Original Article

Can Bone-Specific Alkaline Phosphatase be a Marker of Vascular Calcification in Type 2 Diabetes Mellitus?

Nishant Raizada, Mohammad Aslam, BK Mishra, Diwesh Chawla[1], SV Madhu

Department of Endocrinology, Centre for Diabetes Endocrinology and Metabolism University College of Medical Sciences (University of Delhi) and GTB Hospital, Dilshad Garden, Delhi, [1]Central Research Laboratory, Multi-Disciplinary Research Unit, University College of Medical Sciences (University of Delhi) and GTB Hospital, Dilshad Garden, Delhi, India

Abstract

Background and Aims: Alkaline phosphatase (ALP) enzyme has been linked to vascular calcification. Unexplained elevations in serum ALP levels have been reported in patients with type 2 diabetes mellitus (T2DM). We assessed bone-specific alkaline phosphatase (BAP) levels in patients with T2DM who had unexplained ALP elevations and studied the association between BAP and other markers of vascular calcification. **Methods:** Patients with T2DM who had high serum ALP in the absence of known causes of ALP elevation were studied. The control group was T2DM patients with normal ALP. We measured the serum levels of BAP along with the leptin, fetuin-A, and vitamin K2 levels. Ankle–brachial index (ABI) was also measured in both groups. **Results:** Serum BAP levels were significantly higher in the group with high ALP when compared with the normal ALP group. A significant positive correlation was present between BAP and serum fetuin-A as well as between BAP and Vit K2 levels. There was no correlation between BAP and serum leptin. ABI was comparable between the two groups. **Conclusions:** Patients with T2DM may have unexplained elevation in ALP due to an increase in BAP. Elevation in BAP may be associated with other markers of vascular calcification suggesting an increased risk of vascular calcification.

Keywords: Alkaline phosphatase, bone-specific alkaline phosphatase, fetuin-A, leptin, vascular calcification, vitamin K2

Introduction

Individuals living with type 2 diabetes mellitus (T2DM) are at risk of atherosclerosis. The pathogenesis of atherosclerosis includes the calcification of atherosclerotic plaques. The alkaline phosphatase (ALP) enzyme has been linked to vascular calcification in patients with end-stage chronic kidney disease.[1] Elevated ALP has been associated with increased mortality in patients with coronary artery disease.[2] Unexplained elevations in serum ALP levels have been reported in patients with type 2 diabetes mellitus.[3-5] This elevation was attributed to a rise in bone-specific alkaline phosphatase (BAP). Serum BAP levels are also associated with markers of insulin resistance and metabolic syndrome in normoglycemic individuals.[6] Overall, data on unexplained ALP elevations and BAP in T2DM are scanty. The role of BAP in vascular calcification in T2DM is also not well understood. We assessed BAP levels in patients with T2DM who had unexplained ALP elevations and also studied the association between BAP and other markers of vascular calcification in these patients.

Access this article online

Quick Response Code:

Website: www.ijem.in

DOI: 10.4103/ijem.ijem_418_22

Subjects

This study was conducted at the diabetes clinic of a tertiary care hospital in North India.

Patients with T2DM between 30 and 70 years of age were eligible for the study if their serum ALP levels were elevated. The control group consisted of patients with T2DM who had normal ALP levels. The exclusion criteria included the history of coronary artery disease, ischemic stroke, peripheral arterial disease, chronic liver disease, deranged kidney function

Address for correspondence: Dr. SV Madhu, Director-Professor, Department of Endocrinology, Centre for Diabetes Endocrinology and Metabolism University College of Medical Sciences (University of Delhi) and GTB Hospital, Dilshad Garden, Delhi-110 095, India.
E-mail: drsvmadhu@gmail.com

Submitted: 15-Nov-2022 **Revised:** 30-Jan-2023
Accepted: 13-Feb-2023 **Published:** 14-Apr-2023

How to cite this article: Raizada N, Aslam M, Mishra BK, Chawla D, Madhu SV. Can bone-specific alkaline phosphatase be a marker of vascular calcification in type 2 diabetes mellitus? Indian J Endocr Metab 2023;27:127-32.

test (defined as CKD stage 3 or worse based on eGFR by MDRD equation), deranged liver function tests (defined as any elevation in serum bilirubin, alanine transaminase, or aspartate transaminase above the reference range), elevated parathormone levels, recent fractures (past 3 months), hypothyroidism, pregnancy, or lactation. The study was approved by the institutional ethical committee.

Materials and Methods

We screened the electronic medical records between 2019 and 2021 to identify patients with elevated ALP who did not meet the exclusion criteria. Similarly, patients with normal ALP not meeting the exclusion criteria identified for the control group. These patients were invited to participate in the study after obtaining informed consent. A total of 50 patients with high ALP and 50 with normal ALP were selected. Serum parathormone levels were measured in patients with elevated ALP. Those with serum parathormone levels above the reference range were excluded from the study. We repeated serum ALP levels in all patients to confirm its elevation or normalcy prior to inclusion in the study. Serum ALP levels were measured by AMP method at 30 °C on a Randox Imola autoanalyzer (Randox Laboratories, UK) using commercially available reagents (AP8302, Randox Laboratories, UK). The upper limit of normal for this assay is 90 U/L. ALP levels greater than 90 U/L were defined as high ALP for this study, while levels equal to or less than 90 U/L were defined as normal ALP.

Blood was drawn for measurement of serum BAP, leptin, fetuin-A, and vitamin K2 levels. The serum samples were stored at − 80°C till the analysis. BAP was estimated using ELISA kits (Ostase BAP EIA kit, Immunodiagnostics System Ltd, UK). The intra-assay CV was 3.16%. Similarly, ELISA was done for serum leptin (DRG International USA), serum fetuin-A (Biovendor Laboratorni Medicina a.s. Czech Republic), and vitamin K2 levels (Qayee Biochemicals, Shanghai, China. The intra-assay CV was 3.83% for leptin, 1.04% for fetuin-A, and 2.05% for vitamin K2. Ankle–brachial index (ABI) was measured in all patients using Vista AVS-L500 VA system (Wallach Surgical, USA).

The data were entered in to a Microsoft Excel worksheet and statistical analysis was performed on SPSS version 21 (IBM, USA). The categorical variables were compared using the Chi-square test. Continuous variables were compared using the Student's *t* test if the variables were normally distributed. We performed a log transformation of skewed variables to achieve a normal distribution. The correlation between BAP and other markers was studied by calculating Pearson's coefficient. In case the variable was significantly different between the high and normal ALP groups, we adjusted for the BAP levels by performing a one-way analysis of variance (ANOVA) with BAP as the dependent variable and other variables (which were significantly different between the two groups being studied) as covariates.

The study was approved by Institutional Ethics Committee-Human Research, University College of Medical Sciences, Delhi, India vide letter no IEC-HR/2019/41/144 on 16/10/2019. Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes. The procedures follows the guidelines laid down in Declaration of Helsinki 2008.

Results

A total of 100 patients were studied. Out of these, 50 were in the normal ALP group and 50 were in the high ALP group. The

Table 1: Baseline characteristics of the normal ALP and high ALP group

Parameter	Normal ALP (*n*=50)	High ALP (*n*=50)	*P*
Age (years)	50.4±11.5	49.5±9.9	0.68
Female sex	28 (56%)	26 (52%)	0.84
Duration of diabetes (years)	3.8±5.1	2.40±4.4	0.14
BMI (kg/m^2)	26.8±5.0	26.1±4.6	0.44
Fasting plasma glucose (mg/dl)	204.2±80.3	212.0±80.0	0.63
Postprandial plasma glucose (mg/dl)	292.8±96.3	293.7±81.7	0.96
HbA1c	10.0±2.7% (86.0 mmol/mol)	10.0±2.4% (86.0 mmol/mol)	0.49
Total Chol (mg/dl)	177.5±54.7	185.9±46.4	0.42
TG (mg/dl)	137.0±68.7	154.1±77.1	0.26
HDL (mg/dl)	38.2±10.4	29.7±8.3	<0.001
LDL (calculated) (mg/dl)	101.7±34.4	123.1±38.8	0.006
eGFR (ml/min/1.73 m^2)	97.4±12.1	86.7±16.5	0.001
ALP (U/L)	78.9±9.1	135.8±16.5	<0.001
Calcium (mg/dl)	9.43±0.99	9.47±0.53	0.85
Phosphate (mg/dl)	3.6±0.4	3.8±0.5	0.03
Serum PTH (pg/ml)	35.7±13.6	31.1±9.7	0.06
Serum 25-OH vitamin D	10.57±9.52	10.18±9.50	0.32
ABI Right	1.02±0.07	1.06±0.11	0.19
ABI Left	1.07±0.07	1.08±0.08	0.55

mean ALP levels and the other baseline characteristics of the two groups are shown in Table 1. The groups were comparable in age, sex, duration of diabetes, BMI, and glycaemic status. The calculated LDL values were significantly higher in the high ALP group, while the HDL values were significantly lower. The high ALP group had significantly higher phosphate levels than the normal ALP group. The serum 25 hydroxy vitamin D levels were similar between the two groups. While the eGFR was significantly lower in the high ALP group, the serum PTH levels were higher in the normal ALP group (although not attaining statistical significance). In the normal ALP group, 28% of patients had eGFR between 60 and 90 ml/min/1.73 m^2 while this percentage in the high ALP group was 60%. The results of BAP and other markers of vascular calcification are shown in Table 2.

Ankle–brachial indexes were not significantly different in the two groups. There was no significant correlation between ankle–brachial index values and levels of BAP, vitamin K2, leptin or fetuin-A. On performing one-way ANOVA with BAP as the dependent variable and serum ALP as the covariate, we found that adjusted mean BAP levels were 20.91 µg/L in the normal ALP group and 25.86 µg/L in the high ALP group. The difference in adjusted means was statistically significant. A simple linear regression was calculated to predict serum ALP based on BAP. A significant regression equation was found (F (1,98) =44.23, $P < 0.001$), with an R square of 0.311. The patient's predicted serum ALP is equal to 62.1 + 1.95 (BAP)µg/L when ALP is measured in U/L. The participant's average ALP increased by 1.95 units for every one-unit rise in BAP.

A significant positive correlation between BAP and serum fetuin-A was seen (Pearson's coeff 0.35, $P < 0.001$). [Figure 1] Similarly, a significant negative correlation exists between BAP and Vit K2 levels (Pearson's coeff 0.37, $P < 0.001$). [Figure 2] However, there was no correlation between BAP and leptin levels.

The high ALP group had lower eGFR which may explain the rise in BAP. In view of the significant difference in eGFR in the two groups, we performed two analyses. Firstly, we ran a one-way ANOVA with BAP as the dependent variable and eGFR as the covariate, we found that adjusted mean BAP levels were 18.34 µg/L (SE 1.20, 95% CI 15.9–20.7) in the normal ALP group and 28.44 µg/L (SE 1.20, 95% CI 26.0–30.8) in the high ALP group. The adjusted means were statistically significant ($P < 0.001$). Secondly, we also compared the BAP levels in the normal ALP and the high ALP groups separately for those with eGFR >90 and between 60 and 90. In this analysis, the BAP levels were higher in the high ALP groups in both scenarios as shown in Table 3.

BAP showed a negative correlation with HDL levels (Pearson coefficient − 0.206, $P = 0.04$), while there was no significant correlation with LDL, total cholesterol, and triglycerides. There was no significant correlation between PTH and BAP levels.

Discussion

In this study, we found that patients with type 2 diabetes mellitus and unexplained elevation in ALP have higher BAP levels, lower serum vitamin K2 levels along with fetuin-A levels which tended to be higher. BAP levels were significantly associated with vitamin K2 as well as fetuin-A levels. Unexplained ALP elevations in type 2 diabetes mellitus have been reported the past.[3] Further, BAP levels have also been reported to be elevated in type 2 diabetes.[3-5] Although

Table 2: Results of BAP and other markers of vascular calcification

Parameter	Normal ALP	High ALP	*P*
BAP (µg/L)	18.10±7.68	28.68±8.78	<.001
Leptin (ng/ml)	6.84±6.95	4.92±4.65	0.14
Vitamin K2 (ng/ml)	30.67±6.51	20.71±11.23	<.001
Fetuin-A (µg/ml)	748.9±210.8	839.3±266.3	0.063

Table 3: BAP levels in normal and high ALP groups with eGFR >90 ml/min/1.73 m^2 and eGFR 60-90 ml/min/1.73 m^2

	Normal ALP	High ALP	*P*
eGFR >90 ml/min/1.73 m^2	17.2±6.7	29.2±9.6	<0.001
eGFR 60-90 ml/min/1.73 m^2	20.4±9.4	28.3±8.2	0.01

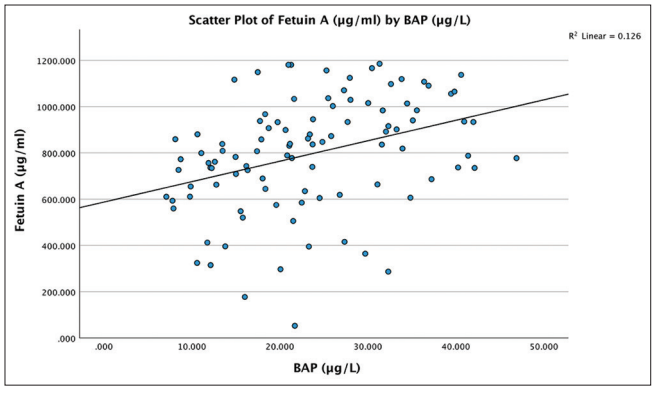

Figure 1: Correlation between serum BAP and serum fetuin-A

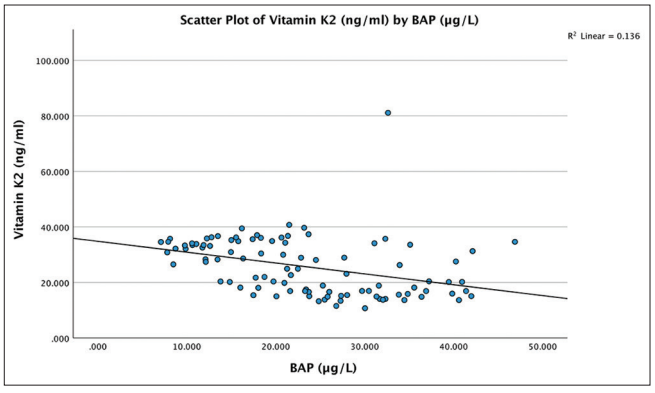

Figure 2: Correlation between serum BAP and serum vitamin K2

there are no recent publications directly addressing this issue, the link between ALP and metabolic syndrome component has been recognized.[6] Hence, an elevation of ALP in type 2 diabetes in the absence of the usual causes is plausible. In fact, we observed seemingly unexplained elevations in ALP in several patients in our diabetes clinic—this led us to explore this topic further. The major component of this elevation in our study is the BAP—which confirms the findings of the previous studies.[3,4] Chronic kidney disease (CKD) is a known cause of ALP elevation but this elevation typically occurs in CKD stages 3 to 5 and occurs in conjunction with secondary hyperparathyroidism. Our study included patients with CKD only if they were CKD stage 1 or 2. Further, elevated PTH was an exclusion criterion in our study. The persistence of the high ALP despite adjusting for eGFR further shows that CKD did not contribute to high ALP in our study. Studies in chronic kidney disease have established that vascular calcification is associated with an elevation in ALP and BAP.[1,7] High ALP levels have been associated with coronary calcification in patients with as well as without established coronary artery disease.[8,9] Patients with diabetes mellitus have higher coronary calcium scores as compared to those without diabetes.[10] In patients with diabetes mellitus, coronary artery calcification predicts future CVD events.[11] In patients with coronary artery disease, elevated ALP has been associated with more severe disease; the patients with diabetes in one such study had high ALP levels.[2] Hence, ALP, presumably its bone-specific component, plays a role in vascular calcification in type 2 diabetes.

The mechanism by which BAP increases vascular calcification is interesting. BAP is expressed in vascular smooth muscle cells.[12] Vascular smooth muscle cells release matrix vesicles which act as a nidus for vascular calcification. Some of these matrix-bound vesicles have high concentrations of BAP, while some contain calcification inhibitors including fetuin-A (non-calcifying vesicles).[13] BAP increases calcification by several methods. BALP inactivates polyphosphates which inhibit calcification, especially pyrophosphate.[14] Inhibition of osteopontin via its dephosphorylation has also been proposed as a mechanism.[15] Further, BAP can hydrolyse organic phosphate esters to release phosphate which is a substrate for calcification.[16] The change in the phosphate to pyrophosphate ratio in the vascular tissue can lead to vascular calcification even if serum phosphate levels are not elevated.[17]

Vitamin K also plays a role in vascular calcification. A vitamin K-dependent protein called matrix GLA protein is an inhibitor of vascular calcification.[18] Studies suggest that vitamin K deficiency results in vascular calcification in chronic kidney disease.[19] Vitamin K antagonists, used as anticoagulant agents, can increase vascular calcification.[20] Vitamin K2 supplementation reduced vascular calcification in animal studies, while in clinical studies, the results have been mixed.[21-23] High vitamin K2 intake may reduce the risk of coronary artery disease and peripheral arterial disease.[24-26] The low vitamin K2 levels in the high ALP group in our study may signify an increased predisposition to vascular calcification.

In our study, fetuin-A was higher in the high ALP group, although the difference was not statistically significant. Fetuin-A is an inhibitor of vascular calcification and has an inverse correlation with calcification in atherosclerotic plaques.[27] Low fetuin-A levels have been associated with vascular calcification in dialysis patients. Fetuin-A inhibits vascular calcification by two processes—firstly, it gets incorporated into matrix vesicles of the vascular smooth muscle cells and prevents their mineralization; secondly, it acts systemically by binding to excess minerals and formation of a fetuin–mineral complex. These fetuin–mineral complexes contribute to the clearance of fetuin-A from circulation.[28] If we assume that high ALP is a surrogate marker for vascular calcification, it would be logical to expect lower fetuin-A in the group with high ALP. However, other explanations for high fetuin-A levels also exist. Fetuin-A is also associated with insulin resistance and atherosclerosis. High fetuin-A levels are associated with an increased risk of diabetes mellitus.[29] Some studies have reported increased cardiovascular disease risk with higher fetuin-A levels in patients with diabetes, while the converse was true for those without diabetes.[30] Diabetes mellitus patients with peripheral arterial disease had higher fetuin-A than those who do not have the peripheral arterial disease.[31] It is possible that fetuin-A is elevated in atherosclerotic states and may reduce later as vascular calcification progresses.

The present study did not find a significant difference in leptin levels in the study groups. Leptin has been shown to be involved in vascular calcification in mouse models. Vascular cells treated with leptin *in vitro*, demonstrated an increase in ALP activity with subsequent differentiation to osteoblastic cells.[32] Elevated leptin levels are associated with aortic calcifications in humans.[33] In one study, plasma leptin levels had a positive association with coronary artery calcification in women.[34] Patients with diabetes had higher leptin levels and greater vascular calcification in lower extremities as compared to controls without diabetes in one study.[35] However, in a larger study with 906 participants biomarkers including leptin did not have any association with coronary artery calcium.[36] The role of leptin in vascular calcification, inflammation, and insulin resistance is complex and requires further research.

Ankle–brachial index has been used as a surrogate marker of vascular calcification and atherosclerosis.[37] Our study population is relatively young with female predominance (many of which may be premenopausal); therefore, clinically detectable vascular calcification may be less in this population. Further, it is well recognized that South Asians are less likely to develop peripheral arterial disease while simultaneously being at a higher risk for coronary artery disease.[38] This can explain the lack of difference in ABI between the groups in our study.

It is now understood that type 2 diabetes mellitus may not be a single disease but rather a conglomeration of multiple

disorders. Novel subgroups of adult-onset diabetes mellitus, each of which has a different long-term complication risk profile, have been identified.[39] Hence, it is safe to say that certain patients with type 2 diabetes mellitus are more likely to develop atherosclerosis as compared to the remainder. This risk may further be modulated by environmental factors. Once the atherosclerotic process has been initiated in at-risk individuals, vascular calcification also starts. The elevation of ALP, probably as a consequence of raised BAP, is a reflection of this process. However, since vascular calcification is not a *sine qua non* of atherosclerosis, all patients with atherosclerosis may not have high ALP. But the presence of seemingly unexplained ALP elevation could signal an underlying atherosclerotic process.

The strength of this study is that it has brought back into focus the unexplained elevations in ALP in type 2 diabetes, which although known for decades, has not been studied recently. The weaknesses of the study include the relatively small sample size. The skewed distribution of results suggests that a larger sample size might have yielded more conclusive results. We did not take into account dietary factors which may influence vitamin K levels. Since South Asians are more predisposed to CAD rather than PAD, the use of coronary artery calcium score instead of ABI would have yielded more precise results.

Unexplained elevations in ALP in type 2 diabetes and its relationship with high BAP and some markers of vascular calcification in an interesting finding are observed. Future research can further elucidate if the severity of atherosclerosis and the presence of atherosclerotic vascular disease can be predicted by simple laboratory tests such as ALP and BAP. It is possible that individuals with elevated ALP and BAP may constitute a subgroup which is at a higher risk for ASCVD. Hence, targeted screening or more intense primary preventive measures can be instituted in these individuals. It is also possible that other markers of vascular calcification such as Vit K 2 may also be used for the same purpose. Considering the significant mortality and morbidity due to macrovascular disease in type 2 diabetes, more research in this area is imperative.

Financial support and sponsorship

This work was supported by an intramural research grant from the Multi-disciplinary Research Unit (MRU), University College of Medical Sciences and GTB Hospital, Dilshad Garden, Delhi, India-110095.

Conflicts of interest

There are no conflicts of interest.

References

1. Shantouf R, Kovesdy CP, Kim Y, Ahmadi N, Luna A, Luna C, *et al*. Association of serum alkaline phosphatase with coronary artery calcification in maintenance hemodialysis patients. Clin J Am Soc Nephrol 2009;4:1106–14.
2. Sahin I, Karabulut A, Gungor B, Avci II, Okuyan E, Kizkapan F, *et al*. Correlation between the serum alkaline phosphatase level and the severity of coronary artery disease. Coron Artery Dis 2014;25:349–52.
3. Maxwell DB, Fisher EA, Ross-Clunis HA, Estep HL. Serum alkaline phosphatase in diabetes mellitus. J Am Coll Nutr 1986;5:55–9.
4. Stěpán J, Havránek T, Formánková J, Skrha J, Skrha F, Pacovský V. Bone isoenzyme of serum alkaline phosphatase in diabetes mellitus. Clin Chim Acta Int J Clin Chem 1980;105:75–81.
5. Foster KJ, Griffith AH, Dewbury K, Price CP, Wright R. Liver disease in patients with diabetes mellitus. Postgrad Med J 1980;56:767–72.
6. Cheung C-L, Tan KCB, Lam KSL, Cheung BMY. The relationship between glucose metabolism, metabolic syndrome, and bone-specific alkaline phosphatase: A Structural equation modeling approach. J Clin Endocrinol Metab 2013;98:3856–63.
7. Ishimura E, Okuno S, Okazaki H, Norimine K, Yamakawa K, Yamakawa T, *et al*. Significant association between bone-specific alkaline phosphatase and vascular calcification of the hand arteries in male hemodialysis patients. Kidney Blood Press Res 2014;39:299–307.
8. Ren Y, Li X, Wang S, Pan W, Lv H, Wang M, *et al*. Serum alkaline phosphatase levels are associated with coronary artery calcification patterns and plaque vulnerability. Catheter Cardiovasc Interv Off J Soc Card Angiogr Interv 2021;97(Suppl 2):1055–62.
9. Panh L, Ruidavets JB, Rousseau H, Petermann A, Bongard V, Bérard E, *et al*. Association between serum alkaline phosphatase and coronary artery calcification in a sample of primary cardiovascular prevention patients. Atherosclerosis 2017;260:81–6.
10. Raggi P, Shaw LJ, Berman DS, Callister TQ. Prognostic value of coronary artery calcium screening in subjects with and without diabetes. J Am Coll Cardiol 2004;43:1663–9.
11. Elkeles RS, Godsland IF, Feher MD, Rubens MB, Roughton M, Nugara F, *et al*. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: The PREDICT study. Eur Heart J 2008;29:2244–51.
12. Shioi A, Katagi M, Okuno Y, Mori K, Jono S, Koyama H, *et al*. Induction of bone-type alkaline phosphatase in human vascular smooth muscle cells: Roles of tumor necrosis factor-alpha and oncostatin M derived from macrophages. Circ Res 2002;91:9–16.
13. Ali SY, Sajdera SW, Anderson HC. Isolation and characterization of calcifying matrix vesicles from epiphyseal cartilage. Proc Natl Acad Sci U S A 1970;67:1513–20.
14. Fleisch H, Bisaz S. Mechanism of calcification: Inhibitory role of pyrophosphate. Nature 1962;195:911.
15. Jono S, Peinado C, Giachelli CM. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. J Biol Chem 2000;275:20197–203.
16. Robison R. The possible significance of hexosephosphoric esters in ossification. Biochem J 1923;17:286–93.
17. Villa-Bellosta R, Wang X, Millán JL, Dubyak GR, O'Neill WC. Extracellular pyrophosphate metabolism and calcification in vascular smooth muscle. Am J Physiol Heart Circ Physiol 2011;301:H61-8.
18. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, *et al*. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997;386:78–81.
19. Levy DS, Grewal R, Le TH. Vitamin K deficiency: An emerging player in the pathogenesis of vascular calcification and an iatrogenic consequence of therapies in advanced renal disease. Am J Physiol Renal Physiol 2020;319:F618–23.
20. Brandenburg VM, Schurgers LJ, Kaesler N, Püsche K, van Gorp RH, Leftheriotis G, *et al*. Prevention of vasculopathy by vitamin K supplementation: Can we turn fiction into fact? Atherosclerosis 2015;240:10–6.
21. Scheiber D, Veulemans V, Horn P, Chatrou ML, Potthoff SA, Kelm M, *et al*. High-dose menaquinone-7 supplementation reduces cardiovascular calcification in a murine model of extraosseous calcification. Nutrients 2015;7:6991–7011.
22. Aoun M, Makki M, Azar H, Matta H, Chelala DN. High dephosphorylated-uncarboxylated MGP in hemodialysis patients: Risk factors and response to vitamin K2, A pre-post intervention clinical trial. BMC Nephrol 2017;18:191.
23. Zwakenberg SR, de Jong PA, Bartstra JW, van Asperen R, Westerink J, de Valk H, *et al*. The effect of menaquinone-7 supplementation on vascular calcification in patients with diabetes: A randomized,

double-blind, placebo-controlled trial. Am J Clin Nutr 2019;110:883–90.
24. Gast GCM, de Roos NM, Sluijs I, Bots ML, Beulens JWJ, Geleijnse JM, *et al*. A high menaquinone intake reduces the incidence of coronary heart disease. Nutr Metab Cardiovasc Dis NMCD 2009;19:504–10.
25. Hariri E, Kassis N, Iskandar J-P, Schurgers LJ, Saad A, Abdelfattah O, *et al*. Vitamin K2-a neglected player in cardiovascular health: A narrative review. Open Heart 2021;8:e001715.
26. Vissers LET, Dalmeijer GW, Boer JMA, Verschuren WMM, van der Schouw YT, Beulens JWJ. The relationship between vitamin K and peripheral arterial disease. Atherosclerosis 2016;252:15–20.
27. Emoto M, Mori K, Lee E, Kawano N, Yamazaki Y, Tsuchikura S, *et al*. Fetuin-A and atherosclerotic calcified plaque in patients with type 2 diabetes mellitus. Metabolism 2010;59:873–8.
28. Komaba H, Fukagawa M. Fetuin-mineral complex: A new potential biomarker for vascular calcification? Kidney Int 2009;75:874–6.
29. Guo VY, Cao B, Cai C, Cheng KK-Y, Cheung BMY. Fetuin-A levels and risk of type 2 diabetes mellitus: A systematic review and meta-analysis. Acta Diabetol 2018;55:87–98.
30. Jensen MK, Bartz TM, Mukamal KJ, Djoussé L, Kizer JR, Tracy RP, *et al*. Fetuin-A, type 2 diabetes, and risk of cardiovascular disease in older adults: The cardiovascular health study. Diabetes Care 2013;36:1222–8.
31. Lorant DP, Grujicic M, Hoebaus C, Brix J-M, Hoellerl F, Schernthaner G, *et al*. Fetuin-A levels are increased in patients with type 2 diabetes and peripheral arterial disease. Diabetes Care 2011;34:156–61.
32. Parhami F, Tintut Y, Ballard A, Fogelman AM, Demer LL. Leptin enhances the calcification of vascular cells: Artery wall as a target of leptin. Circ Res 2001;88:954–60.
33. Szulc P, Amri EZ, Varennes A, Panaia-Ferrari P, Fontas E, Goudable J, *et al*. Positive association of high leptin level and abdominal aortic calcification in men- The Prospective MINOS study. Circ J Off J Jpn Circ Soc 2018;82:2954–61.
34. Iribarren C, Husson G, Go AS, Lo JC, Fair JM, Rubin GD, *et al*. Plasma leptin levels and coronary artery calcification in older adults. J Clin Endocrinol Metab 2007;92:729–32.
35. Chai S, Chen Y, Xin S, Yuan N, Liu Y, Sun J, *et al*. Positive association of leptin and artery calcification of lower extremity in patients with type 2 diabetes mellitus: A Pilot study. Front Endocrinol 2021;12:583575.
36. Mehta A, Patel J, Al Rifai M, Ayers CR, Neeland IJ, Kanaya AM, *et al*. Inflammation and coronary artery calcification in South Asians: The Mediators of Atherosclerosis in South Asians Living in America (MASALA) study. Atherosclerosis 2018;270:49–56.
37. Adragao T, Pires A, Branco P, Castro R, Oliveira A, Nogueira C, *et al*. Ankle--brachial index, vascular calcifications and mortality in dialysis patients. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc-Eur Ren Assoc 2012;27:318–25.
38. Sebastianski M, Makowsky MJ, Dorgan M, Tsuyuki RT. Paradoxically lower prevalence of peripheral arterial disease in South Asians: A systematic review and meta-analysis. Heart 2014;100:100–5.
39. Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, *et al*. Novel subgroups of adult-onset diabetes and their association with outcomes: A data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 2018;6:361–9.