Peer Review File

Article information: Available at https://dx.doi.org/10.21037/atm-22-5358

Reviewer A

Major comment 1: There is a significant lack of evidence for any of the abnormalities/deficiencies to make any evidence-based conclusion on active treatment/intervention and the beneficial effect on vascular calcification. The only recommendation to address is to reduce excess exogenous calcium, otherwise the other disorders are still theoretical (and biologically plausible) but without human controlled studies to support treatment at this point in time. In fact, the authors state in the background of the Abstract that "there is no effective treatment for VC" but yet undertake the narrative review 'to develop a prevention and treatment strategy' for VC in CKD. And the conclusion of the Abstract states "this review adds to clinical practice with the viewpoint of treating CKD-VC through optimized nutrient metabolism management'… but I strongly disagree with this statement. There are associations with VC and nutrient/metabolic abnormalities but no conclusive RCTs to support correcting the disorders is beneficial. The conclusion of the manuscript (page 18) that states "Optimized nutrient metabolism management for CKD is expected to prevent and delay the progression of VC" is not accurate.

Reply 1: Thank you for the valuable comments. This study reviewed nutritional and metabolic factors affecting vascular calcification and the treatment for these nutritional and metabolic disorders. There are insufficient human controlled studies to identify

whether the correction of these nutritional and metabolic disorders improves vascular calcification in chronic kidney disease. Accordingly, we have revised the Title, Abstract, Introduction and Conclusions sections of this paper such that the aim and scope as well as the opinions of this review are clearer and more precise (see Page 1, lines 1-3; Page 2, lines 4 and 5, lines 17-20; Page 3, lines 3-5; Page 4, line 20; Page 5, lines 1 and 2; Page 18, lines 1 and 2).

Major comment 2: There has been a recently published systematic review on interventions in clinical studies that may attenuate VC that is not even referenced in this paper (Xu *et al*, JASN 2022; 33(5): 1011-1032).

Reply 2: We have added the study published by Xu *et al.* (JASN 2022; 33(5): 1011-1032.) to the References (see Page 4, lines 16-18; Page 20, lines 16-18). Thank you for your helpful comment.

Major comment 3: Page 7 – There is a significant discussion about phosphate binders, comparing iron-based to other binders especially, but with no real relation to VC, i.e this is unrelated to the actual topic and narrative review. Essentially the long paragraph that starts “Compared to lanthanum carbonate and sevelamer….” can be deleted altogether from this manuscript as it does not discuss phosphate binders and VC.

Reply 3: We have deleted this paragraph as advised (see Page 7).

Major comment 4: The discussion about iron is extrapolation that and association may be causation. In other words, just because there is an association between iron deficiency and VC, does not mean that 'iron supplementation is beneficial for the prevention and treatment of VC'. There are no studies to support this statement.

Reply 4: The statement "iron supplementation is beneficial for the prevention and treatment of VC" is based on the evidence from in vitro and in vivo studies which are presented in the manuscript (Pages 12, line 16 to Page 13, line 2 "Iron ions bind with…downregulating the expression of Pit-1 and Runx2"). We absolutely agree that the clinical study we referenced only suggests an association between iron deficiency and VC, which does not mean the therapeutic effect of iron supplementation on VC in CKD patients. Accordingly, we have rephrased the sentence "iron supplementation is beneficial for the prevention and treatment of VC" by replacing "is" with "might be" and have noted that the effect of iron supplementation on VC needs to be verified in CKD patients (see Page 13, lines 2-7).

Major comment 5: The discussion on evolocumab doesn't necessarily relate to VC as the association between reduction in cardiovascular events (e.g., cardiovascular death, stroke, myocardial infarction etc) doesn't mean this is association with VC. Also the discussion on page 15 in relation to LDL-C levels and aliroumab is also no necessary in this manuscript as this doesn't relate to VC. In fact, the comment that 'elevated Lp(a)

is found to be an independent risk factor for CKD-VC' is not totally correct as the authors have not presented any evidence/studies/discussion on lipoproteins and VC.

Reply 5: We have deleted the unnecessary descriptions on the association of LDL-C with cardiovascular events and the association of evolocumab or aliroumab with LDL-C or cardiovascular events according to the comment (see Pages 13 and 14). The Lp(a)-lowering effects of evolocumab and aliroumab are remained in the manuscript for the aim of this review. The mechanism underlying Lp(a)-induced vascular calcification is presented in the manuscript (see Page 13, lines 15-20; Page 14, lines 1 and 2). The statement "elevated Lp(a) is found to be an independent risk factor for CKD-VC" has been removed (see Page 14).

Major comment 6: The evidence for protein malnutrition and VC is based on association, and in vivo studies in rats showing low protein diet (but associated high phosphate diet) led to more severe VC. So there is, at this stage, no recommendations about protein intake and VC that can be made.

Reply 6: We absolutely agree with the comment and have stated this point as "However, there is no recommendations about protein intake related to VC prevention in CKD patients" (see Page 15, lines 5 and 6) and "the minimum protein intake for not increasing the risk of VC need to be further identified" (see Page 16, lines 4 and 5) in the revised manuscript. Additionally, we have modified the sentence about the low-

protein high-phosphate diet in uremic rats because one of the two referenced studies did not mention a phosphate component in the low-protein diet (see Page 15, lines 15-18). We apologize for our carelessness.

Major comment 7: Page 17 – the statement that a clinical study showed vitamin K supplements in diabetic patients delayed progression of CAC should also add that this was a post-hoc analysis.

Reply 7: We have revised it as suggested (see Page 17, lines 4-6).

Major comment 8: Table 1 and 2 could be combined. And there should be another Table added that is a summary of all nutrients/interventions discussed.

Reply 8: We have combined Tables 1 and 2 into a new Table 1 and have added a new Table 2 to summarize the discussed nutrition treatments/interventions on vascular calcification in CKD.

Minor comment 1: The word phosphorus should be changed to phosphate to be more accurate.

Reply 1: We have revised the word throughout the manuscript.

Minor comment 2: Page 8 – the description about the follow up of the systematic review of 20 clinical trials (Ref 22) should state that the follow up period of 9 months was a 'mean' follow up.

Reply 2: We have indicated this as suggested (see Page 8, line 7).

Minor comment 3: Page 9: some patients with hypercalcemia have severe secondary hyperparathyroidism (so called tertiary hyperparathyroidism) or have too much calcium/calcitriol supplementation; i.e. not all hypercalcemia is related to nondynamic bone disease.

Reply 3: We have revised the causes of hypercalcemia according to the suggestion (see Page 9, lines 2-5).

Minor comment 4: Page 11 – the abbreviation CPP needs to be expanded; and there should be some mention about what these are for the reader.

Reply 4: The abbreviation CPP are expanded (see Page 10, lines 9 and 10). We have presented the CPP and the mechanism by which CPP regulates vascular calcification (see Page 10, lines 10-19).

Minor comment 5: Was vitamin C considered and looked at with regards to an

association with VC and whether treatment may help?

Reply 5: This is an interesting issue. Many studies have suggested that vitamin C/ascorbic acid protects endothelial cell function and protects against arteriosclerosis through antioxidant stress effects (Collins *et al*. Nutrients. 2021; 13(7): 2330.); however, the risk of cardiovascular disease was not reduced by vitamin C supplementation, as shown in a recent systematic review (Jenkins *et al*. J Am Coll Cardiol. 2021; 77(4): 423-436.). For vascular smooth muscle cells, vitamin C/ascorbic acid is often used as one of the components of high-phosphate medium for calcification induction (e.g., 1. Ciceri *et al*. Nephrol Dial Transplant. 2012; 27(1): 122-7. 2. Kruithof *et al*. J Mol Cell Cardiol. 2021; 156: 95-104. 3. Skafi *et al*. J Cell Physiol. 2019; 234(4): 4825-4839.). Few studies have shown a protective effect of vitamin C against vascular smooth muscle cell calcification or artery calcification in CKD (Ivanov *et al*. Am J Cardiovasc Dis. 2020; 10(2): 108-116.). Thus, we did not add vitamin C/ascorbic acid to the discussion.

Reviewer B

Comment 1: Page 7

a) line 8: "dosage of tablets" should be pill burden.

b) Line 11. FGF-23 should be introduced.

c) Line 18 "compared" should be similar to

d) line 18 data for phosphate lowering of SOH should be given

Reply 1:

a) We have changed "dosage of tablets" to " pill burden" (see Page 7, line 14).

b) We have expanded FGF-23 in terms of the source and function (see Page 7, lines 7-13).

c) What we intend to express is that sucroferric oxyhydroxide is a more stable polynuclear iron mixture than ferric citrate. We apologize for the confusing expression, and we have removed it (see Page 7).

d) We have presented the phosphate-lowering data of sucroferric oxyhydroxide (SOH) (see Page 7, lines 13-16).

Comment 2: Page 8 line 10: remains controversial should be elaborated on.

Reply 2: The controversy regarding the use of iron-based phosphate binders is their effects on iron-related indicators. We have narrated the controversy and changed the word “controversy” to “concern” after consideration (see Page 7, lines 18-20; Page 8, line 1).

Comment 3: Page 9

a) Lines 6-7 actions of 1,25 on phosphate should be described

b) line 8 "reuptake in intestinal and renal tubules" 1,25 leads to a decrease in renal excretion.

c) Line 9 and 10 The sentence "The decreasing urinary..." should be rewritten.

Reply 3:

a) We have made the description that the downregulated 1,25-dihydroxyvitamin D level resulted in reduced calcium and phosphate reuptake in intestinal and renal tubules as well as reduced bone calcium and phosphate released into the circulation (see Page 8, lines 16-20).

b) We have revised this sentence to make it comprehensible. Urinary calcium excretion decreases as a compensatory response to hypocalcemia secondary to reduced calcium and phosphate reuptake in intestinal and renal tubules and may retain the serum calcium level in the normal range (see Page 8, line 20; Page 9, lines 1 and 2).

c) We have rewritten this sentence to make it comprehensible (see Page 8, line 20; Page 9, lines 1 and 2).

Comment 4: Page 12: The authors should discuss the role of inflammation in increasing hepcidin levels.

Reply 4: We have discussed this as suggested (see Page 12, lines 7-12). Thank you for the valuable comment.

Comment 5: Page 13 The authors should discuss the role of inflammation increasing LDL

Reply 5: Current studies have suggested a close positive association between inflammation and LDL-C levels (e.g., 1. Sohrabi *et al*. Trends Mol Med. 2022; 28(1): 1-4. 2. Bernelot *et al*. Eur Heart J. 2017;38(20):1584-1593.). Considering that LDL-C has little effect on VC and the aim of the study (reviewing nutritional and metabolic disorders that regulate VC), we have removed the discussion on LDL-C (see Page 13).