Zinc As A Factor Affecting Serum Calcification Propensity in Patients With Type 2 Diabetes Mellitus

Shinya Nakatani  
Osaka City University  
https://orcid.org/0000-0001-5302-3634

Katsuhito Mori (✉ ktmor@med.osaka-cu.ac.jp)  
Osaka Shiritsu Daigaku

Mika Sonoda  
Osaka Shiritsu Daigaku

Kozo Nishide  
Osaka Shiritsu Daigaku

Hideki Uedono  
Osaka Shiritsu Daigaku

Akihiro Tsuda  
Osaka Shiritsu Daigaku

Masanori Emoto  
Osaka Shiritsu Daigaku

Testuo Shoji  
Osaka Shiritsu Daigaku

Original investigation

Keywords: Serum calcification propensity, Zinc, Diabetes

DOI: https://doi.org/10.21203/rs.3.rs-34107/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

**Background:** Zinc inhibits vascular calcification *in vivo* and *in vitro*. Patients with type 2 diabetes mellitus show hypozincemia and are at an elevated risk of cardiovascular events. Recently, the *in vitro* test (T$_{50}$-test) was developed for the determination of serum calcification propensity. This cross-sectional study investigated the association between serum zinc and T$_{50}$ in type 2 diabetes mellitus patients and the effect of zinc on T$_{50}$ *in vitro*.

**Methods:** The subjects were 132 type 2 diabetes mellitus patients with various kidney function. We measured T$_{50}$ levels by the established nephelometric method.

**Results:** The median (interquartile range) levels of T$_{50}$ and serum zinc were 306 (269 to 332) min, and 80.0 (70.1 to 89.8) µg/dL, respectively. Serum zinc level was significantly and positively correlated with T$_{50}$ ($r_s = 0.219$, $p = 0.012$). This association remained significant in multivariable-adjusted analysis, and was independent of known factors including phosphate, calcium, and magnesium. Renal function and glycemic control were not significantly associated with T$_{50}$. Finally, addition of physiological concentration of exogenous zinc chloride significantly increased the serum T$_{50}$ *in vitro*.

**Conclusions:** This is the first report to investigate the association between serum calcification propensity and zinc levels in type 2 diabetes mellitus patients. Our data suggest that serum zinc is an independent factor that inhibits serum calcification propensity.

Background

Vascular calcification is common and contributes to cardiovascular mortality in patients with type 2 diabetes mellitus [1, 2], and those with chronic kidney disease [3, 4]. The excess cardiovascular morbidity and mortality in those patients could be explained by redistribution and/or overload of calcium and phosphorus as well as imbalanced-calcification regulators [3, 5] in these conditions. The mechanism of vascular calcification is supposed to be due to ectopic deposition of hydroxyapatite [6, 7] induced by increased calcium-phosphorous product (Ca × P) in serum [8, 9], dedifferentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells [10, 11] and accumulation of degenerative extracellular matrix [12]. It is hypothesized that diabetic condition and renal dysfunction share some common causal pathways leading to vascular calcification.

In serum, precipitation of supersaturated calcium and phosphate is prevented by the formation of amorphous primary calciprotein particles (CPPs) [13, 14]. Primary CPPs spontaneously convert into secondary CPPs, containing crystalline hydroxyapatite [13, 14]. The *in vitro* test (T$_{50}$-test) for the determination of serum calcification propensity was developed [15]. This assay measures time required for primary CPPs to transform into secondary CPPs in the presence of supersaturating doses of calcium and phosphate, which increase turbidity of samples. T$_{50}$ can be measured by laser light scatter in turbid samples using nephelometry. Thus, a shorter T$_{50}$ means a higher calcification propensity. Previous
studies have shown that lower $T_{50}$ predicts vascular stiffness progression and all-cause mortality in patients with chronic kidney disease stage 3 and 4 [16], and all-cause mortality and cardiovascular composite endpoint in hemodialysis patients.[17]. A Lower $T_{50}$ was also shown to predict cardiovascular and all-cause mortality in renal transplant recipients [18, 19].

$T_{50}$ is depended on the complex interplay of pro-calcifying (i.e. calcium and phosphate) and anti-calcifying serum components (i.e. magnesium and albumin) [15]. Among them, a higher serum phosphate level was the factor most closely associated with lower $T_{50}$[17, 20]. Phosphate has been reported to induce calcification of VSMCs in vitro [21]. Hyperphosphatemia is a risk factor for vascular calcification and cardiovascular mortality, not only in patients with chronic kidney disease [22], but also in the general population [23]. Thus, suppression of phosphate-induced vascular calcification is clinically important.

Zinc is an essential micronutrient that plays catalytic, structural, and regulatory roles [24]. Recently, zinc was found to inhibit phosphate-induced vascular calcification, in vitro and in vivo [25]. In human, zinc level in blood was reported to be lower in patients with type 2 diabetes mellitus compared to non-diabetic subjects [26-28]. So far, however, the role of zinc in serum calcification propensity is not established.

These previous studies raise the hypothesis that zinc could be one of the factors affecting with serum calcification propensity. To test the hypothesis, we examined the association between serum zinc and $T_{50}$ levels and the effect of increasing zinc concentration on $T_{50}$ in vitro.

**Methods**

**Ethics statement**

This study followed the ethical guidelines for medical and health research involving human subjects by the Japanese Ministry of Health, Labour and Welfare, and the Declaration of Helsinki. This study was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (approval No. 4100). Opt-out option for informed consent was performed as explained in instructions posted on the website of the institution.

**Study design and participants**

This study comprised of two parts. The first part was a cross-sectional study using clinical data derived from our previous study including 143 patients with type 2 diabetes mellitus [29]. The inclusion and exclusion criteria for the clinical study were described as previously [29]. For this analysis, we excluded 11 participants because data of $T_{50}$ and zinc were not available. Finally, 132 patients were included in the present study. The second part was an *in vitro* study in which the effect of increasing zinc concentration on $T_{50}$ assay was examined.

**Determination of calcification propensity**
As previously reported [15], calcification propensity was evaluated by overloading of calcium and phosphate into sera ex vivo. Spontaneous transformation of primary to secondary CPPs can increase turbidity in each serum in the presence of supersaturated solution with time. The light scattering intensity accompanied by progressive turbidness was measured by time-resolved nephelometry. $T_{50}$ was determined one-half maximal transition time, that is, a half-time of transformation from primary to secondary CPPs. According to the original method, we prepared three stock solutions as follows: (1) NaCl solution: 140 mM NaCl, (2) Calcium solution: 40 mM CaCl$_2$+100 mM HEPES+140 mM NaCl pH-adjusted with 10 M NaOH to 7.40 at 37°C, and (3) Phosphate solute on: 19.44 mM Na$_2$HPO$_4$+4.56 mM NaH$_2$PO$_4$+100 mM HEPES+140 mM NaCl pH-adjusted with 10 M NaOH to 7.40 at 37°C. In the 96-well plates, 20 µl of NaCl stock solution and 80 µl of serum were mixed in each well and then shacked for 1 minute. Subsequently, 50 µl of phosphate stock solution and 50 µl of calcium stock solutions were added and shacked for 1 minute, automatically, in the pre-warmed thermo-constant room at 34.5°C. The final concentrations of calcium and phosphate in each sample were 10 mM and 6 mM, respectively. $T_{50}$ was determined in duplicate over 600 minutes per one measurement using a nephelometer (Nephelostar Plus$^R$, BMG Labtech, Ortenberg, Germany).

All serum samples were measured in a blinded manner. Serum samples from healthy volunteers and dialysis patients were also measured as quality control in serum calcification assay. The coefficients of variation (CV) of inter- and intra-assay were 4.4 % and 4.5 % in healthy control serum, 3.2 % and 4.5 % in hemodialysis control serum.

Where indicated, zinc chloride (ZnCl$_2$) (0, 10, and 20 µM) was added to the serum samples, respectively.

**Blood and urine sampling and Measurements**

Serum zinc levels were measured by a commercial laboratory (SRL Co., Ltd., Tokyo, Japan). Renal function was assessed by estimated glomerular filtration (eGFR) using a formula for the Japanese [30]. In this study serum calcium denotes calcium level adjusted for serum albumin according to Payne et al [31]. Urinary albumin to creatinine ratio was calculated as an index of albuminuria. Other measurements were obtained using routine laboratory methods at Osaka City University Hospital.

**Other clinical information**

We collected information on age, sex, height, weight, duration of diabetes, current medications, past history of cardiovascular disease (coronary artery disease, peripheral artery disease, aortic disease, and congestive heart failure requiring hospitalization), smoking habit, and laboratory data by asking the participants and/or by reviewing their medical records.

The diagnosis of type 2 diabetes mellitus was based on medical record and the criteria for diabetes mellitus as defined in the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [32].
Statistics

In clinical study, we summarized continuous variables as medians (interquartile ranges, IQRs) and categorical variables as numbers and percentages. Correlations were analyzed according to the nonparametric Spearman's rank correlation test. Independent associations between the variables and $T_{50}$ were assessed by multiple regression analysis. In in vitro experiments in which $\text{ZnCl}_2$ was added, $T_{50}$ was expressed mean (SD) of the triplicate determinations, and comparison was made by one-way analysis of variance followed by Tukey's test. These statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) or JMP software version 10 (SAS Institute, Inc., Cary, NC, USA). P-values < 0.05 by two-sided tests were considered statistically significant.

Results

Clinical characteristics of the type 2 diabetes patients

Table 1 summarizes the clinical characteristics of the 132 patients with type 2 diabetes mellitus. They all had type 2 diabetes mellitus, although the eligibility criteria did not exclude patients with type 1 diabetes. The age [median (interquartile range)] was 71 (65 to 75) years, and 59.1% of the participants were men. The known duration of diabetes mellitus was 13 (8 to 20) years. Their eGFR [59.0 (37.6 to 73.9) mL/min/1.73 m$^2$] and urinary albumin to creatinine ratio [17 (6 to 212) mg/gCr] showed wide distributions. $T_{50}$ and serum zinc levels were 306 (269 to 332) min, and 80.0 (70.1 to 89.8) µg/dL, respectively.
Table 1
Clinical characteristics of the study participants (n = 132)

| Measurement                                      | Median (IQR) or Percentage |
|--------------------------------------------------|-----------------------------|
| Age (years)                                      | 71 (65–75)                  |
| Male/female, N(%)                                | 78 (59.1) / 54 (40.9)      |
| Body mass index (kg/m²)                          | 24.5 (21.8–27.0)           |
| T₅₀ (min)                                        | 306 (269–332)               |
| eGFR (mL/min/1.73 m²)                            | 59.0 (37.6–73.9)            |
| Creatinine (mg/dL)                              | 0.87 (0.69–1.36)            |
| Blood urea nitrogen (mg/dL)                      | 17 (15–23)                  |
| Serum albumin (g/dL)                             | 4.1 (3.8–4.3)               |
| Fasting plasma glucose (mg/dL)                   | 119 (99–143)                |
| HbA1c (%)                                        | 8.0 (7.1–9.2)               |
| Corrected calcium (mg/dL)                        | 9.4 (9.2–9.7)               |
| Phosphate (mg/dL)                                | 3.8 (3.4–4.2)               |
| Magnesium (mg/dL)                                | 2.1 (2.0-2.3)               |
| Zinc (µg/dL)                                     | 80.0 (70.1–89.8)            |
| Whole PTH (pg/mL)                                | 21.6 (16.4–31.2)            |
| Intact PTH (pg/mL)                               | 36 (27–54)                  |
| Urine albumin to creatinine ratio (mg/gCr)       | 17 (6–212)                  |
| Systolic blood pressure (mmHg)                   | 125 (110 – 38)              |
| Diastolic blood pressure (mmHg)                  | 65 (60–74)                  |
| Current smoker (%)                               | 65 (49.2)                   |
| Use of mediations                                |                             |
| Antihypertensive (%)                             | 79 (59.8)                   |
| Statin (%)                                       | 64 (48.4)                   |
| Insulin (%)                                      | 63 (47.7)                   |

The table gives number and percentage for categorical variables and median (IQR) for continuous variables. Abbreviations are: IQR, interquartile range; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; PTH, para-thyroid hormone.
| Measurement                                          | Median (IQR) or Percentage |
|------------------------------------------------------|-----------------------------|
| Anti-diabetic agent (%)                              | 98 (74.2)                   |
| Complications                                        |                             |
| Retinopathy (%)                                      | 37 (28.2)                   |
| Neuropathy (%)                                       | 66 (50.0)                   |
| Any prior cardiovascular disease (%)                 | 21 (15.9)                   |

The table gives number and percentage for categorical variables and median (IQR) for continuous variables. Abbreviations are: IQR, interquartile range; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; PTH, para-thyroid hormone.

**Correlations between serum calcification propensity and clinical factors**

Table 2 shows the unadjusted correlations between $T_{50}$ levels and various clinical parameters in type 2 diabetes patients. While $T_{50}$ was positively correlated with zinc ($r_s = 0.219, p = 0.012, \text{Fig. 1}$), eGFR ($r_s = 0.199, p = 0.022$), and fasting plasma glucose ($r_s = 0.282, p = 0.001$), it was not significantly correlated with serum magnesium or hemoglobin A1c. $T_{50}$ level was negatively correlated with urinary albumin-creatinine ratio ($r_s = -0.247, p = 0.004$), blood urea nitrogen ($r_s = -0.213, p = 0.011$), and serum phosphate ($r_s = -0.227, p = 0.009$).
### Table 2
Correlation of $T_{50}$ with clinical factors in diabetic patients

| Clinical Variables                  | Correlation with $T_{50}$ | $r_s$      | $p$     |
|-------------------------------------|---------------------------|-----------|---------|
| Age                                 |                           | -0.222    | 0.010   |
| Body mass index                     |                           | 0.013     | 0.886   |
| eGFR                                |                           | 0.199     | 0.022   |
| Creatinine                          |                           | -0.170    | 0.051   |
| **Blood urea nitrogen**             |                           | -0.213    | 0.011   |
| Serum albumin                       |                           | 0.313     | 0.0003  |
| Fasting plasma glucose              |                           | 0.282     | 0.001   |
| HbA1                                |                           | 0.166     | 0.057   |
| Corrected calcium                   |                           | 0.132     | 0.132   |
| **Phosphate**                       |                           | -0.227    | 0.009   |
| Magnesium                           |                           | 0.113     | 0.195   |
| **Zinc**                            |                           | 0.219     | 0.012   |
| Whole-PTH                           |                           | -0.117    | 0.183   |
| Intact-PTH                          |                           | -0.110    | 0.183   |
| **Urine albumin to creatinine ratio**|                         | -0.247    | 0.004   |

Data include the Spearman’s correlation coefficient ($r_s$-value) and the levels of significance ($p$-value) (bolded if $p < 0.05$).

Abbreviations are: eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; PTH, parathyroid hormone.

**Independent association between serum calcification propensity and zinc**

We examined whether serum zinc level was a factor associated with serum $T_{50}$, independent of traditional mineral makers including phosphate, calcium and magnesium, using multiple regression analysis (Table 3). Model 1 included age, sex, any prior cardiovascular disease, current smoking, urinary albumin creatinine ratio, eGFR, corrected calcium, phosphate, magnesium, zinc, and hemoglobin A1c as explanatory variables, with hemoglobin A1c being replaced by plasma glucose in Model 2. Serum Zinc level was found to be associated significantly and positively with $T_{50}$ in both Model 1 ($\beta = 0.213$, $p = 0.038$) and Model 2 ($\beta = 0.229$, $p = 0.024$).
In the present study, we examined the association between serum zinc levels and serum calcification propensity in patients with type 2 diabetes mellitus. Serum zinc level was significantly and positively correlated with $T_{50}$ in the present study. The positive correlation between serum zinc level and $T_{50}$ was

Table 3. Factors associated with serum calcification propensity ($T_{50}$) in 132 type 2 diabetes patients

Data are the standard regression coefficients ($b$ - value) and levels of significance (p-value) (bolded if p < 0.05).

**Abbreviations** are: HbA1c, hemoglobin A1c; $R^2$, multiple coefficient of determination.

### Influence of zinc on serum calcification propensity

To examine whether zinc directly increases $T_{50}$, zinc was added in the serum calcification propensity assay. Addition of exogenous ZnCl$_2$ significantly modified the $T_{50}$ level in pooled serum from healthy subjects (0 µM, 347 ± 0.8 min; 10 µM, 357 ± 5.6; and 20 µM, 379.5 ± 4.2 min; p < 0.001, Fig. 2A), and pooled serum from dialysis patients (0 µM, 156 ± 2.3 min; 10 µM, 163 ± 0.5 min; and 20 µM, 170 ± 0.8 min, p < 0.001, Fig. 2B), respectively.

### Discussion

In the present study, we examined the association between serum zinc levels and serum calcification propensity in patients with type 2 diabetes mellitus. Serum zinc level was significantly and positively correlated with $T_{50}$ in the present study. The positive correlation between serum zinc level and $T_{50}$ was
also shown in the previous study including healthy subjects and patients with chronic kidney disease [25], indicating this correlation is common in various populations. However, so far, whether serum zinc level could be the independent factor associated with serum T<sub>50</sub> were not examined. We showed that serum zinc level was positively associated with T<sub>50</sub> independent of calcium, phosphate, and magnesium. These novel findings suggest that zinc has an important role in suppressing calcification propensity in serum.

We also confirmed that addition of zinc increases T<sub>50</sub>, in vitro assay. The mechanisms underlying zinc inhibits serum calcification propensity were still unclear. Even in the polyethylene glycol hydrogels, not in serum, zinc inhibits transformation from amorphous calcium phosphate (ACP) into hydroxyapatite [33]. In additive -free composite, ACP transformed into brushite within minutes. In contrast, in the presence of zinc, zinc-doped ACP was very stable and did not show any signs of crystallization for up to 20 days. In ACP, zinc iron readily substitutes calcium [34], suppressing crystallization by decreasing solubility [35]. It is thus likely that zinc suppresses the transformation from amorphous primary CPPs into secondary CPPs, containing crystalline hydroxyapatite, in serum.

In a recent study by Voelkl et al, addition of exogenous ZnCl<sub>2</sub> (15 µM) did not improve T<sub>50</sub> in sera from healthy controls and patients on hemodialysis, although serum zinc level was significantly correlated with T<sub>50</sub> in those subjects [25]. The discrepancy between the studies by Voelkl et al by us may be explained by difference in ZnCl<sub>2</sub> concentration. In the present study, we demonstrated that 10 µM ZnCl<sub>2</sub> (= 60.5 µg/dL) did not significantly modify T<sub>50</sub> in serum from hemodialysis patients, which was consistent with the study by Voelkl et al [25]. In contrast, ZnCl<sub>2</sub> concentration of 20 µM (= 131 µg/dL), the upper limit of reference range, could significantly increase T<sub>50</sub> in those subjects. The crystallization inhibition has been reported to be dependent on the zinc concentration in polyethylene glycol hydrogels [33]. Thus, a certain zinc concentration may be required to increase serum calcification propensity.

In the present study, magnesium was also significantly and positively associated with T<sub>50</sub>. Magnesium is one of the known anti-calcifying factors, which improves T<sub>50</sub> in ex vivo [15]. In vitro, magnesium has been reported to prevent phosphate-induced calcification in human aortic VSMC [36]. Similarly, zinc could increase zinc finger protein TNF-α-induced protein 3 (TNFAIP3) expression, which subsequently inhibits NF-kB activation and osteo-/chondrogenic reprogramming, resulting suppression of phosphate-induced VSMC calcification [25]. The findings of zinc on T<sub>50</sub> in the present study, and the above in vitro effects of zinc on phosphate-induced calcification in VSMC were similar to those of magnesium. In addition, recently, a randomized control trial has shown that magnesium supplementation increased T<sub>50</sub> in patients with chronic kidney disease stage 3–4 [37]. Thus, supplementation of zinc, as well as magnesium, might be a potential therapeutic option to attenuate serum calcification propensity and the progression of vasculature calcification. Clearly, however, randomized clinical trials are needed, before such a treatment is recommended.

Albumin is also the anti-calcifying factors associated with T<sub>50</sub> in ex vivo [15]. When zinc and albumin are included simultaneously in multiple regression analysis, the significant associations of both factors with
T\textsubscript{50} turned to be non-significant (data not shown). Serum zinc acts as an extracellular zinc buffer that controls zinc concentration in blood, since approximately 75–80\% of zinc is bound to albumin, accounting for as much as 98\% of the exchangeable fraction of zinc in blood [38, 39]. Serum albumin was significantly and positively correlated with serum zinc levels in the study, thus the confounding effect might explain the results. Measurement of free-zinc ions will be needed to address this issue.

The present study has several limitations. First, the number of subjects examined was relatively small. Second, we cannot be sure whether the findings of this study are applicable to non-diabetic patients. And third, due to the cross-sectional design, we can demonstrate only association, not causality. To confirm the potential benefits of zinc supplementation, further interventional studies are required.

**Conclusions**

In summary, this is the first report to investigate the association between serum calcification propensity and zinc levels in patients with type 2 diabetes mellitus. Serum zinc was found as an independent factor associated positively with T\textsubscript{50}, and zinc has an *in vitro* effect on overall propensity of calcification in serum.

**Abbreviations**

- **CaCl\textsubscript{2}**: Calcium chloride
- **Ca × P**: calcium-phosphorus product.
- **CPPs**: calciprotein particles.
- **eGFR**: estimated glomerular filtration.
- **HEPES**: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
- **NaCl**: Sodium chloride
- **T\textsubscript{50}**: serum calcification propensity.
- **TNFAIP3**: TNF-\(\alpha\)-induced protein 3.
- **VSMCs**: vascular smooth muscle cells.
- **ZnCl\textsubscript{2}**: zinc chloride.

**Declarations**

*Ethics approval and consent to participate*
This study was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (approval No. 4100). Opt-out option for informed consent was performed as explained in instructions posted on the website of the institution.

**Consent for publication**

Not applicable.

**Competing Interests**

The authors declare that they have no conflicts of interest regarding this study.

**Acknowledgements**

The authors thank the participants of this study for providing their medical information. The authors acknowledge the technical assistance of Dr. Masayo Sasagawa from the research laboratory at the Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine.

**Funding**

This study was partly supported by research grants for T.S. from Astellas Pharma (RS01442), Chugai Pharmaceutical Company (AC-1-20150630164707-161208), and Takeda Pharmaceutical Company (15S01790).

**Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Author information**

**Affiliations**

Department of Nephrology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka Japan.

Shinya Nakatani, Mika Sonoda, Kozo Nishide, Hideki Uedono, Akihiro Tsuda, Masanori Emoto

Department of Nephrology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka, Japan.

Katsuhito Mori
Division of Internal Medicine, Dialysis Centre, Inoue Hospital,
16-17, Enoki-machi, Suita, Japan

Mika Sonoda

Department of Vascular Medicine, Osaka City University Graduate School of Medicine,
1-4-3 Asahi-machi, Abeno-ku, Osaka Japan.

Testuo Shoji,

Vascular Science Center for Translational Research, Osaka City University Graduate School of Medicine,
1-4-3 Asahi-machi, Abeno-ku, Osaka Japan.

Testuo Shoji,

**Author's Contributions**

S.N., K.M., and TS. contributed to the concept, design, analysis, interpretation, and writing. M.S., and K.N. contributed to data acquisition and interpretation. H.U., A.T., and M.E. contributed to the concept and interpretation. All authors have read and approved the submitted manuscript.

**Corresponding author**

Correspondence to Katsuhito Mori.

**Additional information**

**Publisher's note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Rights and permissions**

**Open Access:** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit [http://creativecommons.org/licenses/by/4.0/](http://creativecommons.org/licenses/by/4.0/). The Creative Commons Public Domain Dedication
waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

References

1. Raghavan S, Vassy JL, Ho YL, Song RJ, Gagnon DR, Cho K, Wilson PWF, Phillips LS: Diabetes Mellitus-Related All-Cause and Cardiovascular Mortality in a National Cohort of Adults. J Am Heart Assoc. 2019; 8:e011295.

2. Niskanen L, Siitonen O, Suhonen M, Uusitupa MI: Medial artery calcification predicts cardiovascular mortality in patients with NIDDM. Diabetes Care. 1994; 17:1252-1256.

3. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004; 351:1296-1305.

4. Gorriz JL, Molina P, Cerveron MJ, Vila R, Bover J, Nieto J, Barril G, Martinez-Castelao A, Fernandez E, Escudero V et al: Vascular calcification in patients with nondialysis CKD over 3 years. Clin J Am Soc Nephrol. 2015; 10:654-666.

5. London GM: Mechanisms of arterial calcifications and consequences for cardiovascular function. Kidney Int Suppl. (2011) 2013; 3:442-445.

6. Lanzer P, Boehm M, Sorribas V, Thiriet M, Janzen J, Zeller T, St Hilaire C, Shanahan C: Medial vascular calcification revisited: review and perspectives. Eur Heart J. 2014; 35:1515-1525.

7. Avogaro A, Fadini GP: Mechanisms of ectopic calcification: implications for diabetic vasculopathy. Cardiovasc Diagn Ther. 2015; 5:343-352.

8. Houben E, Neradova A, Schurgers LJ, Vervloet M: The influence of phosphate, calcium and magnesium on matrix Gla-protein and vascular calcification: a systematic review. G Ital Nefrol. 2016; 33 (6).

9. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, Jahnen-Dechent W, Weissberg PL, Shanahan CM: Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. J Am Soc Nephrol. 2004; 15:2857-2867.

10. Jablonski KL, Chonchol M: Vascular calcification in end-stage renal disease. Hemodial Int. 2013; 17 Suppl 1:S17-21.

11. Cozzolino M, Gallieni M, Brancaccio D: Vascular calcification in uremic conditions: new insights into pathogenesis. Semin Nephrol. 2006; 26:33-37.

12. Durham AL, Speer MY, Scatena M, Giachelli CM, Shanahan CM: Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. Cardiovasc Res. 2018; 114:590-600.

13. Heiss A, Jahnen-Dechent W, Endo H, Schwahn D: Structural dynamics of a colloidal protein-mineral complex bestowing on calcium phosphate a high solubility in biological fluids. Biointerphases. 2007;
14. Heiss A, DuChesne A, Denecke B, Grotzinger J, Yamamoto K, Renne T, Jahnne-Dechent W: Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. J Biol Chem. 2003; 278:13333-13341.

15. Pasch A, Farese S, Graber S, Wald J, Richtering W, Floege J, Jahnne-Dechent W: Nanoparticle-based test measures overall propensity for calcification in serum. J Am Soc Nephrol. 2012; 23:1744-1752.

16. Smith ER, Ford ML, Tomlinson LA, Bodenham E, McMahon LP, Farese S, Rajkumar C, Holt SG, Pasch A: Serum calcification propensity predicts all-cause mortality in predialysis CKD. J Am Soc Nephrol. 2014; 25:339-348.

17. Pasch A, Block GA, Bachtler M, Smith ER, Jahnne-Dechent W, Arampatzis S, Chertow GM, Parfrey P, Ma X, Floege J: Blood Calcification Propensity, Cardiovascular Events, and Survival in Patients Receiving Hemodialysis in the EVOLVE Trial. Clin J Am Soc Nephrol. 2017; 12:315-322.

18. Keyzer CA, de Borst MH, van den Berg E, Jahnne-Dechent W, Arampatzis S, Farese S, Bergmann IP, Floege J, Navis G, Bakker SJ et al: Calcification Propensity and Survival among Renal Transplant Recipients. J Am Soc Nephrol. 2016; 27:239-248.

19. Dahle DO, Asberg A, Hartmann A, Holdaas H, Bachtler M, Jenssen TG, Dionisi M, Pasch A: Serum Calcification Propensity Is a Strong and Independent Determinant of Cardiac and All-Cause Mortality in Kidney Transplant Recipients. Am J Transplant. 2016; 16:204-212.

20. Bielesz B, Reiter T, Marculescu R, Gleiss A, Bojic M, Kieweg H, Cejka D: Calcification Propensity of Serum is Independent of Excretory Renal Function. Sci Rep. 2017; 7:17941.

21. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM: Phosphate regulation of vascular smooth muscle cell calcification. Circ Res. 2000; 87:E10-17.

22. Block GA, Hulbert-Shearon TE, Levin NW, Port FK: Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: a national study. Am J Kidney Dis. 1998; 31:607-617.

23. Foley RN, Collins AJ, Ishani A, Kalra PA: Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am Heart J. 2008; 156:556-563.

24. Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME: Zinc and human health: an update. Arch Toxicol. 2012; 86:521-534.

25. Voelkl J, Tuffaha R, Luong TTD, Zickler D, Masyout J, Feger M, Verheyen N, Blaschke F, Kuro OM, Tomaschitz A et al: Zinc Inhibits Phosphate-Induced Vascular Calcification through TNFAIP3-Mediated Suppression of NF-kappaB. J Am Soc Nephrol. 2018; 29:1636-1648.

26. Cunningham JJ, Fu A, Mearkle PL, Brown RG: Hyperzincuria in individuals with insulin-dependent diabetes mellitus: concurrent zinc status and the effect of high-dose zinc supplementation. Metabolism. 1994; 43:1558-1562.

27. Kazi TG, Afridi HI, Kazi N, Jamali MK, Arain MB, Jalbani N, Kandhro GA: Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol
Trace Elem Res. 2008; 122:1-18.

28. Makhlough A, Makhlough M, Shokrzadeh M, Mohammadian M, Sedighi O, Faghihan M: Comparing the Levels of Trace Elements in Patients With Diabetic Nephropathy and Healthy Individuals. Nephrourol Mon. 2015; 7:e28576.

29. Sonoda M, Shoji T, Kuwamura Y, Okute Y, Naganuma T, Shima H, Motoyama K, Morioka T, Mori K, Fukumoto S et al: Plasma homocysteine and cerebral small vessel disease as possible mediators between kidney and cognitive functions in patients with diabetes mellitus. Sci Rep. 2017; 7:4382.

30. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A et al: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009; 53:982-992.

31. Payne RB, Little AJ, Williams RB, Milner JR: Interpretation of serum calcium in patients with abnormal serum proteins. Br Med J. 1973; 4:643-646.

32. Expert Committee on the D, Classification of Diabetes M: Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003; 26 Suppl 1:S5-20.

33. Schweikle M, Bjornoy SH, van Helvoort ATJ, Haugen HJ, Sikorski P, Tiainen H: Stabilisation of amorphous calcium phosphate in polyethylene glycol hydrogels. Acta Biomater. 2019; 90:132-145.

34. Gross, K.A , Komarovska, L, Viksna, A: Efficient zinc incorporation into hydroxyapatite through crystallization of an amorphous phase could extend the properties of zinc apatites. Journal of the Australian Ceramic Society. 2013; 49, 129-135.

35. Kanazaki, N, Onuma, K, Trboux, G, Tusumi, S, Ito, A: J Inhibitory Effect of Magnesium and Zinc on Crystallization Kinetics of Hydroxyapatite (0001) Face. Phys. Chem. B. 2000; 104: 4189-4194.

36. Louvet L, Buchel J, Steppan S, Passlick-Deetjen J, Massy ZA: Magnesium prevents phosphate-induced calcification in human aortic vascular smooth muscle cells. Nephrol Dial Transplant. 2013; 28:869-878.

37. Bressendorff I, Hansen D, Schou M, Silver B, Pasch A, Bouchelouche P, Pedersen L, Rasmussen LM, Brandi L: Oral Magnesium Supplementation in Chronic Kidney Disease Stages 3 and 4: Efficacy, Safety, and Effect on Serum Calcification Propensity-A Prospective Randomized Double-Blinded Placebo-Controlled Clinical Trial. Kidney Int Rep. 2017; 2:380-389.

38. Giroux EL, Henkin RI: Competition for zinc among serum albumin and amino acids. Biochim Biophys Acta. 1972; 273:64-72.

39. Handing KB, Shabalin IG, Kassaar O, Khazaipoul S, Blindauer CA, Stewart AJ, Chruszcz M, Minor W: Circulatory zinc transport is controlled by distinct interdomain sites on mammalian albumins. Chem Sci. 2016; 7:6635-6648.

Figures
Figure 1

Correlation between serum zinc and serum calcification propensity (T50). Serum zinc correlated positively with T50. Abbreviations: rs, Spearman's correlation coefficient; P, level of significance.

$r_s = 0.219$
$P = 0.012$
$N = 132$
Figure 1

Correlation between serum zinc and serum calcification propensity (T50). Serum zinc correlated positively with T50. Abbreviations: rs, Spearman's correlation coefficient; P, level of significance.
Figure 2

Influence of zinc on serum calcification propensity (T50). Addition of exogenous 20 µM zinc chloride (ZnCl2) significantly increased T50 compared to those of control (0 µM of ZnCl2 addition) in pooled serum from healthy subjects (A), and pooled serum from dialysis patients (B). *p<0.05; statistically significant versus 0 µM ZnCl2 addition. Abbreviations: ZnCl2; zinc chloride
Influence of zinc on serum calcification propensity (T50). Addition of exogenous 20 µM zinc chloride (ZnCl2) significantly increased T50 compared to those of control (0 µM of ZnCl2 addition) in pooled serum from healthy subjects (A), and pooled serum from dialysis patients (B). *p<0.05; statistically significant versus 0 µM ZnCl2 addition. Abbreviations: ZnCl2; zinc chloride