Postmenopausal Calcium and Vitamin D Supplements Controversy: Do They Increase Cardiovascular Risk?

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Calcium and vitamin D are prescribed in women with osteopenia-osteoporosis to prevent fractures. These bone events increase morbidity and mortality justifying therapy to prevent bone loss. These elderly patients also have cardiovascular risk factors, atherosclerosis and vascular calcifications. Multiple trials demonstrated a relationship between the latter, coronary events and cardiovascular mortality. In these patients, ectopic tissue mineralization may worsen cardiovascular outcome. Calcium intake correlates neither with serum calcium, vascular calcification nor cardiovascular events. However serum calcium-phosphate product is related to vascular calcification and outcome. The main cause for vascular calcification is systemic inflammation and local atherosclerotic process with specific interactions between macrophages and smooth muscle cells. This review examines calcium intake, serum calcium concentration, systemic and vascular inflammation, the mechanisms of vascular calcification and their impact on cardiovascular outcome.

Keywords: calcium and vitamin D supplements, vascular calcifications, cardiovascular risk

Why are calcium and vitamin D supplements so widely prescribed?

Oral calcium-vitamin D supplements are widely prescribed to prevent or to treat osteopenia or osteoporosis in postmenopausal women. Progressive bone loss with low total bone mass and increased risk of fractures is resolved by an optimal intake of calcium and vitamin D that regulates calcium-phosphorus absorption from the gut. Low serum levels of vitamin D are associated with lower serum calcium concentrations, which activate parathormon and leads to calcium resorption from bone tissue to compensate for hypocalcemia.

There are current recommendations for daily intake of both calcium and vitamin D, but they vary slightly between different guidelines. A total daily dose of 1000 mg of calcium and 600 IU of vitamin D are sufficient for bone health [1,2].

Despite the bone benefits obtained from calcium-vitamin D supplements, systemic adverse reactions also occur; kidney stones and cardiovascular (CV) events are among the most clinically relevant [3-5]. It should also be mentioned that these supplements are not sufficient to promote bone health and limit chronic bone loss [6-11]. The mechanism responsible for perceived cardiovascular risk with calcium supplements is the occurrence of vascular calcifications.

Postmenopausal women with lowest bone mineral density are the ones having the highest degree of vascular calcification [12], and osteoporosis and atherothrombosis may concur to shortened survival and quality of life in this patient population. This is precisely the population receiving most calcium-vitamin D supplements. If bone mineral density is usually determined because of the high-perceived risk of fractures in these patients, vascular calcifications are seldom looked for, as far they are more difficult to diagnose in the lack of universal recommendations.

This review is focused on cardiovascular risk induced by an otherwise considered benign treatment with oral supplements to prevent complications of osteopenia-osteoporosis.

Calcium intake

Daily oral calcium intake comes from food containing different amounts of calcium. The most calcium-rich are milk and dairy, but it comes also from bread, cereals, vegetables and fruits. 300 mg of calcium are contained in 240 mL of milk or 29 g of hard cheese per day, while other 200 mg of calcium per day come from other food sources. A daily calcium intake higher than 2000 mg per day is deleterious and associated with adverse effects [13].

Long term follow-up at 11 years of the Framingham Original cohort found an inverse relationship between daily milk consumption (but not for other dairy foods) and the risk of hip fracture [14]. Consequently, the benefit of daily dairy intake is due to vitamin D supplement use; these patients were the only ones showing increased bone mineral density [15].

To produce its effects on bone mass, dietary calcium should be absorbed in serum, a process closely regulated by vitamin D. Serum calcium may induce extra-articular and non-bone effects, such as increased vascular calcification and urinary excretion (fig. 1).

Vascular calcification is a pathological process found in inflammatory vessel wall disease, such as atherosclerosis,

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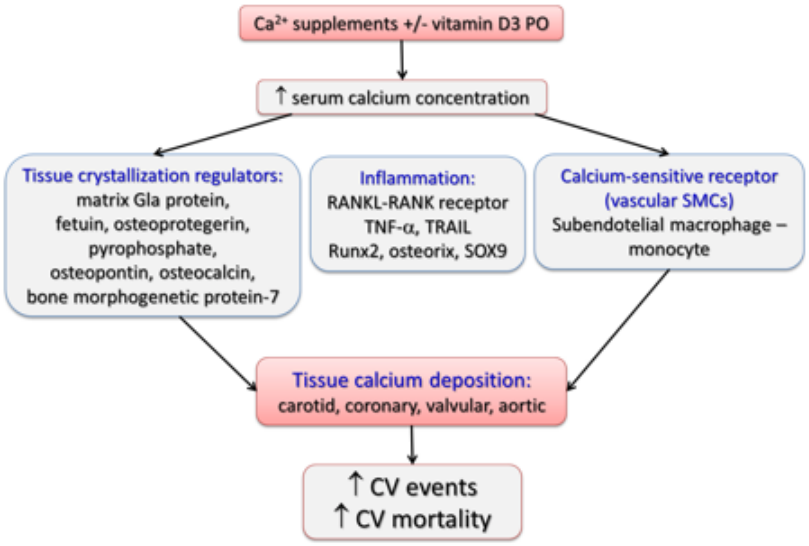

Fig. 1. The proposed mechanism for vascular calcium deposition due to calcium -vitamin D intake. (RANKL -receptor activator of nuclear factor kappa-B ligand, TNFα- tumor necrosis factor alpha, TRAIL - tumor necrosis factor-related apoptosis-inducing ligand, RUNX2 - runt-related transcription factor 2, SMC - smooth muscle cells, CV - cardiovascular)

and is a strong predictor of cardio-vascular complications, independent of traditional risk factors (dyslipidemia, diabetes, hypertension or smoking) [16-18].

Recently, there is consensus that simple calcium intake does not increase coronary calcification [19]. However in the Multi-Ethnic Study of Atherosclerosis, despite high calcium intake was associated with lower risk of atherosclerosis in 5.448 patients without baseline cardiovascular disease, supplements use lead to a higher calcium coronary score [20,21]. An old small trial showed that oral calcium intake does not change serum osteoprotegerin and matrix Gla protein (as markers of tissue crystallization) or urine hydroxyproline excretion [22]. A meta-analysis of 4 randomized trials and 27 observational trials did not confirm an increased cardiovascular risk with calcium or calcium - vitamin D supplements, although those taking more than 2000 mg/day had clearly a higher risk of cardiovascular disease and ischemic heart disease mortality [23]. A large epidemiological study of 41.526 Japanese men followed up for 11 years and 533.692 person-years demonstrated that dietary calcium intake (dairy products) reduced stroke risk, mainly hemorrhagic [24,25].

In the largest available randomized trial, Women Health Initiative (WHI), 36.282 women were randomized to 1000 mg of calcium and 400 UI of vitamin D per day versus placebo. Cardiovascular outcome was a pre-specified secondary endpoint. During 7 years of follow-up calcium and vitamin D supplements did not increase coronary or cerebrovascular risk [26]. Consecutive re-analysis of WHI trial data adjusted for women who were not taking calcium supplements prior to randomization (16.718 patients, 46%) demonstrated however that calcium increased cardiovascular events with a hazard ratio of 1.13 for myocardial infarction or stroke and 1.22 for myocardial infarction and revascularization respectively [27]. A meta-analysis from the same study group performed on 8 different trials identified an increased risk of myocardial infarction in patients randomized to calcium versus placebo (relative risk 1.27; CI 1.01-1.59) and calcium with or without vitamin D versus placebo (relative risk 1.24, CI 1.07-1.45) [28]. Other meta-analyses showed neutral results of calcium supplements on CV disease [23]. The largest meta-analysis performed on 18 trials that enrolled 63.563 patients could not identify an increased coronary heart disease or all-cause mortality risk in elderly women randomized to calcium supplements versus placebo [29]. The main limitation of these meta-analyses is that individual trials were not designed to assess CV outcome as a primary end-point and there were marked differences between study protocols and calcium-vitamin D doses.

In conclusion simple calcium intake, mainly coming from food, does not increase the risk of vascular calcification or cardiovascular risk, while maintaining some effect on reducing the risk of bone loss.

Serum calcium concentration

Going upstream, following the pathogenic scheme from Figure 1, one should consider if serum calcium concentration is a better indicator of vascular calcification and/or cardiovascular risk. It is well known that serum calcium depends on the salt of calcium administered (citrate has the highest serum uptake when compared to gluconate, carbonate or a simple bone meal) [22].

Coronary calcification expressed by the *Agatston score* (CAC score) depended on serum calcium, phosphorus and calcium-phosphorus product, and not on dietary intake of both in a cross-sectional trial in 23.652 Koreans without renal failure or CV diseases [30]. Intestinal absorption of both calcium and phosphorus are tightly regulated, so simple oral intake may have a minor impact on serum levels that may become pathogenic. Similar results were reported in another trial in which abdominal aorta calcifications in 1471 postmenopausal healthy women were positively associated with serum calcium, phosphate and calcium-phosphate product at 5 years follow-up [31]. Dietary calcium intake and calcium supplementation were not associated with changes in abdominal aorta calcifications over 2 to 5 years.

Urinary calcium excretion as an indirect measure of serum calcium concentration was not associated with increased risk of CV events or mortality rates in 903 patients enrolled in the Heart and Soul Study during a mean follow-up of 6 years [32].

In conclusion vascular calcification and CV risk, appear related mainly to serum concentration of calcium, phosphate and calcium/phosphate product and not to oral intake.

The role of inflammation in vascular calcification

Inflammatory reaction in the vessel wall is associated with calcium deposition; calcification of atherosclerotic plaques depends on the degree of cellular inflammation [33]. Oxidized LDL is the most potent pro-inflammatory molecule that accumulates in the subintimal space, activating macrophages and smooth muscle cells. Some trials demonstrated that eicosapentaenoic acid, which blocks lipid oxidation, inhibits osteoblastic differentiation and reduces the degree of vascular calcification [34]. Osteogenic differentiation of simple vascular calcification is modulated by the osteogenic transcription factor Runx2 [35]. The deficiency of this transcription factor leads to a

significant decrease of both osteoclast-like SMCs and macrophages in calcified lesions. This emphasizes the close relationship between inflammation, calcification and bone formation in the vasculature [36-39].

Irrespective of the local cellular mechanisms implicated in vessel wall calcification systemic inflammation of different etiology increases calcium deposition in the vasculature. Female patients with rheumatoid arthritis or systemic lupus erythematosus have higher levels of coronary calcium measured by MSCT when compared with matched controls, after adjustment for other traditional CV risk factors [40,41]. Calcium score in these women was correlated with the degree of systemic inflammation expressed by hsCRP and sICAM-1.

Systemic inflammation is also responsible for the increase of TNF-alpha superfamily members in serum. In the process of vascular calcification two serum glycoproteins are relevant: the receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) [42]. RANKL, a member of TNF-alpha superfamily, and its receptor expressed by osteoclasts promotes osteoclast activation and proliferation. This process is inhibited by OPG a citokine mainly secreted by osteoblasts which functions as a soluble decoy for RANKL. The ratio between RANKL and OPG in serum is the main factor that regulates bone tissue turnover.

Vascular calcification and tissue crystallization regulators

The real pathogenic role of calcium in vascular deposits is found however in the vascular tissue itself in close relationship with serum calcium and phosphate availability as demonstrated above. Vascular calcification is an active chronic phenomenon with common characteristics with bone genesis regulated through a complex interaction between various hormones and cytokines [43,44]. Two different patterns of vascular calcification are recognized: one due to hydroxyapatite deposition in the SMC of the media similar to the endochondral ossification; another due to calcium deposition in the subintimal space and most commonly due to atherosclerosis. It should be mentioned that in advanced atherosclerosis calcium may be found also in the media or adventitia, associated with bone formation in the same manner as with medial SMC calcification. Medial calcification *per primam* is specific to type 2 diabetes mellitus [42], but intimal calcification is also possible as diabetes is a strong risk factor for atherogenesis [45,46].

Heavy peripheral artery calcification is associated with the presence of bone tissue in 6% of vessels examined in specimens obtained after limb amputation [44]. Bone formation in the vessel wall as an extreme manifestation of vascular calcification depends on osteoblastic differentiation of smooth muscle cells and it can follow wall calcium deposition [44]. Mechanisms of vascular calcium deposition and bone formation are similar. Vascular calcification as ectopic mineral metabolism of calcium involves three main cellular types, similar to those in bone generation [47]. Mesenchymal cells such as smooth muscle cells evolve either to fibroblasts in the vessel wall or toward osteoblasts in the bone. Monocytes may evolve toward macrophages in the subendothelial space or toward osteoclasts in the bone tissue. Specific local paracrine factors modulated by inflammatory factors have opposing effects on vessels and on bone. Inflammation decreases bone mineralization while it increases vascular calcification.

Vessel wall calcification is mediated by calcium binding to a calcium-sensing receptor expressed by the smooth muscle cells of the media [48]. Polymorphism of the calcium-sensing receptor gene (i.e. S allele) was related to increased CV mortality and myocardial infarction with 38% and 30% respectively [49]. Thus, calcium may influence evolution of atherosclerosis through its smooth muscle cell receptor.

Vascular calcification depends on matrix protein metabolism related to vitamin K [50], pro-inflammatory chemokines [51,52], oxidized LDL (as a basic promoter of vessel wall inflammatory reaction) [53] and the NFKB ligand signaling system [54]. Addressing both osteoporosis and vascular calcification should be a major step forward into reducing morbidity and mortality of elderly women.

The importance of tissue matrix proteins in vascular calcification is emphasized by the role of matrix Gla protein (MGP). Activation of MGP is due to a carboxylation process and prevents systemic calcification by binding calcium phosphate in the tissues. MGP carboxylation is depending on vitamin K availability [55]. Vitamin K antagonists such as oral anticoagulants impair the activity of both vitamin K-dependent MGP and Bone Gla Protein (BGP or osteocalcin), significantly increasing vascular calcification either in the peripheral [56] or in the coronary arteries [57]. Warfarin-induced systemic calcification can result in adverse clinical effects: besides acceleration of coronary calcification, it promotes atherosclerotic plaque instability [57].

Vascular calcification induced by vitamin-K antagonists is significantly potentiated when administered with vitamin D supplements in an experimental model [58]. Clinically relevant valvular and vascular calcifications were observed in patients treated with warfarin [59]. Because of the increased CV risk due to vascular calcifications induced by vitamin K antagonists, the use of non-VKA anticoagulants may be considered when indicated [60].

Cardiovascular risk associated with arterial calcification

There is clear evidence coming from multiple clinical trials that vascular calcification increases clinical events and mortality risk [61]. Most data were obtained from epidemiological trials that used baseline measurement of coronary artery calcium (CAC) by multi-slice CT (MSCT); coronary calcium is quantified automatically in an operator-independent manner and expressed by the *Agatston score* [62]. CAC score was clearly related to the risk of developing coronary and cardiovascular events in the multiracial MESA trial on 3398 subjects followed up for a median of 7.6 years [63]. In another long-term follow up trial baseline CAC score was correlated with survival mainly in women: 15-year mortality was 23.5% for women and 18% for men with an *Agatston score* higher than 400 [64]. In another trial coronary calcification, added to valvular calcium deposits *and* thoracic and abdominal aortic calcification was most correlated with major coronary heart disease followed by major cardiovascular disease and general mortality in 3586 participants at a median follow-up of 8 years [65,66]. When compared to functional testing CAC score was more sensitive to identify CV events *versus* the ECG exercise test, which was more specific in the PROMISE Trial performed in patients with stable angina [67] (table 1).

Limitations of currently available trials regarding calcium/vitamin D supplements in post-menopausal women

There are inherent limitations of current knowledge to make definite decisions about calcium-vitamin D supplements in post-menopausal women [72].

All available randomized trials are too small, enrolling inhomogeneous patients with various baseline

Trial (reference)	No of patients	Calcium dose/day (mean – mg, source)	Mean FU (years)	Hazard ratio (95% CI)
Hsia [26]	36282	1000 vs placebo (drugs)	7	1.01 (0.79–1.29)
Bonthuis [68]	1529	1261 vs 954 vs 746 (dairy)	14.4	1.73 (0.56–4.54)
Li [69]	23980	1130 vs. 820 (dietary, drugs)	11	1.01 (0.74–1.36)
Van Hemelrijck [70]	20024	1560, 1150 vs. 750, 400 (dietary, supplements)	14.4	0.97 (0.78–1.21)
Khan [71]	41514	1076 vs. 899 (diet)	13	0.89 (0.68–1.15)

Table 1
ADJUSTED HAZARD RATIOS IN STUDIES WHICH CONSIDERED CV DEATH RELATED TO MEAN CALCIUM INTAKE, IRRESPECTIVE OF PATIENT SEX. NO INCREASE IN CV MORTALITY WITH CALCIUM SUPPLEMENTS CAN BE OBSERVE

cardiovascular risk and various treatment duration, medication dosage and follow-up. Different calcium supplements have been used (carbonate, citrullinated, gluconate), known to have different bioavailability. No clear correlations were investigated regarding the dose of orally given calcium, serum calcium concentration, eventual arterial calcium deposits and clinical events, either osteo-articular or cardiovascular. No available trial had cardio-vascular events as primary end-point; all data regarding this issue were obtained from meta-analysis, some with opposing results.

A clinical trial of calcium supplements in post-menopausal women with osteopenia-osteoporosis should enroll 13.500 patients with a mean follow-up of 5 years; such a trial will have an 80% statistical power to identify a 20% difference in the primary endpoint of myocardial infarction, stroke and/or cardiovascular death.

Conclusions

Calcium and vitamin D supplements widely prescribed for osteopenia-osteoporosis in post-menopausal women should be reserved for patients without calcified atherosclerosis and lower cardiovascular risk. When one considers this type of supplements, food supplementation should be preferred versus drugs. The total amount of calcium ingested from all sources should not overcome 1 g/day. A limitation of intake should be considered also because these supplements are seldom sufficient by themselves to prevent bone loss and the risk of fractures.

Cardiovascular disease risk assessment should be performed prior to prescribing long-term calcium-vitamin D supplements. One should consider that vital risk is due to major adverse cardio-vascular events and less to osteopenia or arthritis. Clinical conditions such as treatment with vitamin K antagonists or chronic renal failure on hemodialysis should represent contraindications to these supplements.

This medication should be carefully given to women with high cardiovascular risk and calcified atherosclerosis. Current epidemiological data point to an increased risk of on-treatment events.

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