Vitamin d and prevention of cardiovascular disease and diabetes: Why the evidence falls short. *JAMA*. 2011; 305:2565–2566. [PubMed: 21693745]
76. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin d3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: Randomised double blind controlled trial. *BMJ*. 2003; 326:469. [PubMed: 12609940]
77. Prince RL, Austin N, Devine A, Dick IM, Bruce D, Zhu K. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. *Arch Intern Med*. 2008; 168:103–108. [PubMed: 18195202]
78. Wang L, Manson JE, Song Y, Sesso HD. Systematic review: Vitamin d and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med*. 2010; 152:315–323. [PubMed: 20194238]
79. Hsia J, Heiss G, Ren H, Allison M, Dolan NC, Greenland P, Heckbert SR, Johnson KC, Manson JE, Sidney S, Trevisan M, for the Women's Health Initiative I. Calcium/vitamin d supplementation and cardiovascular events. *Circulation*. 2007; 115:846–854. [PubMed: 17309935]
80. Brazier M, Grados F, Kamel S, Mathieu M, Morel A, Maamer M, Sebert J-L, Fardellone P. Clinical and laboratory safety of one year's use of a combination calcium + vitamin d tablet in ambulatory elderly women with vitamin d insufficiency: Results of a multicenter, randomized, double-blind, placebo-controlled study. *Clinical Therapeutics*. 2005; 27:1885–1893. [PubMed: 16507374]
81. 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]
82. Vieth R. Vitamin d supplementation, 25-hydroxyvitamin d concentrations, and safety. *Am J Clin Nutr*. 1999; 69:842–856. [PubMed: 10232622]

83. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin d deficiency: An important, common, and easily treatable cardiovascular risk factor? *J Am Coll Cardiol*. 2008; 52:1949–1956. [PubMed: 19055985]
84. Autier P, Gandini S. Vitamin d supplementation and total mortality: A meta-analysis of randomized controlled trials. *Arch Intern Med*. 2007; 167:1730–1737. [PubMed: 17846391]
85. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, Bjelakovic M, Gluud C. Vitamin d supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev*. 2011 CD007470. **This recent Cochrane meta-analysis of 50 randomized clinical trials involving nearly 100,000 people provides evidence that vitamin D supplements were associated with modestly lower risk of all-cause mortality. Despite limitations (e.g. population largely older women, formulations vary), this suggests that there may be a net benefit of vitamin D supplementation.
86. Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin d, and the prevalence of metabolic syndrome in middle-aged and older u.S. Women. *Diabetes Care*. 2005; 28:2926–2932. [PubMed: 16306556]
87. Villegas R, Gao Y-T, Dai Q, Yang G, Cai H, Li H, Zheng W, Shu XO. Dietary calcium and magnesium intakes and the risk of type 2 diabetes: The shanghai women's health study. *The American Journal of Clinical Nutrition*. 2009; 89:1059–1067. [PubMed: 19225116]
88. van Dam RM, Hu FB, Rosenberg L, Krishnan S, Palmer JR. Dietary calcium and magnesium, major food sources, and risk of type 2 diabetes in u.S. Black women. *Diabetes Care*. 2006; 29:2238–2243. [PubMed: 17003299]
89. Tremblay A, Gilbert J-A. Milk products, insulin resistance syndrome and type 2 diabetes. *Journal of the American College of Nutrition*. 2009; 28:91S–102S. [PubMed: 19571167]
90. Reid IR. Effects of calcium supplementation on circulating lipids: Potential pharmacoeconomic implications. *Drugs & Aging*. 2004; 21:7–17. [PubMed: 14715041]
91. Li K, Kaaks R, Linseisen J, Rohrmann S. Associations of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the heidelberg cohort of the european prospective investigation into cancer and nutrition study (epic-heidelberg). *Heart*. 2012; 98:920–925. [PubMed: 22626900] **This recent observational study is important because it corroborates some of the recent clinical trial papers that suggested an increased risk of myocardial infarction with calcium supplements, while providing some reassuring evidence that moderate dietary calcium does not appear harmful and may be beneficial.
92. van Mierlo LAJ, Arends LR, Streppel MT, Zeegers MPA, Kok FJ, Grobbee DE, Geleijnse JM. Blood pressure response to calcium supplementation: A meta-analysis of randomized controlled trials. *J Hum Hypertens*. 2006; 20:571–580. [PubMed: 16673011]
93. Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, Ames R, Gamble GD, Grey A, Reid IR. Vascular events in healthy older women receiving calcium supplementation: Randomised controlled trial. *BMJ*. 2008; 336:262–266. [PubMed: 18198394]
94. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: Meta-analysis. *BMJ*. 2010; 341
95. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin d and risk of cardiovascular events: Reanalysis of the women's health initiative limited access dataset and meta-analysis. *BMJ*. 2011; 342 **By carefully re-analyzing the WHI data, and observing that the adverse CVD risk associated with supplements is limited to those not previously taking supplements, these authors expand our understanding of how calcium supplementation may influence CVD risk.
96. Lind L, Skarfors E, Berglund L, Lithell H, Ljunghall S. Serum calcium: A new, independent, prospective risk factor for myocardial infarction in middle-aged men followed for 18 years. *Journal of Clinical Epidemiology*. 1997; 50:967–973. [PubMed: 9291883]
97. Foley RN, Collins AJ, Ishani A, Kalra PA. Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: The atherosclerosis risk in communities (aric) study. *American Heart Journal*. 2008; 156:556–563. [PubMed: 18760141]
98. Jorde R, Sundsfjord J, Fitzgerald P, Bønaa KH. Serum calcium and cardiovascular risk factors and diseases : The tromsø study. *Hypertension*. 1999; 34:484–490. [PubMed: 10489398]

99. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'gostino RB Sr, Gaziano JM, Vasan RS. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med.* 2007; 167:879–885. [PubMed: 17502528]
100. Ross AC, Taylor CL, Yaktine AL, Del Valle HBE. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary reference intakes for calcium and vitamin d: Institute of Medicine. 2010
101. Manson JE, Bassuk SS, Lee IM, Cook NR, Albert MA, Gordon D, Zaharris E, MacFadyen JG, Danielson E, Lin J, Zhang SM, Buring JE. The vitamin d and omega-3 trial (vital): Rationale and design of a large randomized controlled trial of vitamin d and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. *Contemporary Clinical Trials.* 2012; 33:159–171. [PubMed: 21986389] **Trials are needed to establish whether low vitamin D is causally related to CVD risk. Results of the VITAL Trial (design paper listed here) are eagerly awaited.