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Review

Vitamin D and Cardiovascular Disease

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Abstract. *It has long been known from case series that vitamin D excess can lead to atherosclerosis and vascular calcification in humans. In the 1980s, ecological studies provided data that deficient human vitamin D status may also increase the risk of developing cardiovascular disease (CVD). The assumption of a biphasic vitamin D effect on CVD is supported by experimental studies: Numerous studies have demonstrated positive effects of the vitamin D hormone (1,25-dihydroxyviramin D) on the cardiovascular system. However, the effects and mechanisms that lead to vascular calcification by vitamin D excess could also be confirmed. Large prospective observational studies support the hypothesis of a U-shaped association between vitamin D and CVD. These studies indicate that deficient circulating 25-hydroxyvitamin D levels (<30 nmol/l) are independently-associated with increased CVD morbidity and mortality. They also suggest that those circulating 25-hydroxyvitamin D levels, which have long been considered to be safe (100-150 nmol/l), are associated with an increased CVD risk. Meanwhile, numerous randomized controlled trials have investigated the effects of vitamin D supplements or ultraviolet B radiation on biochemical cardiovascular risk markers, cardiovascular physiology, and cardiovascular outcomes. Overall, results are mixed with the majority of studies reporting neither beneficial nor adverse vitamin D effects. Several limitations in the study design, which may have prevented beneficial vitamin D effects, are discussed. In conclusion, it must be stated that the role of vitamin D in the prevention and management of CVD as well as the dose-response relationship of potentially harmful effects still remain to be established.*

Abbreviations: EAR, estimated average requirement of a specific population group; RDA, recommended dietary allowance for a specific population group; UL tolerable upper intake level of a specific population group.

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Vitamin D Physiology and Safety

Vitamin D is unique for humans because it can be produced in the skin, provided that skin exposure to ultraviolet (UV) B radiation is sufficient. Dietary intake is a second, less important, vitamin D source. Because of the ability of the skin to produce vitamin D, many clinicians consider vitamin D a pro-hormone rather than a vitamin.

Skin synthesis of vitamin D has two advantages: it is very effective and toxic effects are usually impossible, since daily skin synthesis of vitamin D reaches a plateau when 15% of the vitamin D precursor, 7-dehydrocholesterol, is converted into vitamin D. Thereafter, vitamin D-inactive substances such as lumisterol and tachysterol are produced. However, in various population groups such as indoor workers, institutionalized patients, veiled women and dark-skinned people living at high geographic latitude skin synthesis of vitamin D is limited or even absent. In these cases, oral vitamin D intake is essential for humans. Due to the increasing number of most of the aforementioned groups, it is likely that in future the vitamin character of vitamin D moves to the forefront of interest.

Generally, essential nutrients like vitamin D show a U-shaped association between oral intake and its biological response (Figure 1): Briefly, an adequate intake guarantees normality of those functions, which depend on the specific nutrient, whereas deficient intake can cause harm. Above the adequate intake range toxic effects may begin to manifest and risk of harm rises again. In case of excessive oral vitamin D intake, the unregulated intestinal vitamin D uptake in combination with the weakly-regulated hepatic 25-hydroxylation of vitamin D can lead to toxic circulating 25-hydroxyvitamin D (25OHD) levels. In extreme cases both deficient vitamin D supply and excess intake can be fatal.

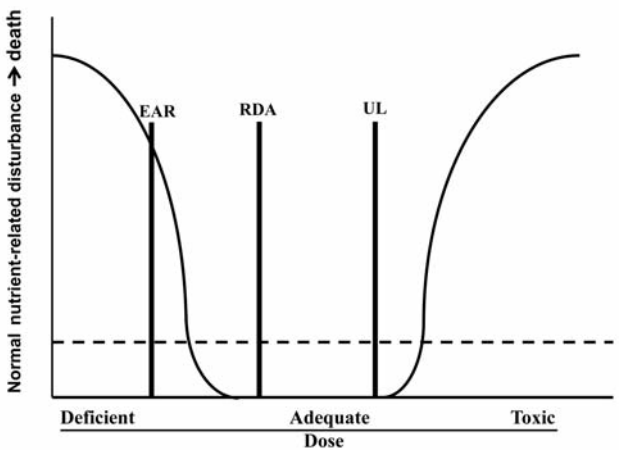

Figure 1. *Dose-response curve for nutrients.*

Vitamin D status can best be assessed by measuring circulating 25OHD levels. The Institute of Medicine (IOM) has classified circulating 25OHD levels of 50 to 125 nmol/l as adequate (divide by 2.496 to convert into ng/ml), levels between 30 and 49.99 as inadequate and levels below 30 nmol/l as deficient, whereas levels >125 nmol/l are classified as potentially harmful (1). Although 1,25-dihydroxyvitamin D (1,25$[OH]_2$D) is the active hormonal form of vitamin D, which is primarily produced in the kidney but also in various extra-renal tissues, circulating 1,25$(OH)_2$D levels are usually homeostatically-regulated and are therefore less valid in assessing human vitamin D status. However, in case of inadequate or deficient vitamin D status, circulating 1,25$(OH)_2$D levels become substrate-dependent, *i.e.* dependent on circulating 25OHD levels (2).

To guarantee an adequate vitamin D supply, the IOM recommends beyond infancy and up to an age of 70 years a daily vitamin D intake of 600 international units (IU; divide by 40 to convert into μg) and for people >70 years, 800 IU daily. The IOM has set the upper tolerable intake level at 4,000 IU daily.

The pivotal role of vitamin D for mineral homeostasis and skeletal health is well-established. The present article gives an overview of vitamin D and cardiovascular disease (CVD) and provides evidence that both vitamin D deficiency and excess supply can cause CVD.

History

The biphasic effect of vitamin D on tissue calcification has long been known: Nearly a century ago, the essential role of vitamin D for bone mineralization and its preventive effect on bone diseases such as rickets and osteomalacia became obvious. Shortly after the discovery of vitamin D it also became evident that oral vitamin D excess is responsible for soft tissue calcification in experimental animals and infants (3). Vitamin D intoxication has been related to infantile hypercalcemia with supravalvular aortic stenosis, mental retardation and craniofacial malformation. Correlative pathological studies also suggested that the intermittently high doses of vitamin D given in former decades for the prevention of rickets were responsible for vascular childhood calcinosis (4). However, in the 1980s a paradigm shift occurred with the observation that UVB radiation, through vitamin D formation, may protect against cardiovascular disease (CVD) (5). The hypothesis was based on ecological data indicating seasonality in coronary heart disease and stroke mortality in Australia, with the highest mortality incidence occurring during wintertime, when skin synthesis of vitamin D is low or even absent. In addition, it became obvious that in the United States mortality from CVD was lowest at high geographic altitude, where UVB radiation is considerable higher compared with sea level. In the 1990s an inverse association between circulating 1,25$(OH)_2$D levels and vascular calcification was reported in two human populations at high and moderate risk for coronary heart disease (6). The hypothesis of cardio-protective vitamin D actions was later on extended to the effect that several apparent paradoxes in the pathogenesis of CVD could be explained by differences in vitamin D status (7). These paradoxes include a low CVD mortality risk despite (i) a high intake of saturated fatty acids, (ii) a high prevalence of cigarette smoking or (iii) a low socioeconomic status. By contrast, a high CVD mortality risk has been reported in populations without the expected risk indicators or despite a very low fat intake.

Experimental Data

It has been hypothesized that vitamin D excess can cause vascular calcification by vascular smooth muscle cell (VSMC) differentiation into osteoblast-like cells either directly or indirectly *via* hypercalcemia and hyperphosphatemia (8). Similarly, it has been assumed that vitamin D deficiency may promote vascular calcification by suppression of inhibitors of VSMC differentiation into osteoblast-like cells (8).

The assumption of a biphasic vitamin D effect on CVD risk is supported by several experimental studies: Briefly, supra-physiological doses of the parent vitamin D substance as well as high doses of 1,25$(OH)_2D_3$ (i) result in a marked increase in aortic calcium and phosphate content, (ii) accelerate cell migration and promote the transition of contractile VSCMs into the osteoblast-like phenotype, (iii) lead to vascular calcification, destruction of elastic fibers, arterial stiffness and (iv) induce left ventricular hypertrophy (9-12). Importantly, studies in fibroblast growth factor-23

(*fgf-23*) null mice (13) demonstrate that phosphate overload seem to be critical for the process of vascular calcification. In these animals, markedly elevated $1,25(OH)_2D$ levels on a phosphate deficient diet had no apparent adverse effects on vascular calcification. The pivotal role of phosphate overload is supported by results in double knockout mice of *klotho*- and the sodium/phosphate co-transporters (14). These animals have normal phosphate levels and also a low risk of vascular calcification, despite the presence of significantly elevated serum calcium and $1,25(OH)_2D$ levels.

In line with the hypothesis of a biphasic effect of vitamin D on CVD, vitamin D receptor (vdr) knockout mice also show several features of premature aging including ectopic calcification and a short lifespan (15). Likewise, vascular calcification can be induced in uremic rats, an experimental model of chronic kidney disease, which is associated with markedly suppressed $1,25(OH)_2D$ levels and supra-physiological parathyroid hormone (PTH) levels (16). Intriguingly, $1,25(OH)_2D_3$ can inhibit tumor necrosis factor alpha (TNFα) converting enzyme, an enzyme that produces secondary hyperparathyroidism, fibrotic and inflammatory lesions to the renal parenchyma and systemic inflammation, which is known to aggravate renal and cardiovascular lesions and enhance the risk of vascular calcification and cardiovascular mortality (17). Notably, vascular calcification and expression of vascular osteoblast differentiation factors can also be induced in low-density lipoprotein receptor (ldl-r)-null mice by feeding a vitamin D deficient diet (18). Similar results were obtained in ApoE-null mice on a vitamin D deficient diet (19).

Vitamin D deficiency may not only influence vascular calcification but also other cardiovascular risk factors: $1,25(OH)_2D_3$ is a negative endocrine regulator of the renin-angiotensin system. The *vdr*-null mice show renin and angiotensin II overexpression leading to hypertension, cardiac hypertrophy and increased water intake (20). Even selective deletion of the vdr gene in cardiac myocytes leads to left ventricular hypertrophy despite normal calcium homeostasis and normal PTH concentrations (21). Another potentially protective vitamin D effect has been reported in macrophages of patients with type 2 diabetes mellitus. In these cells, $1,25(OH)_2D_3$ administration decreases cholesterol uptake and inhibits foam cell formation (22).

Epidemiology

Recently, Wang *et al*. (23) published a meta-analysis of prospective studies on circulating 25OHD and CVD. The analysis included a total number of 19 independent studies, published between 2005 and 2012, with 6,123 CVD cases in 65,994 participants. Comparing the lowest to the highest 25OHD categories, the pooled relative risks was 1.52 (95% confidence interval [CI]: 1.30-1.77) for total CVD, 1.42 (95% CI: 1.19-1.71) for CVD mortality, 1.38 (95% CI: 1.21-1.57) for coronary heart disease and 1.64 (95% CI: 1.27-2.10) for stroke. The CVD risk increased across decreasing 25OHD below approximately 60 nmol/l, with a relative risk of 1.03 (95% CI: 1.00-1.06) per 25 nmol/l decrement in 25OHD. Especially at deficient circulating 25OHD levels, *i.e*. levels below 30 nmol/l, a non-linear increase in CVD risk became obvious. However, there was no clear increase or decrease in CVD risk with 25OHD over 60 nmol/l, based on the few data points. A more recent prospective cohort study in cardiac surgery patients (24) also showed a nonlinear increase of major adverse cardiac and cerebrovascular events (MACCE) until discharge in patients with inadequate and deficient circulating 25OHD levels, respectively, as compared with 25OHD levels of 75 to 100 nmol/l. In detail, compared with the reference group the multivariable-adjusted odds ratio of MACCE was 1.73 (95% CI: 1.01–2.96) at 25OHD levels of 30 to 50 nmol/l and 2.23 (95%CI: 1.31-3.79) at levels below 30 nmol/l. However, at 25OHD levels >100 nmol/l, the odds ratio was again 2.34 (95% CI: 1.12-4.89), indicating a U-shaped association between circulating 25OHD and MACCE. Possible causes for adverse vitamin D effects on the cardiovascular system at 25OHD levels above 100 nmol/l are not well understood. It has been speculated that high circulating 25OHD levels (i) may result in excessive amounts of absorbed calcium leading to an increased risk of CVD events, (ii) may sometimes only reflect low availability of the active vitamin D hormone $1,25(OH)_2D$ leading to deficient rather than excessive vitamin D actions or (iii) may be associated with specific gene variants, which increase the CVD risk independent of vitamin D and may thus only be indicative of an increased CVD risk but not causally related to it (24). Notably, the U-shaped association between circulating 25OHD and clinical outcome in cardiac surgery patients is in general agreement with a huge Israeli data analysis (25). This particular investigation demonstrated an inverse J-shaped association of circulating 25OHD with acute coronary syndrome and mortality. Morbidity and mortality were lowest at 25OHD levels between 50 and 90 nmol/l.

It has been argued that circulating 25OHD levels are only an indicator of solar UVB exposure and not causally linked to CVD risk. Therefore, it is important that in a study by Gotsman *et al*. (26), during a median clinical follow-up of 518 days, vitamin D supplement use was independently associated with reduced mortality in heart failure patients. The inverse association of vitamin D supplement use with mortality was most pronounced in patients with 25OHD levels <25 nmol/l. In another study where patients were followed-up in a cardiovascular practice at a large US academic medical center (27), there was substantial survival benefit among individuals who were on vitamin D supplementation (odds ratio for death 0.39 (95%CI: 0.28-0.53) compared to vitamin D non-users.

Table I. *Summary of randomized controlled vitamin D trials on biochemical and physiological parameters of CVD risk: Daily vitamin D supplements.*

Author,Year, Reference	Mean initial 25OHD level	Number of subjects	Vitamin D	Duaration dose (IU)	End-point	Improvement	Risk
Zittermann, 2009 (34)	30	165	3,333	12 months	Biomarker	Yes	No
Dong, 2010 (35)	34	49	2,000	4 months	Endothelial function	Yes	No
Shab-Bidar, 2011 (36)	39	135	1,000	3 months	Biomarker	Yes	No
Kharlamov, 2012 (37)	<40	109	1,200-1,800	6 months	Cardiac function	Yes	No
Longenecker, 2012 (38)	<50	45	4,000	3 months	Endothelial function	No	Yes[1]
Gepner, 2012 (39)	78	114	2,500	4 months	Endothelial function	No	No
Wood, 2012 (40)	33	265	400 or 1,000	12 months	Biomarker	No	No
Muldowney, 2012 (41)	71	294	200-600	22 weeks	Biomarker	No	No
Salehpur, 2012 (42)	37	77	1,000	12 weeks	Biomarker	Yes	No
Yiu, 2013 (43)	53	100	5,000	12 weeks	Endothelial function	N	No

[1]Insulin sensitivity impaired.

Randomized Controlled Trials

During recent years, various randomized controlled trials (RCTs) (28-37) have investigated the effects of daily vitamin D supplements on biochemical cardiovascular risk markers such as lipid parameters and inflammatory markers, endothelial function measured by brachial artery flow-mediated vasodilation and cardiac function measured by left ventricular ejection fraction and New York heart association functional class (Table I). Daily vitamin D doses ranged between 200 and 5,000 IU/d (5-125 μg/d). Generally, results are mixed. Beneficial effects were reported in 5 out of 10 studies (50%). Five out of 6 studies with initial 25OHD levels ≤ 40 nmol/l demonstrated beneficial vitamin D effects on CVD risk markers, whereas none of the studies with initial 25OHD >40 nmol/l reported such an effect. Most importantly, with the exception of one study in HIV-infected patients that reported an impaired insulin sensitivity in the vitamin D group compared with the placebo group (32), none of the other studies reported any risks or adverse effects of the vitamin D supplements. It cannot be ruled-out that the vitamin D effect on insulin sensitivity in HIV-infected patients was a chance finding, since the mean increment in 25OHD in the vitamin D group was only 12.5 nmol/l and thus rather low. However, it may also be that the disease has influenced the vitamin D effect on glucose metabolism.

Table II summarizes the results of studies where bolus administration or UVB exposure was used to assess the vitamin D effect on biochemical risk markers and endothelial function (38-51). Bolus doses ranged from 20,000 IU weekly up to 300,000 IU monthly. Again, results are mixed. Four out of 13 studies (31%) reported beneficial effects. In all 4 studies, initial 25OHD levels were ≤40 nmol/l. One of two other studies with initial 25OHD levels ≤40 nmol/l reported no beneficial effect and the other study reported mixed results. None of the 13 studies reported any risk or adverse vitamin D effect.

There is, however, some evidence that bolus administration of vitamin D should be used cautiously in patients without deficient 25OHD levels: In a pooled data analysis in 928 subjects receiving vitamin D supplements of 20,000-40,000 IU *per* week or placebo for 6-12 months (52), baseline circulating 25OHD level in those given vitamin D was 55.9 nmol/l and the mean increase was 82.4 nmol/l. Compared to the placebo group there was in the vitamin D group at the end of the studies a slight, but significant increase in hemoglobin A_{1C}, C-reactive protein and in those with low baseline high-density lipoprotein-cholesterol and circulating 25OHD levels less than 50nmol/l a slight decrease in serum HDL-cholesterol, demonstrating that vitamin D bolus administration does not improve biochemical markers of CVD risk in subjects without vitamin D deficiency. If anything, the effect of 20,000-40,000 IU vitamin D weekly bolus administration was negative.

One of the most important CVD risk factors is hypertension (53). In 2009, Witham *et al*. (54) performed a meta-analysis of RCTs on vitamin D and blood pressure. They came to the conclusion that in patients with initial systolic blood pressure >140 mmHg vitamin D decreased systolic blood pressure non-significantly by –3.65 mmHg (95%CI:–8.00; 0.74) and diastolic blood pressure significantly by –3.05 (95%CI:–5.50; –0.60). However, this meta-analysis has the limitation that the number of included patients was small (n=445). A more recent meta-analysis (55), which was based on more than 1,700 patients, reported a non-significant reduction in systolic and diastolic blood pressure of only –0.94 mmHg (95%CI:–2.98; 1.10) and –0.52 mmHg (95%CI:–1.18; 0.14), respectively. Unfortunately however, this meta-analysis was not restricted to patients with elevated blood pressure.

While RCTs on risk markers may give important insights into potential mechanisms of vitamin D actions on the cardiovascular system, only those RCTs which investigate

Table II. *Summary of randomized controlled vitamin D trials on biochemical and physiological parameters of CVD risk: Bolus Administration of Vitamin D or UVB exposure.*

Author, Year, Reference	Mean initial 25OHD level	Number of subjects	Vitamin D	Duaration dose (IU)	Endpoint	Improvement	Risk
Sugden, 2008 (44)	40	34	100,000 D_2/once	8 weeks	Endothelial function	Yes	No
Tarcin, 2009 (45)	25	46	300,000 D_2/monthly	3 months	Endothelial function	Yes	No
Witham, 2010 (46)	21	61	100,000/200 000 D_2/once	12 weeks	Endothelial function	No	No
Jorde, 2010 (47)	56	330	20,000-60,000 D_3/weekly	6-12 months	Biomarker	No	No
Harris, 2011 (48)	34	57	60,000 D_3/monthly	4 months	Endothelial function	Yes	No
Scragg, 2011 (49)	42	119	UVB/twice weekly	12 weeks	Biomarker	No	No
Witham, 2012 (50)	38	58	100,000 D_2/once	8-16 weeks	Endothelial function	Yes at 8 weeks No at 16 weeks	No No
Stricker, 2012 (51)	41	65	100,000 D_3/once	1 month	Endothelial function	No	No
Sokol, 2012 (52)	<50	90	50,000 D_2/weeky	12 weeks	Endothelial function	No	No
Ponda, 2012 (53)	<40	151	50,000 D_3/weeky	8 weeks	Biomarker	No	No
Witham, 2013 (54)	49	159	100,000 D_3/3 monthly	12 months	Endothelial function	No	No
Witham, 2013 (55)	42	75	100,000 D_2/2 monthly	4 months	Endothelial function	No	No
Rahimi-Ardabili, 2013 (56)	40	50	50,000 D_3/20 daily	2 months	Biomarker	Yes	No
Witham, 2014 (57)	41	68	100,000 D_3/2 monthly	6 months	Cardiac function	No	No

clinically relevant end-points can demonstrate whether or not vitamin D is effective in preventing CVD events. An update of a Cochrane collaboration review (56) came to the conclusion that in RCTs with predominantly elderly people (>70 years) vitamin D supplement use is able to reduce total mortality by 6% (95%CI: 2%-9%). However, this effect was primarily based on the reduction of cancer mortality, whereas no significant effect was observed on CVD mortality (relative risk: 0.98, 95%CI: 0.90-1.07). More recently (57), it has been suggested that vitamin D supplementation does not reduce clinical outcomes, including CVD risk, in unselected community-dwelling individuals by more than 15% and that future trials with similar designs are unlikely to alter these conclusions (57). Nonetheless, we should bear in mind that many RCTs on vitamin D and CVD have important limitations: First, many RCTs and meta-analyses of RCTs do not, or not adequately, take study adherence into account, although poor study adherence is of clinical relevance and may influence study results substantially (58). Second, many patients included in vitamin D supplementation trials were not vitamin D deficient (*i.e.* circulating 25OHD levels not below 30 nmol/l). Therefore, a strong vitamin D effect on CVD prevention cannot be expected. Third, vitamin D is a negative endocrine regulator of the renin-angiotensin aldosterone system (20). In Western societies, medications such as diuretics and angiotensin- converting enzyme inhibitors/angiotensin II receptor blockers are frequently prescribed to patients who are at an increased risk for CVD events and may thus interact with vitamin D actions on CVD risk. In addition, statins, which are also frequently prescribed in Western societies, are considered to have vitamin D agonistic effects (59) and may thus influence potential CVD effects of vitamin D supplements. Fourth, many control groups cannot be considered as real placebo groups since most individuals in the control groups are able to get their vitamin D by skin synthesis. Fifth, in several RCTs the vitamin D group was also supplemented with high amounts of calcium. There is however convincing evidence that high amounts of supplemental calcium may increase CVD risk (60-62) and may thus adversely affect potentially beneficial vitamin D effects on CVD risk (63). Finally, many RCTs do not take physical activity into account, although it is well known that calcium and vitamin D metabolism differ markedly in physically active and inactive individuals (64), which may also influence CVD risk.

Given the various limitations of published RCTs, the potential of vitamin D in reducing CVD outcomes may be more pronounced than currently assumed, at least in specific subgroups. Results of adequately designed RCTs with sufficient statistical power are therefore still warranted.

Outlook

There are currently several ongoing trials with CVD as one of the main outcomes. In total, more than 40,000 participants aged 50 years and over will be included in these studies, which are performed in the United States, New Zealand and Europe (65). First results can be expected in 2017. Hopefully, these studies will provide more clarity about the role of vitamin D on CVD risk.

Conclusion

There is evidence from experimental studies that vitamin D has important beneficial effects on parameters of CVD risk, but may also lead to vascular calcification in case of excessive oral intake. Cohort studies that investigated the association of circulating 25OHD levels with CVD outcomes support the assumption of a U-shaped or inverse J-shaped association between vitamin D status and CVD risk. Notably, results of these studies fit well together with those circulating 25OHD values the IOM has classified deficient and potentially harmful, respectively.

RCTs have the advantage that they can provide reliable scientific evidence concerning potential vitamin D effects on CVD risk. Trials on biochemical and physiological CVD risk markers have the additional advantage that adequate statistical power is usually already achieved with relatively low numbers of study participants. Nevertheless, published results are mixed. Based on these data, one can speculate that relevant vitamin D effects on CVD outcome, if any, may only occur if initial circulating 25OHD levels are in the deficiency range. Regarding clinical trials, various limitations may contribute to the fact that so far no significant effects of vitamin D supplements have been demonstrated on clinically-relevant CVD outcomes. Fortunately, no serious side-effects have been seen at daily vitamin D doses up to 5,000 IU or at oral bolus administration of up to 300,000 IU monthly. Nevertheless, results of a pooled data analysis of RCTs indicate some negative effects of weekly vitamin D administration of 20,000 to 40,000 IU on biochemical cardiovascular risk markers. Importantly, the mean 25OHD increment in that data analysis resulted in circulating 25OHD levels the IOM has classified potentially harmful (>125 nmol). Harmful vitamin D effects in case of bolus administration but beneficial effects in case of daily administration have also been reported in studies on vitamin D and bone health (66-68). In total, data from RCTs on vitamin D and CVD risk support the IOM classification of deficient and potentially harmful circulating 25OHD levels.

In summary, it must be stated that the role of vitamin D in the prevention and management of CVD still remains to be established. In addition, the dose-response relationship of potentially harmful vitamin D effects on the cardiovascular system has to be studied *in extenso*.

References

1 Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ and Shapses SA: The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab *96*: 53-58, 2011.

2 Zittermann A: Vitamin D and disease prevention with special reference to cardiovascular disease. Prog Biophys Mol Biol *92*: 39-48, 2006.

3 Jahreis G and Hesse V: Vitamin D-induced tissue calcinosis and arteriosclerosis changes. I: A contribution to the 60 year history of vitamin D research with special reference to childhood. Padiatr Grenzgeb *29*: 203-211, 1990 [In German].

4 Kobylinski S, Hafer B and Kohde G: Correlative pathologic studies on the role of vitamin D in vascular calcinosis in childhood. Zentralbl Allg Pathol *129*: 137-147, 1984 [In German].

5 Scragg R: Seasonality of cardiovascular disease mortality and the possible protective effect of ultra-violet radiation. Int J Epidemiol *10*: 337-341, 1981.

6 Watson KE, Abrolat ML, Malone LL, Hoeg JM, Doherty T, Detrano R and Demer LL: Active serum vitamin D levels are inversely correlated with coronary calcification. Circulation *96*: 1755-1760, 1997.

7 Zittermann A, Schleithoff SS and Koerfer R: Putting cardiovascular disease and vitamin D insufficiency into perspective. Br J Nutr *94*: 483-492, 2005.

8 Huybers S and Bindels RJ: Vascular calcification in chronic kidney disease: new developments in drug therapy. Kidney International *72*: 663-665, 2007.

9 Niederhoffer N, Lartaud-Idjouadiene I, Giummelly P, Giummelly P and Atkinson J: Calcification of medial elastic fibers and aortic elasticity. Hypertension *29*: 999-1006, 1997.

10 Price PA, Buckley JR and Williamson MK: The amino bisphosphonate ibandronate prevents vitamin D toxicity and inhibits vitamin D-induced calcification of arteries, cartilage, lungs and kidneys in rats. J Nutr *131*: 2910-2915, 2001.

11 Rebsamen MC, Sun J, Norman AW and Liao JK: 1alpha,25-dihydroxyvitamin D3 induces vascular smooth muscle cell migration *via* activation of phosphatidylinositol 3-kinase. Circ Res *91*: 17-24, 2002.

12 Bas A, Lopez I, Perez J, Rodriguez M and Aguilera-Tejero E: Reversibility of calcitriol-induced medial artery calcification in rats with intact renal function. J Bone Miner Res *21*: 484-490, 2006.

13 Stubbs JR, Liu S, Tang W, Zhou J, Wang Y, Yao X and Quarles LD: Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. J Am Soc Nephrol *18*: 2116-2214, 2007.

14 Razzaque MS: The dualistic role of vitamin D in vascular calcifications. Kidney Int *79*: 708-714, 2011.

15 Tuohimaa P: Vitamin D and aging. J Steroid Biochem Mol Biol *114*: 78-84, 2009.

16 Tamagaki K, Yuan Q, Ohkawa H, Imazeki I, Moriguchi Y, Imai N, Sasaki S, Takeda K and Fukagawa M: Severe hyperparathyroidism with bone abnormalities and metastatic calcification in rats with adenine-induced uraemia. Nephrol Dial Transplant *21*: 651-659, 2009.

17 Dusso A1, Arcidiacono MV, Yang J and Tokumoto M: Vitamin D inhibition of TACE and prevention of renal osteodystrophy and cardiovascular mortality. J Steroid Biochem Mol Biol *121*: 193-198, 2010.

18 Schmidt N, Brandsch C, Kühne H, Thiele A, Hirche F and Stangl GI: Vitamin D receptor deficiency and low vitamin D diet stimulate aortic calcification and osteogenic key factor expression in mice. PLoS One *7*: e35316, 2012.

19 Ellam T, Hameed A, ul Haque R, Muthana M, Wilkie M, Francis SE and Chico TJ: Vitamin D deficiency and exogenous vitamin D excess similarly increase diffuse atherosclerotic calcification in apolipoprotein E knockout mice. PLoS One *9*: e88767, 2014.

20 Li YC1, Kong J, Wei M, Chen ZF, Liu SQ and Cao LP: 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest *110*: 229-238, 2002.

21 Chen S, Law CS, Grigsby CL, Olsen K, Hong TT, Zhang Y, Yeghiazarians Y and Gardner DG: Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy. Circulation *124*: 1838-1847, 2011.

22 Oh J, Weng S, Felton SK, Bhandare S, Riek A, Butler B, Proctor BM, Petty M, Chen Z, Schechtman KB, Bernal-Mizrachi L and 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 *120*: 687-698, 2009.

23 Wang L, Song Y, Manson JE, Pilz S, März W, Michaëlsson K, Lundqvist A, Jassal SK, Barrett-Connor E, Zhang C, Eaton CB, May HT, Anderson JL and Sesso HD: Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. Circ Cardiovasc Qual Outcomes *5*: 819-829, 2012.

24 Zittermann A, Kuhn J, Dreier J, Knabbe C, Gummert JF and Börgermann J: Vitamin D status and the risk of major adverse cardiac and cerebrovascular events in cardiac surgery. Eur Heart J *34*: 1358-1364, 2013.

25 Dror Y, Giveon SM, Hoshen M, Feldhamer I, Balicer RD and Feldman BS: Vitamin D levels for preventing acute coronary syndrome and mortality: evidence of a nonlinear association. J Clin Endocrinol Metab *98*: 2160-2167, 2013.

26 Gotsman I, Shauer A, Zwas DR, Hellman Y, Keren A, Lotan C and Admon D: Vitamin D deficiency is a predictor of reduced survival in patients with heart failure; vitamin D supplementation improves outcome. Eur J Heart Fail *14*: 357-366, 2012.

27 Vacek JL, Vanga SR, Good M, Lai SM, Lakkireddy D and Howard PA: Vitamin D deficiency and supplementation and relation to cardiovascular health. Am J Cardiol *109*: 359-363, 2012.

28 Zittermann A, Frisch S, Berthold HK, Götting C, Kuhn J, Kleesiek K, Stehle P, Koertke H and Koerfer R: Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. Am J Clin Nutr *89*: 1321-1327, 2009.

29 Dong Y, Stallmann-Jorgensen IS, Pollock NK, Harris RA, Keeton D, Huang Y, Li K, Bassali R, Guo DH, Thomas J, Pierce GL, White J, Holick MF and Zhu H: A 16-week randomized clinical trial of 2000 international units daily vitamin D_3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. J Clin Endocrinol Metab *95*: 4584-4591, 2010.

30 Shab-Bidar S, Neyestani TR and Djazayery A: Efficacy of vitamin D3-fortified-yogurt drink on anthropometric, metabolic, inflammatory and oxidative stress biomarkers according to vitamin D receptor gene polymorphisms in type 2 diabetic patients: a study protocol for a randomized controlled clinical trial. BMC Endocr Disord *11*: 12, 2011.

31 Kharlamov AN, Perrish AN, Gabiskiĭ IaL, Ronne Kh and Ivanova E: Vitamin D in the treatment of cardiorenal syndrome in patients with chronic nephropathy. Kardiologiia *52*: 33-44, 2012 [Article in Russian].

32 Longenecker CT1, Hileman CO, Carman TL, Ross AC, Seydafkan S, Brown TT, Labbato DE, Storer N, Tangpricha V and McComsey GA: Vitamin D supplementation and endothelial function in vitamin D deficient HIV-infected patients: a randomized placebo-controlled trial. Antivir Ther *17*: 613-621, 2012.

33 Gepner AD1, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N and Stein JH: A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. PLoS One *7*: e36617, 2012.

34 Wood AD, Secombes KR, Thies F, Aucott L, Black AJ, Mavroeidi A, Simpson WG, Fraser WD, Reid DM and Macdonald HM: Vitamin D3 supplementation has no effect on conventional cardiovascular risk factors: a parallel-group, double-blind, placebo-controlled RCT. J Clin Endocrinol Metab *97*: 3557-3568, 2012.

35 Muldowney S, Lucey AJ, Hill TR, Seamans KM, Taylor N, Wallace JM, Horigan G, Barnes MS, Bonham MP, Duffy EM, Strain JJ, Cashman KD and Kiely M: Incremental cholecalciferol supplementation up to 15 μg/d throughout winter at 51-55° N has no effect on biomarkers of cardiovascular risk in healthy young and older adults. J Nutr *142*: 1519-1525, 2012.

36 Salehpour A, Shidfar F, Hosseinpanah F, Vafa M, Razaghi M, Hoshiarrad A and Gohari M: Vitamin D_3 and the risk of CVD in overweight and obese women: a randomised controlled trial. Br J Nutr *108*: 1866-1873, 2012.

37 Yiu YF, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Lau CP, Cheung BM and Tse HF: Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. Atherosclerosis *227*: 140-146, 2013.

38 Sugden JA, Davies JI, Witham MD, Morris AD and Struthers AD: Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabet Med *25*: 320-325, 2008.

39 Tarcin O1, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O and Akalin S: Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab *94*: 4023-4030, 2009.

40 Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD and Struthers AD: The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. Diabetologia *53*: 2112-2119, 2010.

41 Jorde R, Sneve M, Torjesen P and Figenschau Y: No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D3 for 1 year. J Intern Med *267*: 462-472, 2010.

42 Harris RA, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, Shah Y, Zhu H and Dong Y: Vitamin D_3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. Am J Hypertens *24*: 557-562, 2011.

43 Scragg R, Wishart J, Stewart A, Ofanoa M, Kerse N, Dyall L and Lawes CM: No effect of ultraviolet radiation on blood pressure and other cardiovascular risk factors. J Hypertens *29*: 1749-1756, 2011.

44 Witham MD, Dove FJ, Sugden JA, Doney AS and Struthers AD: The effect of vitamin D replacement on markers of vascular health in stroke patients – a randomised controlled trial. Nutr Metab Cardiovasc Dis *22*: 864-870, 2012.

45 Stricker H, Tosi Bianda F, Guidicelli-Nicolosi S, Limoni C and Colucci G: Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral arterial disease: a randomised controlled pilot study. Eur J Vasc Endovasc Surg *44*: 307-312, 2012.

46 Sokol SI, Srinivas V, Crandall JP, Kim M, Tellides G, Lebastchi AH, Yu Y, Gupta AK and Alderman MH: The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease. Vasc Med *17*: 394-404, 2012.

47 Ponda MP, Dowd K, Finkielstein D, Holt PR and Breslow JL: The short-term effects of vitamin D repletion on cholesterol: a randomized, placebo-controlled trial. Arterioscler Thromb Vasc Biol *32*: 2510-2515, 2012.

48 Witham MD, Dove FJ, Khan F, Lang CC, Belch JJ and Struthers AD: Effects of vitamin D supplementation on markers of vascular function after myocardial infarction--a randomised controlled trial. Int J Cardiol *167*: 745-749, 2013.

49 Witham MD, Adams F, Kabir G, Kennedy G, Belch JJ and Khan F: Effect of short-term vitamin D supplementation on markers of vascular health in South Asian women living in the UK – a randomised controlled trial. Atherosclerosis *230*: 293-299, 2013.

50 Rahimi-Ardabili H1, Pourghassem Gargari B and Farzadi L: Effects of vitamin D on cardiovascular disease risk factors in polycystic ovary syndrome women with vitamin D deficiency. J Endocrinol Invest *36*: 28-32, 2013.

51 Witham MD, Ireland S, Houston JG, Gandy SJ, Waugh S, Macdonald TM, Mackenzie IS and Struthers AD: Vitamin D therapy to reduce blood pressure and left ventricular hypertrophy in resistant hypertension: randomized, controlled trial. Hypertension *63*: 706-712, 2014.

52 Jorde R, Strand Hutchinson M, Kjærgaard M, Sneve M and Grimnes G: Supplementation with High Doses of Vitamin D to Subjects without Vitamin D Deficiency May Have Negative Effects: Pooled Data from Four Intervention Trials in Tromsø. ISRN Endocrinol *2013*: 348705, 2013.

53 Thompson AM1, Hu T, Eshelbrenner CL, Reynolds K, He J and Bazzano LA: Antihypertensive treatment and secondary prevention of cardiovascular disease events among persons without hypertension: a meta-analysis. JAMA *305*: 913-22, 2011.

54 Witham MD, Nadir MA and Struthers AD: Effect of vitamin D on blood pressure: a systematic review and meta-analysis. J Hypertens *27*: 1948-1954, 2009.

55 Kunutsor SK, Burgess S, Munroe PB and Khan H: Vitamin D and high blood pressure: causal association or epiphenomenon? Eur J Epidemiol *29*: 1-14, 2014.

56 Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, Bjelakovic M and Gluud C: Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev 1: CD007470. doi: 10.1002/14651858, 2014.

57 Bolland MJ, Grey A, Gamble GD and Reid IR: The effect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis. Lancet Diabetes Endocrinol *2*: 307-320, 2014.

58 Avenell A1, MacLennan GS, Jenkinson DJ, McPherson GC, McDonald AM, Pant PR, Grant AM, Campbell MK, Anderson FH, Cooper C, Francis RM, Gillespie WJ, Robinson CM, Torgerson DJ, Wallace WA and RECORD Trial Group: Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D(3) and/or calcium (RECORD trial). J Clin Endocrinol Metab *97*: 614-622, 2012.

59 Grimes DS: Are statins analogues of vitamin D? Lancet *368*: 83-86, 2006.

60 Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD and Reid IR: Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ *341*: c3691, 2010.

61 Xiao Q, Murphy RA, Houston DK, Harris TB, Chow WH and Park Y: Dietary and supplemental calcium intake and cardiovascular disease mortality: the National Institutes of Health-AARP diet and health study. JAMA Intern Med *173*: 639-646, 2013.

62 Michaëlsson K, Melhus H, Warensjö Lemming E, Wolk A and Byberg L: Long term calcium intake and rates of all cause and cardiovascular mortality: community based prospective longitudinal cohort study. BMJ *346*: f228, 2013.

63 Wang L, Manson JE, Song Y and Sesso HD: Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. Ann Intern Med *152*: 315-323, 2010.

64 Zittermann A, Börgermann J, Gummert JF and Pilz S: Future directions in vitamin D and cardiovascular research. Nutr Metab Cardiovasc Dis *22*: 541-546, 2012.

65 Kupferschmidt K: Uncertain verdict as vitamin D goes on trial. Science *337*: 1476-1478, 2012.

66 Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D and Nicholson GC: Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA *303*: 1815-1822, 2010.

67 Rossini M, Adami S, Viapiana O, Fracassi E, Idolazzi L, Povino MR and Gatti D: Dose-dependent short-term effects of single high doses of oral vitamin D(3) on bone turnover markers. Calcif Tissue Int *91*: 365-369, 2012.

68 Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, Lyons RA, Flicker L, Wark J, Jackson RD, Cauley JA, Meyer HE, Pfeifer M, Sanders KM, Stähelin HB, Theiler R and Dawson-Hughes B: A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med *367*: 40-49, 2012.

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