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Vitamin D and Cardiovascular Diseases: Causality

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GRAPHICAL ABSTRACT

Beneficial Effects of Vitamin D in Cardiovascular Diseases
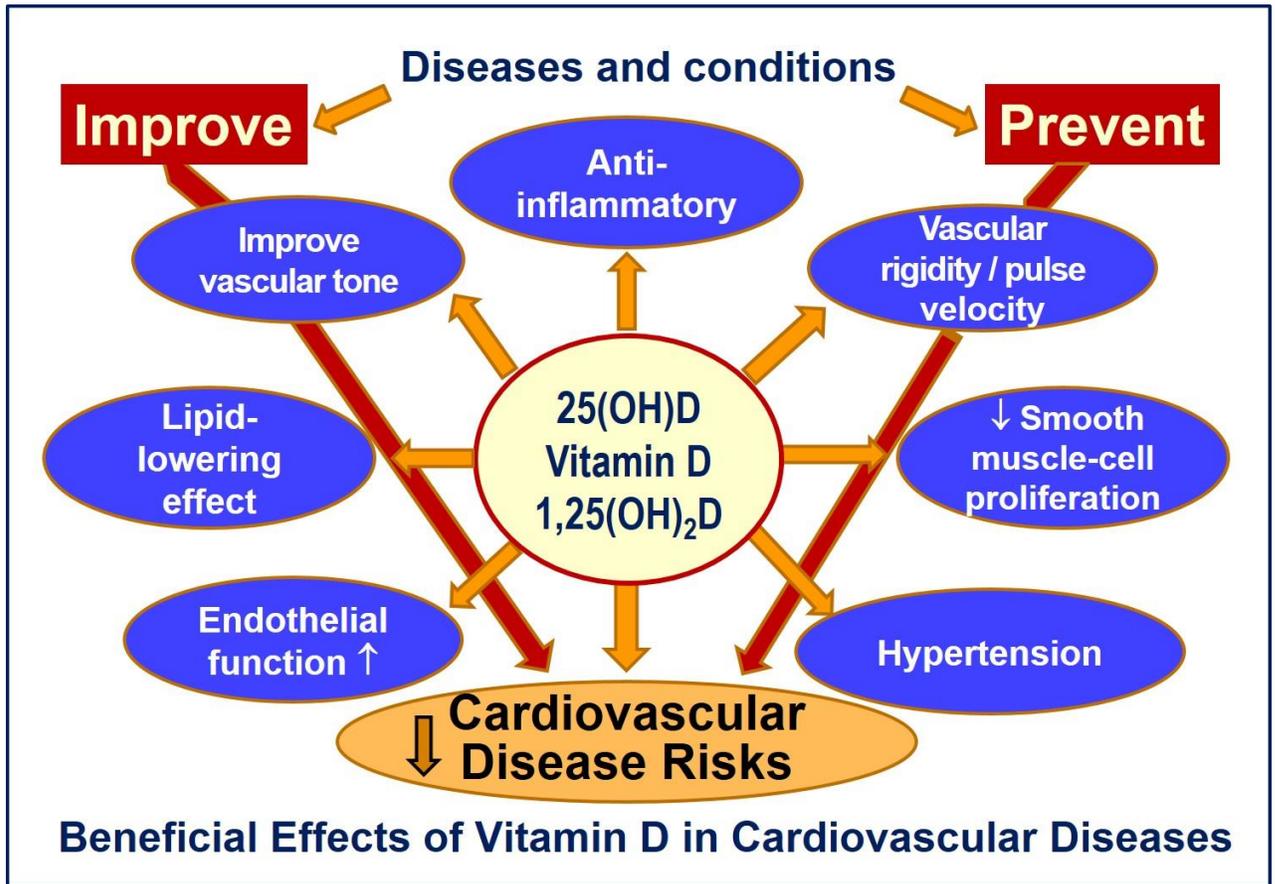
Highlights:

- Vitamin D attenuates several cardiovascular-related diseases (CVD)

- Epidemiological and biological data indicate vitamin D has beneficial effects on CVD risk reductions

- The few RCTs available also indicate vitamin D has beneficial effects on CVD, but some studies are non-conclusive

- There are no longer-term, adequately powered, randomized, vitamin D clinical studies available with cardiovascular primary end points

- CVD benefits appear to occur when the serum 25(OH)D levels are above 30 ng/mL

- The optimum serum 25(OH)D levels that facilitates beneficial pleiotropic effects are between 30 and 60 ng/mL.

ABSTRACT:

Vitamin D regulates blood pressure, cardiac functions, and endothelial and smooth muscle cell functions, thus playing an important role in cardiovascular health. Observational studies report associations between vitamin D deficiency and hypertension and cardiovascular-related deaths. Peer-reviewed papers in several research databases were examined, per the guidelines of the Preferred Reporting Items for Systematic Reviews, using key words that address the relationship between vitamin D and cardiovascular disease. Correlations and interpretations were made considering the risks–benefits, broader evidence, and implications. This review analyzed current knowledge regarding the effects of vitamin D on the cardiovascular system. $1,25(OH)_2D$ and related epigenetic modifications subdue cellular inflammation, improve overall endothelial functions, reduce age-related systolic hypertension and vascular rigidity, and attenuate the actions of the renin–angiotensin–aldosterone system. Most observational and ecological studies support 25(OH) vitamin D having protective effects on the cardiovascular system. However, the association of vitamin D deficiency with cardiovascular diseases is based primarily on observational and ecological studies and thus is a matter of controversy. Adequately powered, randomized controlled clinical trial data are not available to confirm the association. Thus, to test the hypothesis that correction of vitamin D deficiency protects the cardiovascular system, well-designed, statistically powered, longer-term clinical trials are needed in persons with vitamin D deficiency. Nevertheless, the available data support that adequate vitamin D supplementation and sensible sunlight exposure to achieve optimal vitamin D status are important in the prevention of cardiovascular disease and other chronic diseases.

Keywords

1.0   INTRODUCTION:

Vitamin D is a multifunctional pro-hormone postulated to have widespread actions in humans. Poor vitamin D status is common in institutionalized people, those with darker skin, those taking medications that accelerate the catabolism of vitamin D, those with gastrointestinal malabsorption diseases, those who are obese, and among older people. Poor vitamin D status results in osteomalacia, osteoporosis, and increased risks of falls and fractures and is associated with a variety of non-communicable diseases that lead to poor health outcomes. The prevalence of hypovitaminosis D is high among the populations of the United Arab Emirates, Saudi Arabia, Oman, and other Middle Eastern countries, especially among women (1, 2). In general, dark-skinned persons are at high risk of being deficient in vitamin D compared with their white-skinned counterparts.

Vitamin D deficiency has been associated with other serious consequences, including an increased risk of common cancers and autoimmune, infectious, and cardiovascular diseases (CVDs) (3). Many lifestyle factors, including smoking, alcohol consumption, and lack of physical activity; malnutrition; metabolic abnormalities, such as diabetes, insulin resistance, and obesity; and excessive stress have a negative impact on the risk for CVDs. In addition, endogenous and exogenous conditions, behavioral consequences, environmental conditions, and epigenetic influences have major effects on the development of CVD.

Conditions and issues such as pollution, consumption of contaminated water and food, infectious and parasitic diseases, climate change, and deficiencies in micro-nutrients (vitamins and minerals) have deleterious effects on CVD (4, 5). Vitamin D has anti-inflammatory and anti-mitotic actions that facilitate stabilizing the endothelium, one of the key explanations for its cardiovascular-protective effects (6, 7). In the human body, approximately 80% of the daily vitamin D requirement (D_3) should be generated via skin after exposure to ultraviolet B (UVB) rays from the sun (8, 9).

Dietary vitamin D consists of D_2 and D_3, but the diet provides insufficient amounts of vitamin D. Therefore, in the absence of supplements, a significant portion of vitamin D in humans needs to come from exposure to solar UVB (2, 10) or individuals are likely to experience

vitamin D deficiency. Figure 1 illustrates the routes of generation and key sites of activation of vitamin D; 25-hydroxylation in the liver generates 25-hydroxy vitamin D [25(OH)D] and 1α-hydroxylation in renal tubules produces its active secosteroid hormone, $1,25(OH)_2D$.

< Figure 1 >

1.1 Reliability issues related to vitamin D assays:

Although several excellent attempts have been made globally to standardize vitamin D assays, the reliability of the generated data remains a concern. With the continued interest surrounding vitamin D assays, it is a crucial to optimize, validate, and standardize assay methods of 25(OH)D and its metabolites (11). Doing so would minimize variability and misinterpretations (12).

Because of increasing clinical demands and requests for measurements, it is essential for clinical laboratories to be able to handle high volumes of 25(OH)D testing. Consequently, several automated assays for the measurement of 25(OH)D have become available in recent years (11). However, ongoing concern exists about the variability, (in)accuracy, and specificity of automated 25(OH)D assays in comparison with the results of those from the isotope dilution, solid-phase extraction liquid chromatography/tandem mass spectrometry (ID-XLC-MS/MS) method (12-15).

These automated 25(OH)D assays are based on enzyme linked immunosorbent assay (ELISA), radio immunoassay (RIA), and chemiluminescence immunoassay (CLIA). Examples of these commercially available assays include Architect, Centaur, iSYS, Liaison, and Elecsys, and Diasorin. Their results can be compared with extraction-based assays, such as ID-XLC-MS/MS and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. However, these automated assays show greater deviations and variabilities in measured 25(OH)D concentrations than are seen with the ID-XLC-MS/MS method (11, 14-16).

In one systematic analysis, compared with the LC-MS/MS method, the IDS-iSYS 25(OH)D and ORGENTEC $25(OH)D_3/D_2$ assays demonstrated mean relative biases of 16.3% and 17.8%, whereas the IDS-iSYS 25(OH)D assay showed a mean bias of 1.5% with

approximately 20% inter-assay variability (17). Meanwhile, the measurement of circulating vitamin D metabolites, such as 3-epimer and 24,25-$(OH)_2$D, remains a research tool with little benefit to practicing physicians.

As a part of the effort to attain standardization, the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland) Standard Reference Materials (SRM) has been distributed to participating laboratories. To minimize variabilities and maintain the accuracy of testing, Certification of Standard Reference Materials, the development and application of a standardization protocol, and the Vitamin D External Quality Assurance Survey (DEQAS) have been developed (13, 18).

The use of such quality control procedures has markedly improved the standardization, accuracy, and comparability of methods (13); all clinical laboratories that measure 25(OH)D are encouraged to join this quality assurance program. When choosing a method to use, each laboratory must balance the factors of turnaround time, convenience, cost, and the specificity and accuracy of the information obtained (12). Therefore, when interpreting the results of 25(OH)D measurements, careful consideration of the measurement method used by the laboratory and its participation in a validated quality control program such as DEQAS are needed (16). When a scientific paper is published, readers assume that vitamin D assays have been carried with all of the aforementioned precautions taken to assure the validity of data.

1.2 Role of vitamin D in the cardiovascular system:

The roles of vitamin D in the regulation of bone-mineral metabolism (19-24), the musculoskeletal system (25-27), and fracture prevention (28-32) have been well established. Recent data suggest associations between hypovitaminosis D and metabolic syndrome, diabetes, hypertension, and immune diseases (33-36). Vitamin D deficiency is also an independent risk factor for CVDs (6, 7, 37-41). In addition, ecologic and observational studies point toward an association between serum 25(OH)D levels and impaired general health, CVD risk factors (37, 39), CVD mortality, and cancer mortality (37, 39, 42).

Cross-sectional studies report inverse associations between less sun exposure [and thus low serum 25(OH)D levels] and increased risks of myocardial infarction (MI), stroke, heart failure, and peripheral vascular diseases (43-51). Through multiple mechanisms, vitamin D plays an important role in cardiovascular protective effects, including regulation of blood pressure and vascular smooth muscle cell functions, modulation of vascular tone, and maintenance of healthy endothelium (51-53). Having low serum 25(OH)D levels over longer periods increases the vulnerability to many diseases, including CVDs (54, 55).

In humans, vitamin D levels fluctuate with the seasonality, with the lowest levels observed during the winter (56, 57), which is also a time with increased incidences of ischemic heart disease and myocardial infarctions (MI) (58). The seasonal fluctuations in mortality (i.e., decreased CVD-related deaths in the summer and increased deaths in the winter and among those who live in higher latitudes) likely are related to the lower serum vitamin D levels during winter, which provide less protection for cardiovascular tissues (59).

Nitric oxide (NO) is a rapidly acting, endogenous vasodilator (60, 61). Human skin contains photo-labile NO derivatives, such as nitrite and S-nitroso thiols. Following UVA irradiation, via non-enzymatic mechanism, NO is released by these compounds (62). One study reported that the UVA irradiation of human skin caused a significant lowering of blood pressure (62). In addition, the UVA-induced release of NO in human skin has been demonstrated using confocal fluorescence microscopic studies of skin pre-labeled with the NO-imaging probe diaminofluorescein 2 diacetate in an NO-synthase independent but dose-dependent manner (57). These data suggest that low UV exposure-induced NO liberation in subcutaneous tissues may contribute to reduced mortality and lowering of blood pressure after exposure to sunlight (57).

1.3   Inflammation, vascular calcification, and hypovitaminosis D:

Pro-inflammatory cytokine plasma matrix metalloproteinase-9 concentrations are higher in patients with CVD (6, 63, 64). Vitamin D deficiency is also associated with higher circulating concentrations of the inflammatogenic matrix metalloproteinase-9, which influences vascular wall remodeling and calcification, whereas vitamin D supplementation decreases serum matrix metalloproteinase-9 concentrations by 68% (65).

Some of the beneficial effects of vitamin D in reducing the risk of CVD appear to mediate through the reductions in inflammation (6), the risks of insulin resistance metabolic syndrome, type 2 diabetes (T2D), and vascular calcification (66-69). Meanwhile, hypercalcemia and hyperphosphatemia increase vascular calcification (70-73).

Vascular calcification is an active and complex process that involves numerous mechanisms (71, 74). Calcium deposition in arterial walls worsens existing renal failure and increases premature deaths (75). In persons with renal failure, vitamin D deficiency and hyperphosphatemia further enhance the likelihood of vascular calcification (70, 71, 74, 76, 77). The occurrence of vascular calcification enhanced in persistent hypocalcemia and hypercalcemia (70, 76, 77) significantly increases cardiovascular-related mortality (78-80).

However, the condition is not necessarily driven by calcium intake, serum calcium levels, or vitamin D (72). Vascular calcification leads to increases in arterial stiffness and pulse wave velocity, thus increasing susceptibility to CVD and mortality (75, 81, 82). In addition, in patients receiving dialysis, vascular reactivity is significantly correlated with serum vitamin D and PTH levels (52). Figure 2 illustrates the role of vitamin D and vitamin D receptor (VDR) and the pathways of vascular calcification that lead to increased mortality(83).

< Figure 2 >

Vascular calcification reduces the elasticity of blood vessels with consequent impairment of hemodynamic variables. These lead to the development of arterial hypertension, endothelial abnormalities and atheroma formation, cardiac hypertrophy, ischemic heart disease, MI, peripheral artery disease, and increased mortality (2, 68, 84, 85).

1.4 Potential adverse effects from supplemental vitamin D:

Although hypercalcemia and hypercalciuria are common presentations of vitamin D overdose, nephrocalcinosis can develop in susceptible individuals with any prolonged hypercalcemia (e.g., hyperparathyroidism), or following consumption of high doses of vitamin D (e.g., more than 40,000 IU vitamin D daily) for a long duration. Nevertheless, evaluation of more than 20,000, serum 25(OH)D levels performed at the Mayo Clinic, USA,

those with a 25(OH)D levels higher than 50 ng/mL, except one others had normal serum calcium levels (86).

A reported in Canadian adults, who those who consumed up to 20,000 IUs of vitamin D_3 daily, increased their serum 25(OH)D levels up to 60 ng/mL (150 nmol/L), but they fund had no evidence of toxicity as evident by hypercalcemia or hypercalciuria (87). In another study, those who were treated with 50,000 IUs of vitamin D_2 once in 2-weeks (equivalent to ~3,300 IU/day) up to 6-years maintained their 25(OH)D concentrations at optimal range—between 40-60 ng/mL (100-150 nmol/L) without having vitamin D toxicity (88). In general, it is unlikely that a person develops adverse effects (except allergy) of vitamin D, if the doses are less than 20,000 IU a day and serum 25(OH)D levels are consistently less than 100 ng/mL (250 nmol/L).

1.5   Epigenetics:

Both genetic and epigenetic determinants, such as sun exposure, environmental toxins, and dietary factors, can alter vitamin D metabolism and its response, which may manifest as suboptimal, aberrant, or non-classical in clinical presentations. Modification of expression by epigenetic mechanisms and dietary substances includes vitamin D (89-91). In addition, the downstream regulatory pathways in vitamin D signaling and VDR polymorphism can lead to wide variability in outcomes among different populations.

Some of the unexplained findings associated with vitamin D deficiency and other diseases may be attributable to epigenetic changes in vitamin D and its enzyme system, as well as toxic and nutritional influences (e.g., changes in vitamin D, vitamin B-complex status, VDR polymorphisms, and the homocysteine and methylenetetrahydrofolate reductase genes).

In addition to the known genomic and non-genomic regulation of vitamin D, the epigenetic mechanisms modify the functions and thus outcomes of this key hormone (89-94). Modulations of activity can occur through the vitamin D activating enzymes of the human CYP27B1 (1α-hydroxylase) and CYP24A1 (24-hydroxylase) genes and polymorphism of other cytochrome P450 enzymes.

Vitamin D is essential for the functioning of many genes, so hypovitaminosis D negatively affects certain physiological processes, including brain development and DNA repair (95-97). For example, several reports suggest that prenatal and early postnatal vitamin D deficiency increases the risk for autism; the likely mechanisms include impaired brain development and increased *de novo* mutations (94, 98). Through epigenetic mechanisms, human immunodeficiency virus (HIV) causes podocyte injury through the downregulation of VDR and the activation of the renin–angiotensin system (92, 99).

European researchers have observed that certain psychiatric disorders, such as schizophrenia, are three times more common in dark-skinned immigrants and their second-generation offspring than in native-born white people (99). The reasons given have centered on epidemiological biases and psychological factors, such as racism, abuse, or social defeat, with no biological hypotheses having been tested. These European researchers have suggested that changes in sun exposure and diet and stress associated with immigration (cultural shock) may explain the increased risk for psychosis in immigrants (99).

Epigenetic changes secondary to diet or stress and changes in gene expression can influence vitamin D-related outcomes, including increasing the risk for psychosis, CVD, allergy, autoimmune disease, and certain cancers (93, 100). Vitamin D insufficiency may explain why dark-skinned immigrants experience psychosis when moving to high latitude countries, and maternal hypovitaminosis during pregnancy may increase the risks in the second generation (99).

It is known that $1,25(OH)_2D$ has anti-tumor properties; it is degraded by the product of the CYP24A1 gene, which is downregulated in human prostate cancer (and cancers of the thyroid, lung, rectum, colon) by unknown mechanisms (90, 93, 101, 102). Although the CYP24A1 expression is inversely correlated with promoter DNA methylation in prostate cancer cell lines, repression of CYP24A1 gene expression in human prostate cancer cells is mediated by promoter DNA methylation and repressive histone modifications (101). These changes can be attributable to the cancer prevention (or exacerbation) properties of $1,25(OH)_2D$ and explain the higher incidence of prostate cancer observed in those with

increased fluctuations of intracellular $1,25(OH)_2D$ levels (i.e., the U-shape curve described in some patients with prostate cancer) (103-105).

2.0   CLINICAL STUDY DATA:

Many randomized clinical trials (RCTs) and prospective and cross-sectional studies report statistically significant inverse associations between 25(OH)D levels and CVD risk factors in various populations, locations, and circumstances (106).

2.1   Case–control studies:

In a community-based, case–control study, the relationship between the plasma level of 25(OH)D and MI was investigated. Analyses of blood samples were performed in 179 patients who presented to emergency rooms with MI within 12 hours of the onset of symptoms. This group was compared with an age- and sex-matched control group. Those who experienced MI had significantly lower mean $25(OH)D_3$ levels than did control subjects (12.8 versus 14.4 ng/mL; p = 0.017, albeit both groups were vitamin D deficient); the case–control differences were greatest in the winter and spring. The authors of the study concluded that having higher serum 25(OH)D levels was protective against MI, with a relative risk of 0.43 (95% CI = 0.27, 0.69). Overall, a decrease in MI risk was observed in those with higher serum vitamin D_3 levels during all four seasons (107).

In another case–control study, subjects with serum 25(OH)D levels of less than 15 ng/mL had twice the incidence of MI than did those with levels greater than 30 ng/mL (108). This difference can be explained by the vascular-protective effects of 25(OH)D and by improving endothelial cells and vascular smooth muscle cell functions through the intracellular action of $1,25(OH)_2D$ (109-111). The Framingham Offspring Study also reported that those with serum 25(OH)D levels of greater than 30 ng/mL had a 50% reduction in cardiovascular events (49).

Large cohort analyses report a strong association between vitamin D status and cardiovascular deaths and all-cause mortality (84, 112-114). Several other studies confirm inverse associations of serum 25(OH)D levels with the prevalence of coronary artery

disease, peripheral vascular diseases, and heart failure (45, 46, 51, 108, 113, 115, 116). Most research supports that serum 25(OH)D levels greater than 30 ng/mL (75 nmol/L) [range between 30 and 80 ng/mL (200 nmol/L)] are protective against CVDs and are associated with a lower risk of experiencing congestive heart failure (117-122), hypertrophic cardiomyopathy (123), vascular calcification (124) and atherosclerosis (49, 108, 115).

The relationships between vitamin D, parathyroid hormone (PTH), and sudden cardiac deaths also have been evaluated. Among 267 study participants, 11.7% who had secondary hyperparathyroidism [i.e., elevated serum PTH and low 25(OH)D levels] had a twofold higher risk of sudden cardiac death [hazard ratio: 2.2 (95% CI: 1.2–4.1); p = 0.02] compared with participants with normal serum PTH and 25(OH)D levels (125).

2.2   Cross-sectional studies:

Several cross-sectional studies have reported an inverse correlation between serum 25(OH)D levels and CVD mortality. For example, in one study, after adjustment for potentially confounding variables, the hazard ratios (HR) for CVD mortality were correlated with serum 25(OH)D levels. In the lowest quartile of 25(OH)D (<17.2 ng/mL), the HR was 3.2; in the second quartile (17.3 to 23.2 ng/mL), the HR was 2.4; and in the third quartile (>23 ng/mL), the HR was 2.3 (126). The higher the serum 25(OH)D levels (even within the deficient and insufficient range), the smaller the risk of CVD mortality.

A cross-sectional analysis was performed of serum 25(OH)D in 195 healthy 20- to 40-year-old European adults with a BMI between 27.5 and 32.5, intact PTH, and biomarkers of CVD risk. Serum 25(OH)D levels of 11% of the study participants were below 10 ng/mL, and those individuals had a higher relevance of metabolic syndrome. In addition, 66% of Icelandic, 43% of Irish, and 30% of Spanish young adults had serum 25(OH)D concentrations below 20 ng/mL (127).

In a vitamin D supplementation study, improvement of several cardiovascular functions was reported (114, 128). With death used as a dependent variable in a logistic regression analysis, relationships were demonstrated between serum 25(OH)D levels and coronary

artery disease [odds ratio (OR), 2.5; confidence interval (CI), 1.9–3.2]; diabetes mellitus (OR, 1.7; CI, 1.3–2.2); cardiomyopathy (OR, 3.1; CI, 2.2–4.4); and hypertension (OR, 1.6; CI, 1.3–2.1) (129). These data further strengthen the hypothesis that vitamin D is cardio-protective. However, other studies have shown no such beneficial effects with vitamin D treatment (130-132).

One large cross-sectional analysis of data reported that, after adjusting for age, gender, body mass index, education level, residence location, and region, the subjects with the lowest serum 25(OH)D levels [less than 10 ng/mL (< 25 nmol/l)] had a twofold higher prevalence rate of CVD compared with subjects with serum levels more than 30 ng/mL (75 nmol/l) (39). In addition, the prevalence of other risk factors for CVD (including higher waist circumference, fasting glucose, low-density lipoprotein cholesterol, and triglyceride levels; and lower high-density lipoprotein cholesterol levels) also was higher among subjects in the lowest category than among those in the highest category (39).

In comparison to RCTs, case-controlled, cross-sectional, and epidemiological studies are likely to have confounding factors. Small scale RCTs and case reports of children with rickets (133, 134) have generated the hypothesis of a causal link between vitamin D and CVD. However, well-designed [i.e., longer duration studies following normalization of serum 25(OH)D levels] and clinical trials with adequate statistical power showing the prevention of CVD after vitamin D supplementation have not been reported yet (135).

2.3 Randomized clinical study data on cardiovascular diseases:

A prospective case–control study of more than 18,000 men reported a statistically significant correlation between low 25(OH)D levels and increased risk for MI, even after adjustments were made for traditional CVD risk factors (108). The association of vitamin D status with cardiovascular mortality rates was investigated in a clinical study of 11,000 persons, (129). The mean age of the study group was 58 ± 15 years, body mass index was 30 ± 8 kg/m^2, and the mean serum 25(OH)D level was 24 ± 14 ng/mL.

Those with serum 25(OH)D levels below 30 ng/mL had a significantly higher incidence of CVD, including hypertension, coronary artery disease, cardiomyopathy, congestive heart

failure, peripheral arterial diseases, stroke, and diabetes. At the end of the 5.5-year study period, mortality was two times higher in those with serum 25(OH)D levels below 30 ng/mL than in those with serum 25(OH)D levels ≥ 31 ng/mL (129).

Serum 25(OH)D levels have been found to be inversely correlated with CVD risk factors, such as dyslipidemia, hypertension, and diabetes mellitus (106). In addition, several prospective, observational, and cohort studies have reported associations between higher serum 25(OH)D levels and lower risk of CVD (51, 129, 136) and healthful genotypic effects on serum lipids (137). The cardiovascular risks were lower in those with serum 25(OH)D levels above the minimal levels recommended by the Endocrine Society guidelines (30 ng/mL) (114, 138, 139).

Other studies have confirmed the association between vitamin D status and CVD hard end points (107, 117, 129, 140-148). In addition, the rates of vitamin D deficiency in people increase with the distance they live from the equator, which is also associated with increased prevalence of diabetes and CVDs (149). For example, higher rates of ischemic heart disease are reported in countries further from the equator (and those who are living at higher altitudes), where people are exposed to less UVB and thus have a high prevalence of hypovitaminosis D (150). Although the evidence is indirect, the increased rates of CVDs in these countries have been attributed to the high prevalence of vitamin D deficiency in a population that experiences less exposure to sunlight (151, 152).

A Mendelian randomization analysis of 95,766 participants in the Copenhagen City Heart Study examined the associations of genetically low 25(OH)D concentrations with increased mortality attributable to various causes adjusted for common risk factors (153). A multivariable adjusted hazard ratio for a 20 nmol/L (8 ng/mL) of plasma 25(OH)D levels was related to increased mortality.

The multivariable adjusted hazard ratio for those with a plasma 25(OH)D concentration less than 20 nmol/L, all-cause mortality was 1.19 [95% confidence interval (CI), 1.14 to 1.25); cardiovascular mortality, 1.18 (95% CI, 1.09 to 1.28); cancer mortality, 1.12 (95% CI, 1.03 to 1.22); and for other mortality 1.27 (95% CI, 1.15 to 1.40). Although the genetic and observational odds ratios were significant for cancer and other mortalities [1.13 (CI, 1.03 to

1.24)], but not for cardiovascular mortality [0.77 (CI, 0.55 to 1.08)] (153). The author concluded that the genetically low 25(OH)D levels are causally associated with cancer and other mortality, but the association with cardiovascular mortality could be a confounder.

2.4 Meta-analyses data:

A meta-analysis of 18 RCTs using vitamin D doses of 300 to 2,000 IU/day reported prevention in or reduction of the incidence of several disorders, including fractures, bone loss, falls, colorectal cancers, CVDs, and all-cause mortality (51, 154-157). In addition, clinical studies support a role for vitamin D (109, 158) in maintaining cardiovascular health through the direct action of the vitamin on cardiomyocytes and the indirect actions through the circulating hormone and calcium (159, 160).

Data also suggest that decreased serum vitamin D levels or abnormalities of VDR through a number of mechanisms enhance vascular calcification and increase the risk for premature death (72, 74, 76, 77). The positive effects of supplementation with vitamin D and correlations between serum 25(OH)D concentrations and the cardiovascular system (including hazard ratios) are not linear (Figure 3). The greatest beneficial effects are demonstrable in those with lower concentrations of 25(OH)D levels, with little or no effect seen for supplementation in those with 25(OH)D levels greater than 30 ng/mL (72, 77, 157)(157, 161).

< Figure 3 >

Another meta-analysis examined the blood levels of 25(OH)D and concluded that levels are lower in patients with peripheral artery disease than in those with normal leg circulation (162). The authors of that meta-analysis examined six case–control studies (n = 6,418 individuals) that fulfilled the inclusion criteria. The analysis reported that blood 25(OH)D levels of those with peripheral artery disease were significantly lower than levels in the control group standardized mean difference (SMD or SSMD, a measure of effect size) -0.32; 95% CI, -0.58, -0.05; p = 0.02. The lower levels of circulating 25(OH)D were associated with peripheral artery disease, particularly in patients with critical limb ischemia.

The authors concluded that low 25(OH)D levels may contribute to the development of advanced disease (162).

Overall meta-analyses of RCTs showed no consistent pattern or effects on endothelial function, blood pressure, left ventricular functions, myocardial infarction, stroke, or CV-associated mortality (135, 163). However, some of these trials were poorly designed (164) and were underpowered to detect differences of the intervention.

2.5 The need for adequately powered, well-designed vitamin D RCTs:

There is a dearth of well-designed, adequately powered RCTs testing vitamin D hypotheses related to extra-musculoskeletal diseases. Despite the need, a trial that is close to an ideal has yet to be conducted. Because of the inferior study designs plus selection bias, not one of the three currently conducted large RCTs, including the Vitamin D and Omega-3 Trial (VITAL) (165), even after a cost of more than $20 million, is likely to generate data to resolve ongoing issues related to the plausible benefits of vitamin D.

Many of the vitamin D RCTs are based on assumptions, guidelines, and pharmaceutical RCTs, the latter of which assume that the intervention agent (e.g., vitamin D or a drug) is the only source and that there is a linear dose–response relation; neither assumption is accurate (166, 167). The main goal of vitamin D research is to determine the relation between serum 25(OH)D concentration and health outcomes. Thus, the RCTs that are systematic reviews and meta-analyses of vitamin D_3 should include only trials that begin with subjects with hypovitaminosis D and for which participants in the treatment group had adequate doses of vitamin D to increase 25(OH)D concentrations to a level at which measurable beneficial effects could be expected (168). In the absence of such conditions and based on selection and other biases, some meta-analyses have reached erroneous conclusions (135, 169).

The guidelines recommended by Robert Heaney for all nutrient intervention RCTs, including those for vitamin D_3, are based on biological response to nutrients and should be followed (170). The goal of RCTs and systematic reviews should be to determine the relationships between the 25(OH)D concentration and health outcomes and not to

determine whether giving a specific amount of vitamin D_3 has any health benefit (166, 168); otherwise, the conclusions made can be misleading. Some of the conditions that need to be incorporated into such a study design are illustrated in Table 1.

<Table 1 >

*Rationale: Because of the genetic and epigenetic variabilities, approximately 20% of persons with hypovitaminosis-associated diseases need blood 25(OH)D levels greater than 50 ng/mL (125 nmol/L) to achieve adequate amounts of the vitamin in their responsive cells. Any degree of renal impairment would aggravate this [http://www.vitamindwiki.com/tiki-index.php?page_id=813]. Additional suggestions to improve RCTs have been made by other authors (167, 168, 170-173).

Having an adequately powered, well-designed, long-term RCT is the only way to answer the multitudes of questions and come up with definitive conclusions regarding the effectiveness of vitamin D in non-skeletal tissues/systems in those who are deficient. Individual variability in absorption, catabolism, and responsiveness (174), as well as the epigenetic issues, creates situations in which direct dose–response relationships may not exist in some people supplemented with vitamin D. Therefore, study sample sizes need to increase to accommodate the individual variability of responsiveness.

3.0 Vitamin D deficiency and cardiovascular diseases:

3.1 Cardiovascular diseases and serum vitamin D levels:

In a prospective, 4-year, follow-up clinical study, men and women with low vitamin D concentrations (<15 ng/mL) were three times more likely to have hypertension than were those with higher (>30 ng/mL) 25(OH)D levels (175). In addition, in the Framingham Heart Study, patients with low vitamin D concentrations (<15 ng/mL) had a 60% higher risk of heart disease than did those with higher concentrations (49). The same data demonstrated that subjects with low vitamin D concentrations were twice as likely to have MIs than were those with higher concentrations (>30 ng/mL) (108).

$1,25(OH)_2D$ has a negative feedback effect on production and serum levels of PTH. Active vitamin D also indirectly suppresses biosynthesis and secretion of renin, thus suppressing angiotensin II (176-178). The PRIMO study demonstrated that treatment with the vitamin D metabolite paricalcitol normalized heart-wall thickness and left-ventricular end-diastolic pressure (179), which is postulated to occur through suppression of angiotensin II. Most evidence supports an association between 25(OH)D deficiency and increased risk for morbidities associated with CVD and mortality, even in the absence of chronic kidney disease (108, 115, 180-183). A meta-analysis of all-cause mortality found 36 ng/mL to be the level at which there was no further reduction in mortality for higher values (184).

3.2 Role of vitamin D in subduing the renin–angiotensin system:

Exposure to UVB decreases blood pressure, presumably by increasing circulatory $1,25(OH)_2D_3$ levels and indirectly suppressing the production and secretion of renin. In addition, $1,25(OH)_2D$, PTH, and calcium have direct effects on cardiovascular tissue and functions (120-122). Data also support the idea that low vitamin D status is a contributory factor in the pathogenesis of congestive cardiac failure (117) and leads to increased cardiovascular-related mortality (49, 185-189). Taken together these data suggest that correcting vitamin D deficiency, rather than pursuing treatments with expensive cardiac drugs, should be a starting point in a treatment regimen.

Although evidence indicates the existence of biological associations that link low vitamin D with endothelial dysfunction and CVD, no RCT evidence demonstrates that CVDs are prevented after vitamin D supplementation (69, 84). Therefore, from the hypotheses generated by published observational studies, adequately powered clinical trials should be designed and carried out in individuals with serum vitamin D levels less than 20 ng/mL to test the hypothesis that vitamin D supplementation [elevating and maintaining the serum 25(OH)D levels above 30 ng/mL] has cardiovascular-protective effects (114, 139). Such studies would also provide information on the optimal serum 25(OH)D levels needed to achieve such beneficial end points.

Figure 4 illustrates the negative correlations of serum 25(OH)D levels and survival. A relationship is evident when serum 25(OH)D levels are less than 30 ng/mL (<75 nmol/L)

(190-192). The mortality (reported in quartiles) data shown in Figure 4 are significantly different even after adjusting for a number of variables, including age, sex, diabetes mellitus, smoking status, hypertension, HDL cholesterol levels, and glomerular filtration rate (193).

< Figure 4 >

3.3 Vitamin D effects in the cardiovascular system—biological evidence:

The National Health and Nutrition Examination Survey (NHANES) reported a link between vitamin D deficiency and atherosclerosis. Low serum 25(OH)D levels were associated with a higher prevalence of peripheral arterial disease (194) and decreased levels of high-density lipoprotein cholesterol-associated apolipoprotein A-I (195).

In a randomized, placebo-controlled intervention study in postmenopausal women, vitamin D supplementation was shown to have a beneficial effect on the elastic properties of the arterial wall (196). Another study confirmed that the pulse wave velocity was shown to decrease as serum 25(OH)D and $1,25(OH)_2D$ levels increased ($p < 0.001$ for both) (197).

The target of calcineurin and the transcription factor nuclear factor of activated T-cell (NFAT) also have been linked to the development of cardiac hypertrophy. Studies reveal that isoproterenol treatment *in vitro* of neonatal rat cardiac myocytes resulted in myocyte hypertrophy and increased myocyte-enriched calcineurin-interacting protein-1 (MCIP1) expression. Co-administration of $1,25(OH)_2D_3$ resulted in a dose-dependent favorable reduction in MCIP1 expression (198). Vitamin D and the VDR also have been implicated in the support of normal endothelial function. Figure 5 illustrates meta-analyses data from prospective clinical studies on the relationship between the relative risks of CVD and mean serum levels of 25(OH)D (190-192).

< Figure 5 >

3.4 Endothelial dysfunction, inflammation, and vitamin D deficiency;

Endothelial dysfunction is associated with decreased vasodilatory ability and creation of pro-inflammatory and prothrombotic unhealthy states of the endothelium (199). Endothelial dysfunction plays a key role in many cardiovascular disorders, including the pathogenesis of atherosclerosis, initiation and progression of plaque formation (199), and increased arterial stiffness (69, 200). Supplementation of patients with vitamin D led to a statistically significant decrease in arterial stiffness compared with placebo (201, 202) and a reduction of the mean pulse wave velocity from 5.41 m/s (SD, 0.73) at baseline to 5.33 m/s (SD, 0.79) (p = 0.031) (203).

Endothelial dysfunction leads to the development of CVD. 3-hydroxy-3-methyl-glutaryl-co-enzyme A (HMG-CoA) reductase inhibitors (statins) are known to stabilize endothelium. Other studies have reported associations between vitamin D deficiency and endothelial dysfunction (68). Improvement with endothelial functions has been reported after supplementation with vitamin D or its analogs. For example, Gardner and colleagues tested the effects of a bioactive analog of $1,25(OH)_2D_3$, paricalcitol, in preventing cardiac hypertrophy in rats infused with moderate doses of angiotensin II (800 ng/kg/min) over a 2-week period.

Infusion of angiotensin II led to increased blood pressure, myocyte hypertrophy, expression of hypertrophy-sensitive fetal genes (i.e., atrial natriuretic peptide, B-type natriuretic peptide, and alpha skeletal actin gene expression), and increased cardiac interstitial fibrosis with augmented procollagen 1 and 3 expression. In each case, co-administration of paricalcitol (intraperitoneal injection of 300 ng/kg every 48 h) resulted in partial reversal of the negative effects of angiotensin II (204).

3.5 Hypertension, the renin–angiotensin system, and vitamin D:

Endothelial cells also express the 1α-hydroxylase enzyme 1,25(OH)$_2$D and have nuclear VDR (205). In addition, vitamin D decreases the expression of the renin gene and facilitates the control of blood pressure (94, 206). Interactions and downstream activations of VDR lead to a number of key physiological processes related to the cardiovascular system, including suppression of the renin–angiotensin–aldosterone system, regulation of cell apoptosis, and suppression of inflammation (205). Table 1 illustrates the relationship between blood pressure and serum 25(OH)D levels in a cross-sectional study of 2,722 individuals in the United States (207).

< Table 2 >

Several studies have suggested that the protective effect of vitamin D on the heart is exerted by suppressing the renin–angiotensin hormone system (118, 208). In addition to attenuating the renin–angiotensin–aldosterone system, 1,25(OH)$_2$D suppresses cellular inflammation in cardiac cells and endothelial and smooth muscle cells. Downregulation of the renin–angiotensin system would re-establish cardiovascular homeostasis, serum electrolytes, and intravascular volume.

Other observational studies have suggested vitamin D levels are inversely related to blood pressure (175, 209). Nevertheless, smaller and shorter duration studies using vitamin D have failed to demonstrate a relationship between supplementation and blood pressure (210-212). The potential mechanism for the link between vitamin D and high blood pressure involving inhibition of the renin–angiotensin–aldosterone was derived from *in vitro* and *in vivo* animal studies (119, 208). For example, data from the VDR-knockout mouse models suggest that the modulation of the renin–angiotensin system by vitamin D is involved in the development of left ventricular hypertrophy (118, 119, 208). Figure 6 illustrates the interactions of 1,25(OH)$_2$D with the renin–angiotensin–aldosterone system in blood pressure control.

< Figure 6 >

Many observational studies have suggested protective effects of exogenous 25(OH)D on CVD. These data warrant the establishment of appropriate national policies to recommend higher levels of safe sun exposure, together with food fortification and dietary and vitamin D supplementation (213, 214). The IOM committee evaluated only clinical trials in creating its report in 2011 (i.e., a partial evidence), and its public health-related recommendations are applicable only to North Americans; thus, it cannot be considered an authority. Nevertheless, it cautioned against such a policy, citing "insufficient" evidence (215).

Vitamin D knockout mice have an up-regulated renin–angiotensin system, as demonstrated by sustained increased angiotensin II renin mRNA associated with elevated blood pressure (118). In addition, genetic studies in mice revealed that vitamin D signaling inhibits the renin–angiotensin–aldosterone activity, working through suppressing transcription of the renin gene (109, 118, 208). Consequently, vitamin D plays a key regulatory role in blood pressure homeostasis (176-178). There is also evidence to suggest that optimization of serum 25(OH)D levels would attenuate the age-associated increase of systolic blood pressure (118, 216).

Vitamin D deficiency increases blood pressure and cardiac hypertrophy in rodents. In addition, $1,25(OH)_2D_3$ and its analogs have been shown to reverse myocyte hypertrophy *in vitro* and cardiac hypertrophy *in vivo* in the Dahl rats treated with agonists, spontaneous hypertensive rats, spontaneous hypertensive heart failure-prone rats, and the 5/6th nephrectomy model of chronic renal failure. A variety of CVDs, including congestive heart failure, MI, coronary artery disease, and peripheral vascular disease, have been linked to vitamin D deficiency.

3.6 Higher 25(OH)D levels are associated with lower all-cause mortality:

A meta-analysis of 11 observational studies of 60,000 individuals reported a risk reduction of 29% in level of mortality over a period of approximately 10 years for the highest versus the lowest category of 25(OH)D level (217, 218). Comparing graded levels of intake, the

reduction in risk was 14% for an increase of 5 ng/mL, 23% for an increase of 10 ng/mL, and 39% for an increase of 20 ng/mL of plasma levels of 25(OH)D, starting from a median of ~11 ng/mL. The participants who started with the lowest levels of serum 25(OH)D had greater benefits from additional vitamin D than did those who started with higher serum levels (i.e., 30–40 ng/mL).

In addition to ethnic-based differences, relationships between blood vitamin D levels and the risk of mortality in the general population have been described (219). African Americans in the United States had increased rates of vitamin D deficiency, an independent risk factor for cardiovascular and all-cause mortality (220). In parallel, excess CVD morbidity and premature mortality observed in the African American community in particular is a striking example of racial and ethnic disparity in health outcomes. It is hard to reduce or prevent racial-based healthcare disparity in the absence of rectifying vitamin D deficiency in the African American community.

4.0   DISCUSSION:

The heart and vasculature are important targets of vitamin D actions, and the activated VDR plays an important role in regulating cardiovascular function (221). 1-α-hydroxylase enzyme and VDR are present in vascular endothelial and smooth muscle cells, cardiac myocytes, and cardiac fibroblasts. Complete deletion of the VDR gene in mouse leads to hyporeninemic hypertension and cardiac hypertrophy (118).

Despite food fortification programs in most countries, vitamin D intakes are low in many groups, in part because of their unique dietary patterns, such as low milk consumption, vegetarian diets, limited or no use of dietary supplements, or changes away from their traditional food consumption (222). Food fortification and use of supplements can significantly increase population vitamin D intakes across all ages (222).

Groups of people that are at greatest risk for vitamin D deficiency include housebound, institutionalized, older and/or disabled people; dark-skinned people; night-shift and indoor workers; and those who lack skin exposure to sunlight for any reason (223). There are no

unreasonable risks from intake of less than 4,000 IU per day of vitamin D_3, 50,000 IU taken every other week, or from a population serum 25(OH)D level of 40 to 60 ng/mL.

The dietary or vitamin D supplements dose-dependently increase serum vitamin D levels (224). It has been reported that the dietary vitamin D needed to maintain serum 25(OH)D above 32 ng/mL (80 nmol/L) in adults during the winter is 41.1 µg a day (approximately 1,650 IU/day) (224). Older adults are more prone to vitamin D deficiency than are younger adults. With less than 15 minutes/day of sun exposure, older adults need a consistent daily oral intake of 24.7 µg (1,000 IU) to maintain their serum 25(OH)D levels above 20 ng/mL (50 nmol/L) and 38.7 µg (1,600 IU) per day to maintain levels above 32 ng/mL (80 nmol/L) (225).

Most of the clinical evidence supports the idea that having adequate 25(OH)D (i.e., more than 30 ng/mL) reduces cardiovascular risk (68). This is mediated through several mechanisms, including lowering blood pressure, calcification of arteries, and inflammation; reducing the levels of matrix metalloproteinase; and decreasing the incidence and severity of chronic kidney disease (6, 49, 226), diabetes, viral and bacterial infections, and infectious respiratory diseases (118, 221). In fact, blood vitamin D levels can be used as a surrogate marker for the prevalence of several diseases, including CVDs (67).

The findings in this review are similar to those in a review of the evidence based on Hill's criteria for causality in a biological system (227). That review also identified the paucity of clinical trials as being the major obstacle to the acceptance of the hypothesis that vitamin D reduces the risk of CVD. As noted by Bradford Hill (228), not all criteria need to be satisfied for causality to be claimed, but the more that are, the greater the likelihood of causality.

Collectively, the data presented in this review demonstrate that the VDR/vitamin D endocrine system plays a key role in the maintenance of cardiovascular homeostasis (69). Overall, data also suggest that normalizing the levels of the body's vitamin D stores will have important public health outcomes (84), cost savings, and will help control the incidences and prevalence of CVDs.

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Figure Legends:

Figure 1: Metabolic activation of vitamin D:

The generation of pre-vitamin D in the skin from the precursor 7-dehydrocholesterol, following skin exposure to UVB is illustrated. Pre-vitamin D together with the vitamin D absorbed via the gastrointestinal tract are transported to the liver, where 25-hydroxylase enzyme (CYP24A1) converts it to 25(OH)D, the body's storage form of vitamin D. 1α-hydroxylase enzyme (CYP27B1) is predominantly located in renal tubules (also present in other cells, such as in macrophage); converts 25(OH)D into its active hormonal form, 1,25(OH)D. Any excess vitamin D is converted to an inactive metabolite through 24-hydroxylation.

Figure 2:   Low activation of vitamin D receptors, increases the risk of arterial calcification and cardiovascular-related mortality:

Potential pathways showing the effects of low vitamin D levels enhancing vascular calcification and thus, increasing cardiovascular mortality [adapted from Andress, 2006 (83)].

Figure 3:   The risks of cardiovascular diseases decrease with increasing blood 25(OH)D levels:

Meta-analysis of CVD relative risks versus serum 25(OH)D levels from five case-controlled studies from Germany, Mexico, the United Kingdom and the United States [modified from (157, 161)]. Dashed lines indicate the plateaued hazard ratio at serum vitamin D levels of 75 nmol/L (30 ng/mL).

Figure 4: Higher blood vitamin D levels, increase the survival rate:

Vitamin D levels and cardiovascular mortality. Summarized data from meta-analysis of several prospective studies indicating an inverse relationship between serum 25(OH)D levels and relative risks for CVDs (114, 139). The hazard ratio for CVD mortality in the upper three 25(OH)D quartiles compared with the lowest quartile of serum vitamin D are presented: 5.38 (95% CI, 1.28–14.34; $p < 0.001$) [modified from Pilz et al, 2009 (193)].

Figure 5: Increasing blood vitamin D levels, decrease the relative risk of cardiovascular diseases:

Summarized data from meta-analysis of several prospective studies indicating an inverse relationship between serum 25(OH)D levels and relative risks for CVDs. A relationship is evident when the serum 25(OH)D levels are below 30 ng/mL (< 75 nmol/L) (190-192). Linear trend test: RR=1.03 (95% CI; 1.00-1.06) per each, 25 nmol/L decrements in blood 25(OH)D levels. Dashed lines indicate the plateaued relative risk at 1, when the mean serum vitamin D levels are at 75 nmol/L (30 ng/mL).

Figure 6: Physiological levels of vitamin D, decrease blood pressure and improve vascular functions:

Interactions of vitamin D metabolite with the renin–angiotensin system, leading to control of blood pressure and intravascular volume distributions [modified from Wimalawansa, 2012 (2)].
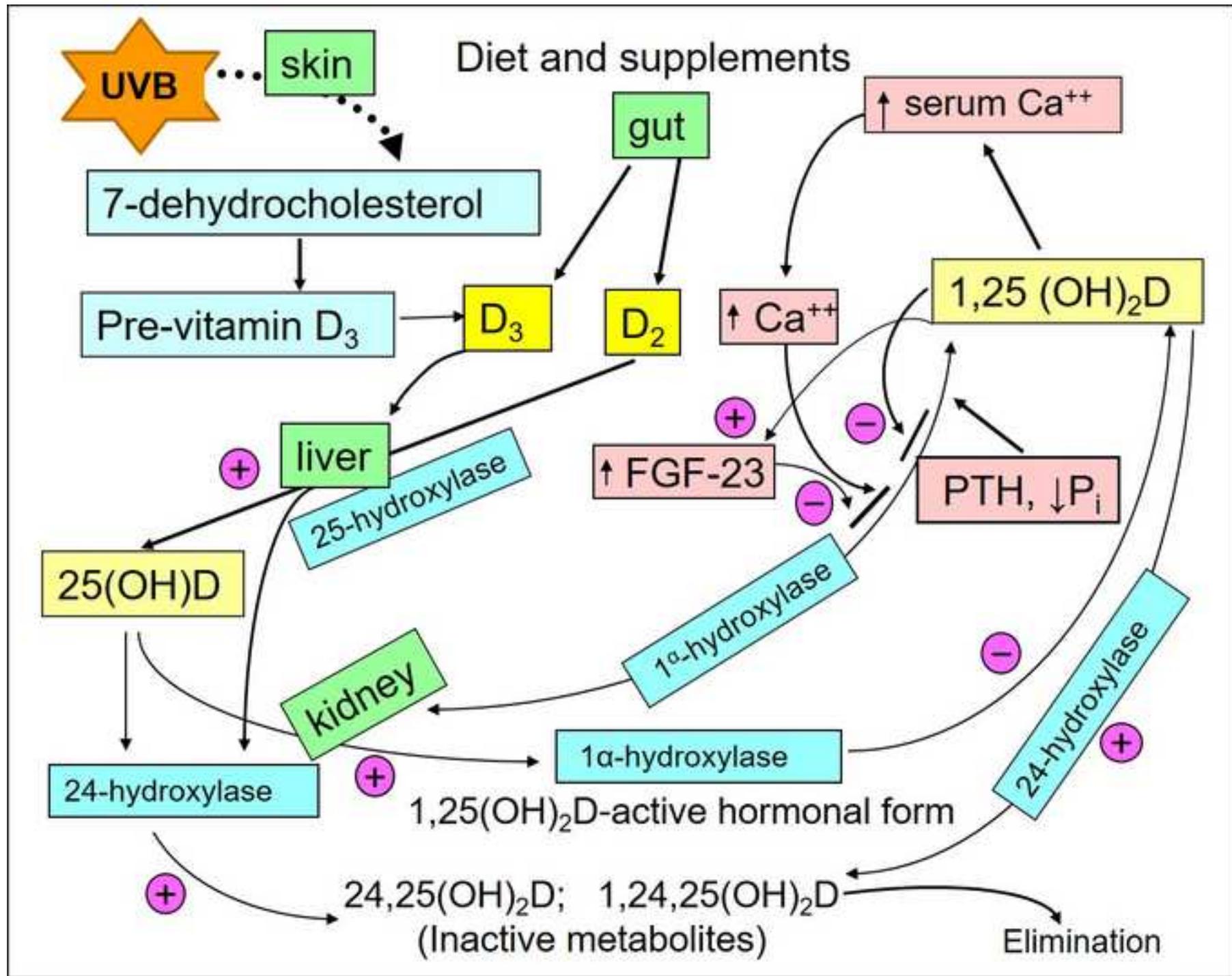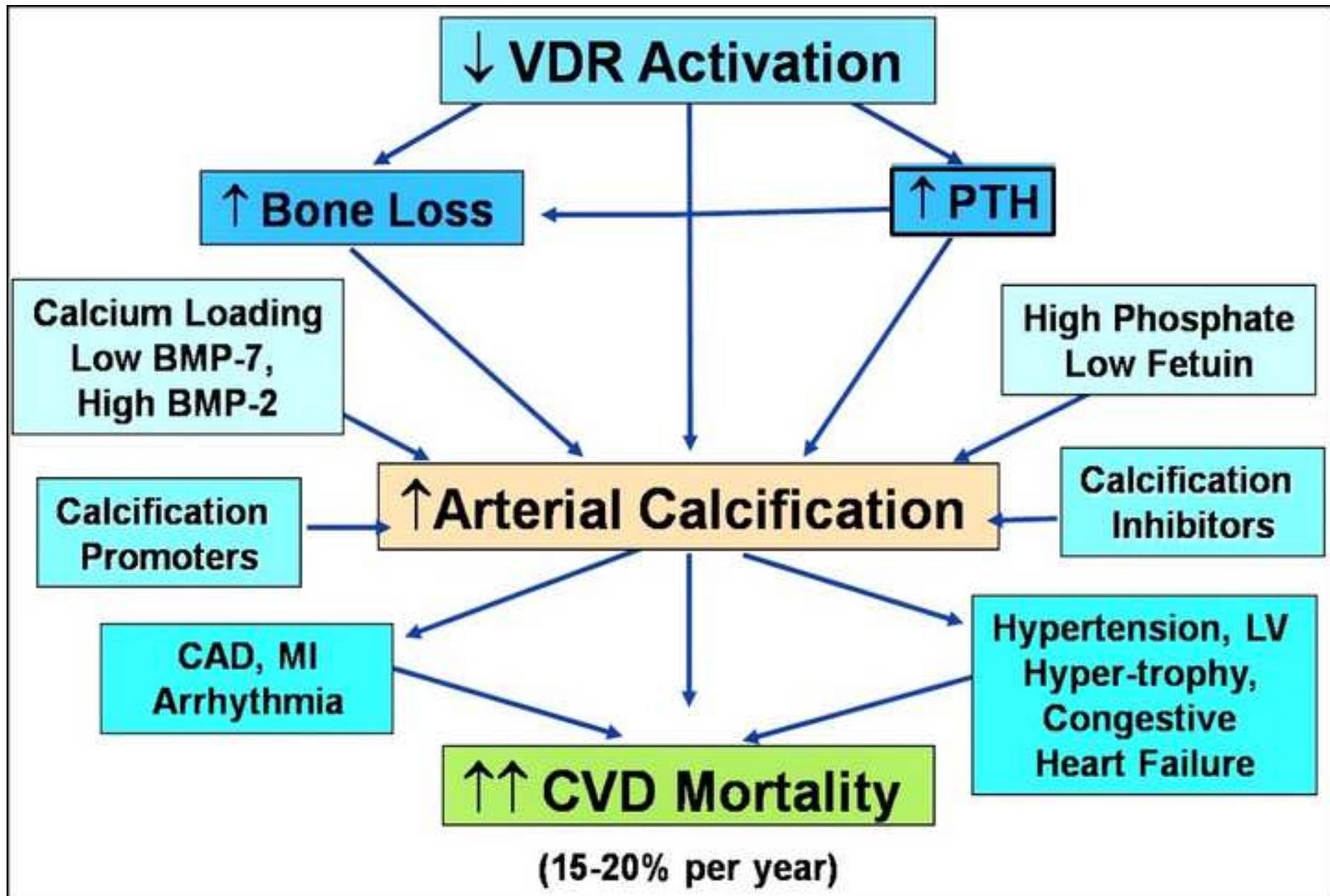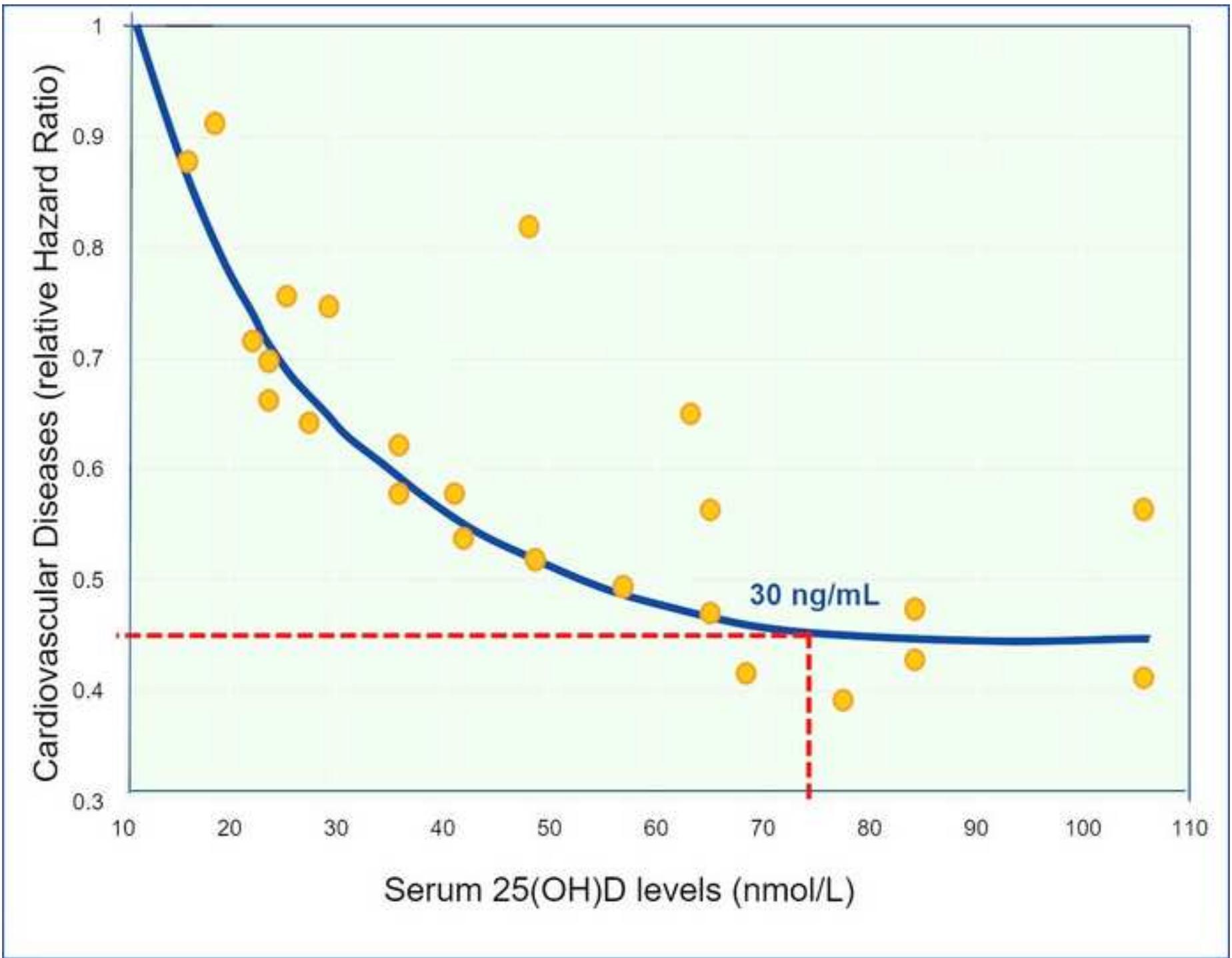
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure shows a bubble plot with y-axis "Relative Risk (RR) of Cardiovascular Diseases" ranging from 0 to 5, and x-axis "Serum 25(OH)D Concentrations (nmol/L)" ranging from 0 to 140.

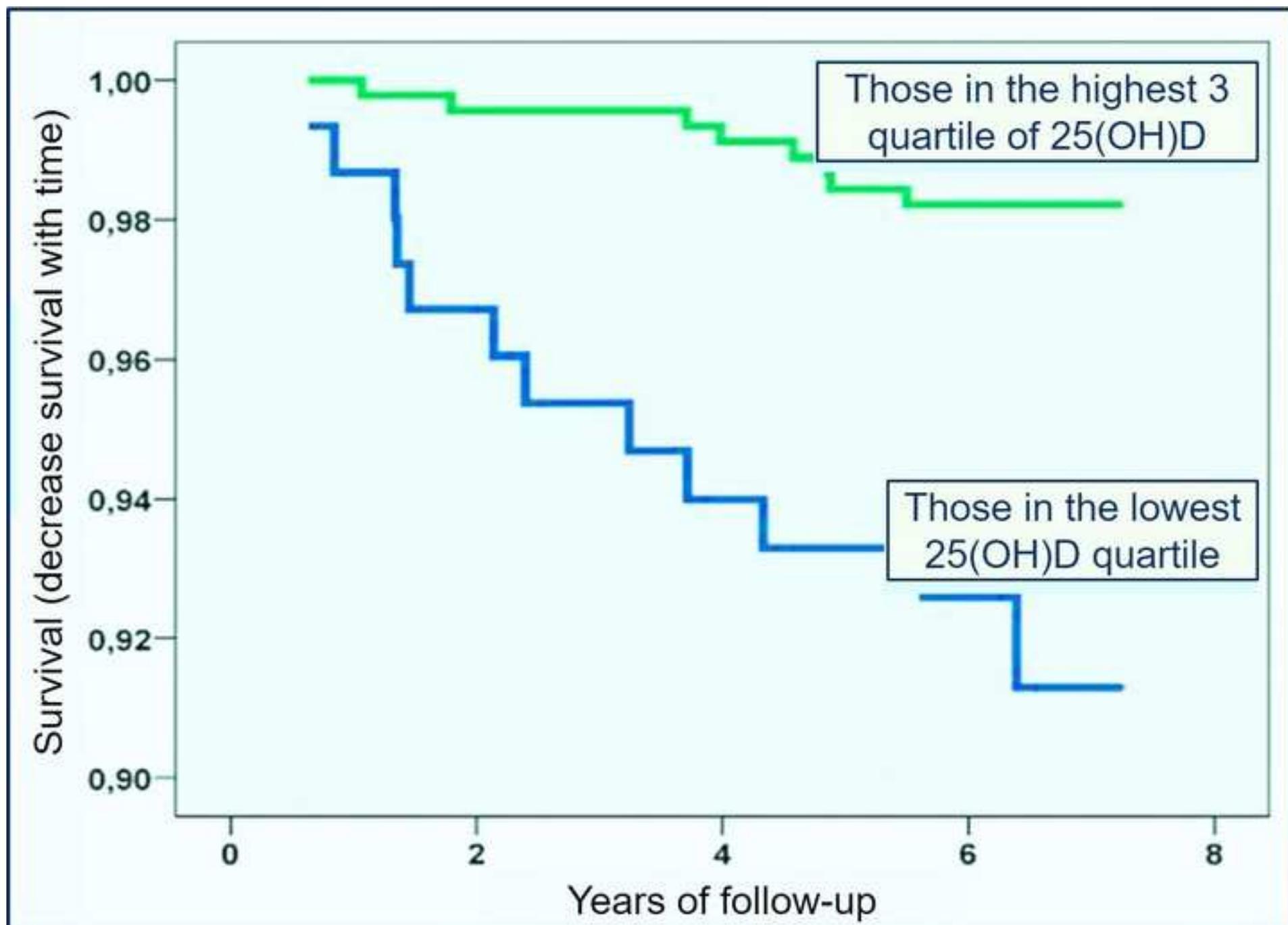Linear trend test: RR=1.03 (95% CI; 1.00-1.06) per 25 nmol/L decrements in 25(OH)D level
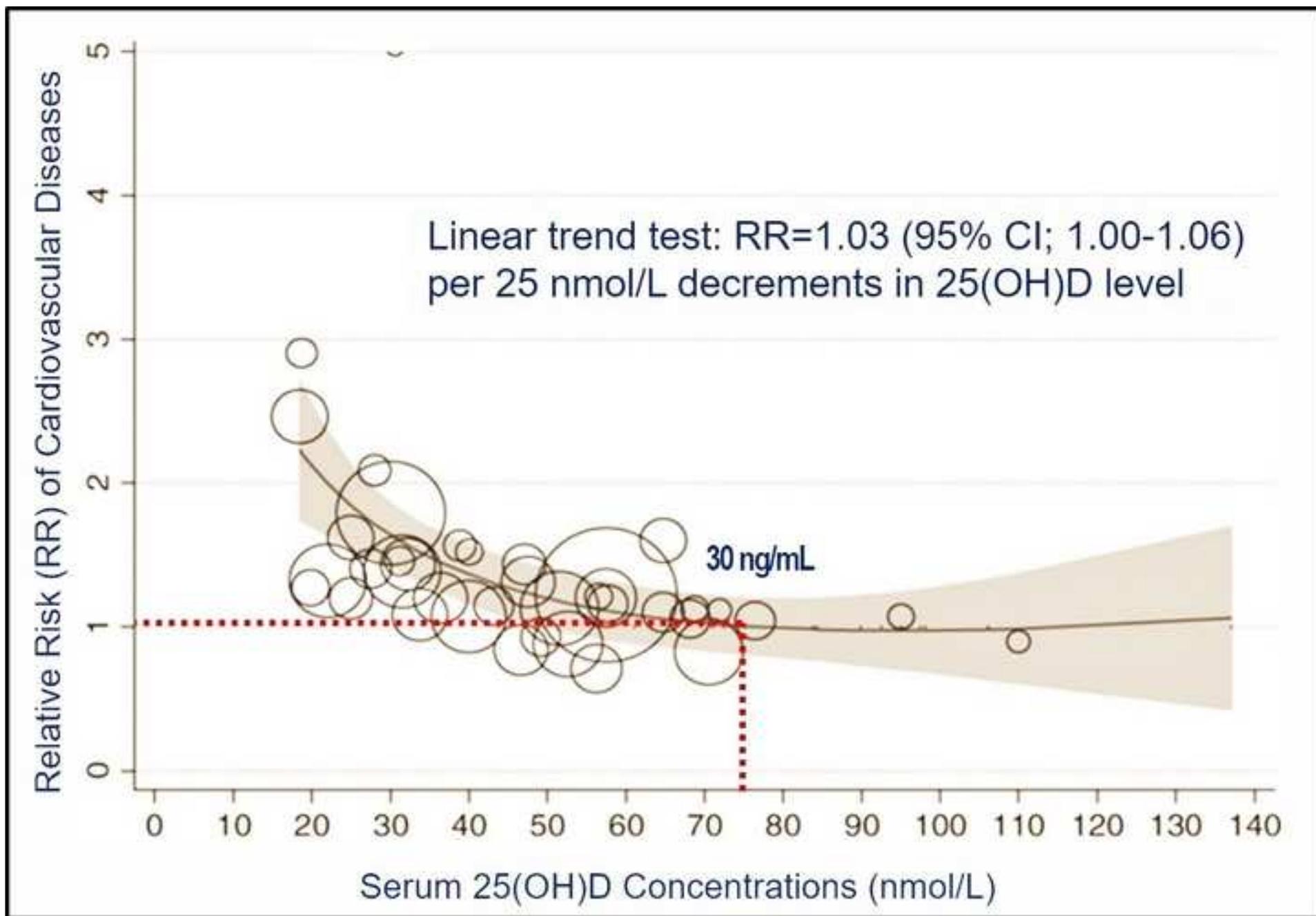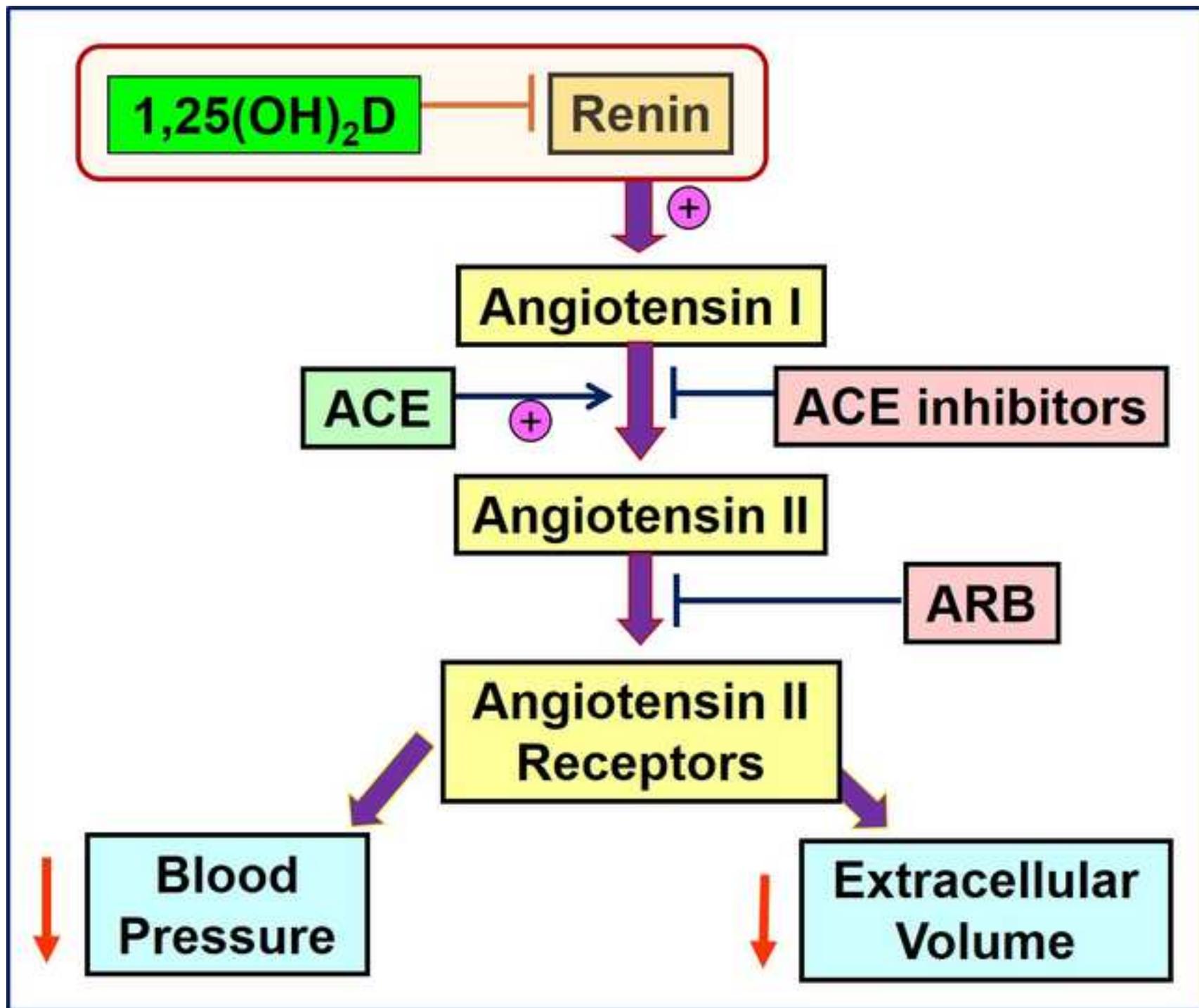
30 ng/mL

Figure 6

Table 1: Guidance to randomized, control, vitamin D clinical trials, study design:

Criteria	Characteristics and conditions that must be accomplished	Clarifications (caveats)
1	RCT must be adequately powered for meaningful statistical analysis and to make conclusions	Valid, statistical power analysis is essential during the designing of RCT
2	Participants in study groups must have the disease being studied (e.g., particular CVD); alternatively, for those with deficiency (insufficiency) researchers can determine whether or not the disease develops over the course of the study	All study participants need to have hypovitaminosis D; i.e., deficiency [serum 25(OH)D levels below 20 ng/mL] or severe deficiency [levels below 10 ng/mL], depending on the disease situation
3	Study end point must be based on hard end points	Reduction of morbidity or mortality; decreased incidence of the disease being investigated, etc.
4	The dose of vitamin D must be adequate for the study participants to achieve blood 25(OH)D levels greater than 30 to 40 ng/mL.* This is essential not missing the treatment effects.	Some participants may need higher daily doses of vitamin D, as much as 5,000 IU/day; *Caveat:* Because of the feeling of well-being, it is possible that subjects may determine that they are in the treatment arm.

5	An alternative, practical, and cost-effective regimen is to administer oral vitamin D, 50,000 IU once a week (monitored at 3-month intervals) to achieve the desired blood 25(OH)D levels.	Safety monitoring: serum and urinary calcium levels to identify hypercalcemia and hypercalciuria (very rare adverse effects at these doses).
6	Predetermined serum 25(OH)D levels must be achieved and maintained within the expected range; one clinic visit can be replaced with a home vitamin D testing kit	For example, dose titrated to achieve serum levels to more than 30 ng/mL (75 nmol/L), with levels maintained between 30 and 50 ng/mL throughout the study (maintenance doses).
7	Measure 25(OH)D, serum calcium and urinary calcium levels at baseline and every 3 months; only subjects with low blood 25(OH)D concentration should be enrolled–(health outcome relationships).	Assure the desired blood level is achieved and maintained within the expected range, avoiding adverse effects; alternatively, investigators could look at results as a function of baseline 25(OH)D, not dose or blood levels of 25(OH)D achieved.
8	Other objective measurements such as echocardiography or Brain naturetic peptide.	These tests should be performed before and after the treatment period.
9	Trial duration of at least 2 years, preferably between 2 and 5 years. This is more feasible at institutions such as those for individuals with developmental disabilities.	Some of these clinical trials could be completed by 1 year, if the protocol includes "loading doses" of vitamin D [i.e., achieving the

	However, compliance is compromised with longer durations, but this problem can be minimized by using a loading dose regimen at the onset and a smaller dose thereafter.	desired serum 25(OH)D levels within the first few weeks]. Proper maintenance of blood levels throughout the study is essential.
10	The placebo group ideally should remain deficient (most people in the community otherwise will remain untreated with vitamin D). However, provision of vitamin D in a regimen of 400 IU/day is unlikely to alter the study outcomes.	*Note:* Cochrane rejects studies that have any amount of extra/supplementary vitamin D in the placebo group. The baseline and the achieved maintenance levels of blood 25(OH)D are more important than the vitamin D dose itself.
11	If the ethic committee objects, the Institute of Medicine (IOM) recommended dose could be administered in the placebo group.	For example, 600 IU vitamin D per day could be given to the placebo group. However, this may interfere with the trial outcomes and statistics \.
12	Because the participants are likely to have concomitant nutritional deficiencies that are not detected by commonly used blood tests, both groups should be supplemented with other micronutrient supplements, such as vitamin K2, magnesium, boron, zinc, and omega-3.	Some cofactors are required for paracrine effects of 25(OH)D and $1,25(OH)_2D$, a combination of cofactors should be administered to both the treatment and placebo groups to assure that there are no other deficiency-induced confounding effects.

| 13 | Conclusions based on study data with "faulty study designs" would not resolve the current issues or the understanding.

Future RCTs should focus on vitamin D-deficient individuals and apply more objective and standardized hard end point outcome measurements. | To date, all vitamin D intervention meta-analyses have used studies with differing sizes, population groups, varied baseline vitamin D levels, and study durations. Thus, interpretations and conclusions from these meta-analyses are subject to questions. |

Table 2: Relationship between the rate of hypertension and serum 25(OH)D levels*

	25(OH)D Concentration (nmol/L)			
	<38	38–75	75–99	>100
Hypertension	52%	41%	27%	20%
Odds ratio	2.7 (1.4–5.2)	2.0 (1.5–2.6)	1.3 (1.2–1.6)	1.00

*After Bandahar et al, 2004 (207).