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# Deoxycholic Acid, a Metabolite of Circulating Bile Acids, and Coronary Artery Vascular Calcification in CKD

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## Abstract

**Background—**Vascular calcification is common among patients with chronic kidney disease (CKD), and it is associated with all-cause and cardiovascular disease mortality. Deoxycholic acid (DCA), a metabolite of circulating bile acids, is elevated in CKD and induces vascular mineralization and osteogenic differentiation in animal models.

**Study Design—**Cohort analysis of clinical trial participants

**Setting & Participants—**One hundred twelve patients with moderate to severe CKD (eGFR, 20–45 mL/min/1.73 $m^2$) who participated in a randomized controlled study to examine the effects of phosphate binders on vascular calcification.

**Predictor—**Serum deoxycholic acid

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Supplementary Material

Table S1: Association of baseline deoxycholic acid >58 ng/mL with baseline BMD.

Table S2: Association of baseline log-transformed deoxycholic acid with baseline BMD.

*Note:* The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

**Outcomes—**Baseline coronary artery calcification (CAC) volume score and bone mineral density (BMD), and change in CAC volume score and BMD after nine months.

**Measurements—**Deoxycholic acid was assayed in stored baseline serum samples using liquid chromatography–tandem mass spectrometry, CAC was measured using a GE-Imitron C150 scanner, and BMD was determined using abdominal computed tomography scans with calibrated phantom of known density.

**Results—**Higher serum DCA levels were significantly correlated with greater baseline CAC volume and lower baseline BMD. After adjusting for demographics, co-existing illness, body mass index, eGFR and circulating markers of mineral metabolism including serum calcium, phosphorus, vitamin D, parathyroid hormone, and fibroblast growth factor 23, a serum DCA level >58 ng/mL (the median) was positively associated with baseline CAC volume ($\beta = 0.71$; 95% CI, 0.26–1.16; $p = 0.003$) and negatively associated with baseline BMD ($\beta = -20.3$; 95% CI, −1.5 to −39.1; $p = 0.04$). Serum DCA level >58 ng/mL was not significantly associated with change in CAC volume score after nine months ($\beta = 0.06$; 95% CI, −0.09 to 0.21; $p = 0.4$). The analysis for the relationship between baseline DCA levels and change in BMD after nine months was not statistically significant, but was under-powered.

**Limitations—**The use of non-fasting serum samples is a limitation as DCA levels may vary based on the time of day and dietary intake. Few trial participants with complete data to evaluate the change in CAC volume score (n = 75) and BMD (n=59). No data on changes in DCA levels over time.

**Conclusions—**Among patients with moderate to severe CKD, higher serum levels of DCA were independently associated with greater baseline CAC volume score and with lower baseline BMD.

### Keywords

vascular calcification; deoxycholic acid (DCA); biomarker; chronic kidney disease (CKD); bone mineral density (BMD); coronary artery calcification (CAC); circulating bile acid; mineral metabolism; cardiovascular disease

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Many people live with chronic kidney disease (CKD); in the United States, the overall prevalence of CKD is about 14%.[1] CKD carries significant morbidity and mortality.[2] Among Medicare patients, the adjusted mortality rate among those with CKD is 118/1000 person-years compared to 48/1000 person-years among those without CKD.[3] Many patients with CKD are more likely to have a cardiovascular disease event or die than to progress to end-stage renal disease (ESRD).[2] Indeed, for those with CKD, cardiovascular disease represents an outsized factor associated with poor outcomes. Not only is there a greater prevalence of traditional cardiovascular risk factors compared to those with normal kidney function,[4] but CKD also confers non-traditional cardiovascular risk factors such as abnormal mineral metabolism, anemia, malnutrition, increased oxidative stress, inflammation, and volume overload.[5] A well-established cardiovascular risk factor in CKD is systemic and coronary artery calcifications (CACs), which are highly prevalent among both non–dialysis-dependent[6] and dialysis-dependent[7] CKD patients. Among those with non–dialysis-dependent CKD, CAC has been shown to increase the risk of all-cause mortality, cardiovascular disease events and mortality, and hospital admission.[8–10] Likewise, among

patients with dialysis-dependent CKD, CAC is associated with vascular dysfunction,[11] a risk factor for cardiovascular disease, and is an independent and incremental predictor of all-cause mortality.[12]

Vascular calcification occurs when hydroxyapatite crystals deposit in the intimal or medial layer of arteries. It is an actively regulated process[13] involving various signaling pathways. Many clinical factors are associated with vascular calcification: advancing age, diabetes, kidney function decline, inflammatory states, and rare genetic conditions. In CKD, inflammatory cytokines and abnormal mineral metabolism, especially hyperphosphatemia, induce vascular calcification,[14] however, other mechanisms are also implicated.[15]

Circulating bile acid levels are elevated in CKD.[16,17] Furthermore, the composition of bile acids is perturbed, characterized by a decrease in the proportion of the primary bile acid, cholic acid, and an increase in the proportion of the secondary bile acid, deoxycholic acid (DCA).[16] DCA is directly toxic when applied to vascular smooth muscle cells.[18] Bile acids and their nuclear receptor, farnesoid X receptor (FXR), regulate key processes such as lipid and glucose metabolism,[19] and FXR is found in numerous tissues including liver, kidney, intestine, macrophages, and vasculature.[19] Activation of FXR attenuates vascular calcification in a CKD model,[20] reduces atherosclerotic plaque formation in animal models,[21,22] and reduces levels of circulating DCA.[22] Thus, a plausible hypothesis is that elevated levels of circulating DCA in CKD promote vascular calcification through reduced FXR activation and direct vasculature toxicity.

Low bone mineral density (BMD) and fracture are strongly related to vascular calcification and poor cardiovascular outcomes. Among postmenopausal women and older men, vascular calcification is linked with low BMD[23,24] and increased fracture rate.[25–27] Likewise osteoporosis severity is proportionally associated with cardiovascular disease event risk.[28] Similar associations among vascular calcification, low BMD, and osteoporosis have been observed in ESRD[29–33] and CKD[34,35] cohorts. Precise mechanisms that underlie these observations are still unclear, however, vascular calcification and bone remodeling share many of the same signaling pathways and genes, and may be mediated by age, inflammation and, in the case of CKD, by mineral metabolism abnormalities.[36]

Understanding the mechanisms associated with the development of vascular calcification and decreased BMD will identify potential treatment targets and strategies. Since activation of FXR is associated with reduced DCA levels and attenuated vascular calcification, we hypothesized that elevated circulating DCA levels among CKD patients would be associated with more severe vascular calcification and decreased BMD. This is a *post hoc* analysis of the randomized controlled trial Effects of Phosphate Binders in Moderate CKD.[37] Herein, we report results from a cross-sectional and longitudinal analysis of patients with CKD stages 3b-4 (estimated glomerular filtration rate [eGFR], 20–45 ml/min/1.73 $m^2$) examining the association of baseline DCA levels with baseline CAC volume scores, a measure of vascular calcification, and baseline BMD, as well as the association of baseline DCA levels with change in CAC scores and BMD after nine months.

# METHODS

## Participants

The details of the study that assessed the effects of phosphate binding on serum markers of mineral metabolism, vascular calcification, and lumbar BMD were described previously.[37] Briefly, 148 participants with CKD stages 3b-4 (eGFR 20–45 ml/min/1.73 $m^2$ calculated with the MDRD [Modification of Diet in Renal Disease] Study equation) and phosphorus 3.5–6.0 mg/dL were randomized to calcium acetate, lanthanum carbonate, sevelamer, or matching placebo and followed up for nine months. The primary end point was change in serum phosphorus, and secondary end points included changes in serum parathyroid hormone (PTH), fibroblast growth factor 23 (FGF-23), active vitamin D (1,25-dihydroxyvitamin D [1,25$[OH]_2$D]), urine phosphorus, and fractional excretion of phosphorus as well as change in vascular calcification scores (coronary arteries, thoracic, and abdominal aorta), and change in lumbar BMD. The study was approved by the Schulman Institutional Review Board (Cincinnati, OH) and registered at ClinicalTrials.gov (study number: NCT00785629). The Colorado Multiple Institution Review Board protocol number is 09-0870. All study participants provided written documentation of informed consent. Procedures were in accordance with the ethics standards of the institutional review board and the Declaration of Helsinki.

A subset of participants with available serum and complete baseline information for CAC and lumbar BMD were included in the present cross-sectional analysis, resulting in a final cohort of 112 participants. Additionally, there were available data in 75 of the 112 participants for a longitudinal analysis examining the relationship between baseline DCA levels and change in CAC volume score after nine months; only 59 participants had complete data to examine the relationship between baseline DCA levels and change in BMD after nine months.

## Variables

Non-fasting, baseline stored serum samples were assayed for the predictor variable, circulating DCA levels, using liquid chromatography–tandem mass spectrometry (LC-MS/MS) as previously described.[22] In brief, human serum (100 μL) was diluted in 300 μL of cold acetonitrile containing 3 ng of D6-DCA (Cambridge Isotope Laboratory) as internal standard. The mixture was passed through a Phree phospholipid removal plate (Phenomenex). The eluate was evaporated with nitrogen gas stream, then redissolved in 100 μL of 10mM ammonium acetate buffer (pH 8.0)/methanol (1:1, v/v). A 10 μL aliquot of each sample solution was then injected into LC–electrospray ionization –MS/MS system (QTRAP 3200, SCIEX) for analysis. The outcome variables were baseline total CAC volume score and lumbar BMD. The CAC volume score was obtained using a GE-Imitron C150 scanner and a standard protocol as previously described.[38] Atherosclerotic calcium was defined as a plaque area ≥1 $mm^2$ with a density of ≥130 Hounsfield units. Total calcium volume score was derived by the sum of all lesion volumes in cubic millimeters.[39] A single experienced investigator (?.?.) performed all image assessments. Lumbar BMD was determined using abdominal computed tomography scans with a calibrated phantom of

known density (Image Analysis QCT 3D PLUS, Columbia, KY). Measurements of BMD were performed in a 5-mm-thick slice of trabecular bone from each vertebra (L2 to L4).

Covariates were measured at the time of enrollment. All other clinical chemistry analyses were performed by Quest Diagnostics (Denver, CO). $1,25(OH)_2D$ assays were performed using a commercially available radioimmunoassay (Diasorin Inc., Stillwater, MN). Intact FGF-23 was measured using a sandwich immunoassay (Kainos, Japan).

### Statistical Analysis

Approximately normally distributed continuous variables are described with mean ± standard deviation while variables with skewed distributions are described with median and interquartile range. Categorical variables are described with frequency and percentage. The median DCA level was 58 ng/mL. Subjects with DCA levels greater than the median of 58 ng/mL were compared with participants with DCA levels less than or equal to the median of 58 ng/mL. To evaluate differences in baseline characteristics between these two groups, Fisher's exact test was used for categorical variables and the Wilcoxon rank-sum test was used for continuous variables. Spearman correlations were used to investigate the association of circulating DCA levels with CAC volume score, lumbar BMD, eGFR, and markers of mineral metabolism including serum levels of calcium, phosphorus, PTH, $1,25(OH)_2D$, and FGF-23.

Multivariable linear regression models were used to examine the cross-sectional association of DCA levels with CAC volume score and lumbar BMD at baseline. Longitudinal analyses using multivariable linear regression models were used to examine the relationship between baseline DCA levels and the change in CAC volume score and BMD after nine months. DCA levels were modeled as a categorical variable > or ≤ 58 ng/mL (the median) and were log-transformed and used as a continuous variable. Given the skewed distribution of CAC volume score, its values were logarithmically transformed for the purposes of this analysis. We used a combination of criteria based on subject-matter considerations and biological understanding to designate five sets of covariates for inclusion in the regression models: unadjusted; model 1, adjusted for age; model 2, adjusted for model 1 + sex, race, body mass index (BMI), diabetes, hypertension, coronary artery disease; model 3, adjusted for model 2 + eGFR; model 4, adjusted for model 3 + serum calcium and phosphorus; and model 5, adjusted for model 4 + serum PTH and FGF-23. In the longitudinal analysis of DCA levels and change in CAC volume score and BMD at nine months, treatment assignment (active, which included treatment with either calcium acetate, lanthanum carbonate, or sevelamer versus placebo) was added to model 5 in order to account for the effect of treatment group. A p-value of $<0.05$ was considered to be significant. All statistical analyses were performed with SAS software, version 9.4 (SAS Institute Inc, Cary, NC)

## RESULTS

### Study Participants

Demographics and baseline characteristics in the total cohort and by DCA level > or ≤ the median of 58 ng/mL are shown in Table 1. A total of 112 subjects were analyzed. Forty-

eight percent of the total cohort was male, 78% was white, and the mean age was 66 ± 12 (standard deviation) years. The mean eGFR was 31.5 ± 8.7 ml/min/1.73 $m^2$ and mean BMI was 31.5 ± 7.3 $kg/m^2$. There was a high prevalence of comorbidity among the cohort: 56% had diabetes, 98% had hypertension, 22% had coronary artery disease, and 89% had hyperlipidemia. The median CAC volume score was 246 (interquartile range [IQR], 43.0–743.0), mean BMD was 113.1 ± 40.4 $g/cm^2$, and median DCA level was 58.4 (IQR, 29.2–111.8) ng/mL. Among those with DCA level > 58 ng/mL, the median DCA level was 111.8 (IQR, 80.3–168.3) ng/mL and among those with DCA ≤ 58 ng/mL it was 28.7 (IQR, 16.5–41.7) ng/mL. There were few statistically significant differences between the two DCA groups. However, the group with DCA > 58 ng/mL was significantly older than the group with DCA ≤ 58 ng/mL (68 ± 10 and 63 ± 13 years, respectively; p = 0.04) and had significantly lower phosphate levels (4.1 ± 0.4 and 4.3 ± 0.4 mg/dL, respectively; p = 0.04). Although not statistically significant, there was nominally greater prevalence of diabetes, coronary artery disease, hyperlipidemia, and higher BMI among those with DCA level > 58 ng/mL. The median CAC volume score was significantly higher among those with DCA > 58 ng/mL compared to those with DCA ≤ 58 ng/mL (474.0 [IQR, 126.5–1017.5] and 143.0 [IQR, 0.0–525.0], respectively) while the mean BMD was significantly lower among those with DCA > 58 ng/mL compared to those with DCA ≤ 58 ng/mL (99.6 ± 33.7 and 125.5 ± 42.4 $g/cm^2$, respectively).

In the total cohort, DCA positively correlated with CAC volume score (r = 0.29; p = 0.009) and negatively correlated with BMD (r = −0.32; p = 0.004). DCA did not significantly correlate with eGFR (r = −0.08; p = 0.5) or any of the markers of mineral metabolism including serum levels of calcium (r = 0.09; p = 0.4), phosphorus (r = 0.06; p = 0.6), PTH (r = −0.12; p = 0.2), $1,25(OH)_2D$ (r = 0.05; p = 0.7), or FGF-23 (r = −0.09; p = 0.3).

## Cross-sectional Analyses

In cross-sectional unadjusted analyses, baseline DCA level > 58 ng/mL positively and significantly associated with baseline log-transformed CAC volume score (β = 0.74; 95% confidence interval [CI], 0.29–1.19; p = 0.002). In the final model that adjusted for age, sex, race, BMI, comorbidities, and serum calcium, phosphorus, PTH, and FGF-23, the association did not change (β = 0.71; 95% CI, 0.26–1.16; p = 0.003; Table 2). When log-transformed and modeled as a continuous variable, baseline DCA was also significantly associated with baseline log-transformed CAC volume score (β = 0.68; 95% CI, 0.29–1.07; p =0.007) in the fully adjusted model.

Meanwhile, baseline DCA level > 58 ng/mL was negatively associated with baseline BMD in cross-sectional analyses. In the unadjusted model the β was −26.0 (95% CI, −43.1 to −8.9; p = 0.004). The estimate was only somewhat attenuated in the final model: β = −20.3 (95% CI, −39.1 to −1.5; p = 0.04; Table 3). Likewise, when baseline DCA was log-transformed and modeled as a continuous variable, there was a significant association with baseline BMD in the unadjusted model (β = −28.0; 95% CI, −45.2 to −10.8; p = 0.002). In the final model, after adjustment for all covariates, the estimate was only slightly attenuated: β = −25.9 (95% CI, −45.7 to −6.1; p = 0.01). There was no association between DCA level > 58 ng/mL and baseline eGFR.

### Longitudinal Analyses

Seventy-five subjects had complete data to perform longitudinal analyses examining the association of baseline DCA level >58 ng/mL and change in log-transformed CAC volume score. After nine months of follow-up, the median (unlogged) change in CAC volume score was 12.0 (IQR, −3.0 to −84) and median percentage change was 10.4% (IQR, −4.9%-33.0%). In longitudinal unadjusted analyses, baseline DCA level >58 ng/mL was not significantly associated with change in log-transformed CAC volume score after nine months (β = 0.04; 95% CI, −0.07 to 0.15; p = 0.8). In the fully adjusted model, which included age; sex; race; BMI; comorbidities; serum calcium, phosphorus, PTH, and FGF-23; and treatment group, the association did not change and remained non-significant (β = 0.06; 95% CI, −0.09 to 0.21; p = 04; Table 4). Similarly, when baseline DCA was log-transformed and modeled as a continuous variable, there was no significant association with change in log-transformed CAC volume score after nine months (β = 0.04; 95% CI, −0.12 to 0.20; p = 0.6) in the fully adjusted model.

Only 59 subjects had complete data available to perform longitudinal analyses examining the association of baseline DCA level (either as a categorical variable at a cutpoint of 58 ng/mL, or as a log-transformed continuous variable) with change in BMD after nine months. Furthermore, the change in BMD after nine months of follow-up was small, with a median absolute change of 0.5 (IQR, −4.6 to 8.9) and a percent change of 0.4% (IQR, −4.8% to 8.0%). There was no statistically significant association between baseline DCA levels and change in BMD after nine months (Tables S1 and S2, provided as online supplementary material). However, given the small number of subjects with available data, these analyses were limited by lack of power.

## DISCUSSION

In this analysis of patients with CKD stages 3b-4, we found that DCA levels > the median of 58 ng/mL and higher log-transformed DCA levels, modeled as a continuous variable, were independently associated with greater log-transformed CAC volume score at baseline. Conversely, DCA, when modeled as a categorical variable (> the median level of 58 ng/mL) and as a log-transformed continuous variable, was associated with lower baseline lumbar BMD. Notably, in the cross-sectional analyses, the relationship between higher DCA levels (>58 ng/mL and log-transformed levels) and log-transformed CAC volume score remained strong even when the model was adjusted for markers of mineral metabolism including calcium, phosphorus, PTH, and FGF-23. When taken together with data from experimental studies, our findings suggest that elevated DCA is a novel mechanism for the development of and potential biomarker for vascular calcification in CKD.

In the main trial, assignment to active treatment (either calcium acetate, lanthanum carbonate, or sevelamer carbonate) was associated with an increase in CAC volume score at nine months.[37] In the longitudinal analyses controlling for treatment assignment (active versus placebo) reported here, there was no statistically significant relationship between baseline DCA levels and change in log-transformed CAC volume score nor change in BMD after nine months. It is important to note that the change in CAC volume score and BMD was small. In fact, the median change in annualized total CAC volume score was <30% in

the main trial and 10.4% in our analyses. Furthermore, there were only 75 subjects available with nine-month follow-up CAC volume scores (59 with nine-month BMD) and complete data, so our analysis is likely underpowered. We did not perform linear regression analyses based on specific treatment group (calcium acetate versus lanthanum carbonate versus sevelamer versus placebo) due to small numbers in each group, leading to power and precision limitations. To better assess whether DCA levels predict or are related to CAC volume score progression, a prospective observational study is needed, ideally without randomization to treatment that is known to affect the progression of CAC volume score.

Emerging data implicate endoplasmic reticulum (ER) stress as a novel mechanism for vascular calcification. ER stress occurs when unfolded proteins in the ER lumen activate signal transduction pathways that influence disease processes, including vascular calcification.[40,41] DCA is a novel mediator of ER stress in vascular smooth muscle cells. When vascular smooth muscles cells were treated with various bile acids, DCA, but not other bile acids, induced mineralization and osteogenic differentiation via ER stress.[18] These data suggest a mechanistic role of DCA in the pathogenesis of vascular calcification and support the results we report here.

In animal models, abnormalities in ER stress signaling components were associated with decreased BMD.[42–44] Furthermore, among humans with osteoporosis, ER molecular chaperones in osteoblasts were down-regulated and there was a decreased ER stress response compared to in apparently healthy controls.[45] Taken together, these data suggest that the ER stress response may be related to decreased BMD. However, the link between ER stress, vascular calcification, and BMD has not been investigated.

Vascular calcification develops early in the course of CKD,[8–10] well before any overt change in serum markers of mineral metabolism such as calcium and phosphorus. Presently, computed tomography is the gold standard for detection of vascular calcification and used mainly in research. If a practitioner screens for vascular calcification, the 2009 NKF-KDOQI (National Kidney Foundation–Kidney Disease Outcomes Quality Initiative) guidelines recommend the use of plain lateral abdominal radiography.[46] Regardless of modality, the best currently available diagnostic techniques expose CKD patients to radiation doses and are of high cost, which are not insignificant for this group of frequent health care utilizers.[47] Therefore, a reliable serum biomarker would be useful for clinical diagnosis and for research purposes. The results of our study suggest that DCA could be a potential biomarker for vascular calcification in CKD given its association with CAC independent of eGFR and other markers of mineral metabolism.

Moreover, the mechanistic role that DCA plays in ER stress and vascular calcification suggest it may be a potential treatment target as well. Indeed, alterations in gut flora may reduce the conversion of cholic acid to DCA.[48] Interventions that change the composition of the gut microbiome, thereby potentially lowering DCA levels, could be tested with the aim to improve measures of vascular calcification. Further investigation is required.

Strengths of this study include a fair number of participants with CKD, measurements of CAC and BMD, numerous co-variables including markers of mineral metabolism, and

available data to examine both cross-sectional and longitudinal relationship between DCA levels (levels >58 ng/mL and log-transformed levels) and log-transformed CAC volume score and BMD. Despite these and other strengths, this study has some important limitations. As an observational cohort study, causality cannot be concluded. DCA levels were only measured at baseline, thus, insight into how DCA changes over time is limited. Other biochemical bone markers, such as alkaline phosphatase, were not collected. The majority of study participants were white (78%) and older (66 years) so our results may not be applicable to other races/ethnicities and younger cohorts. Levels of DCA were measured in non-fasting serum samples; DCA levels are known to vary based on time of day and meal intake.[48] We did not observe a significant association of DCA and eGFR, however, only a limited range of eGFR was studied. Longitudinal analyses, which evaluated the association of baseline DCA levels (levels >58 ng/mL and log-transformed levels) with change in log-transformed CAC volume score and BMD over 9 months were limited by small numbers (n = 75 and n = 59, respectively) and are underpowered. Finally, despite adjustments for potential confounders, residual confounding may still be present.

In conclusion, higher DCA levels (levels >58 ng/mL and log-transformed levels) were independently associated with vascular calcification as measured by log-transformed CAC volume score and lower lumbar BMD at baseline among subjects with moderate to severe CKD. Baseline DCA levels (levels >58 ng/mL and log-transformed levels) were not significantly associated with change in log-transformed CAC volume score or BMD after nine months, however, the number of subjects with complete data to perform these analyses and the change in CAC volume score and BMD were both very small. Our findings suggest that elevated DCA in CKD via an ER stress mechanism is a novel mechanism for the development of and potential biomarker for vascular calcification. Prospective studies to determine whether DCA levels predict CAC volume score progression are needed. Furthermore, lowering DCA levels may reveal a way to mitigate vascular calcification in CKD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Table 1

Baseline Characteristics

| | All (N = 112) | DCA > 58 ng/mL (n = 56) | DCA ≤ 58 ng/mL (n = 56) | P |
|---|---|---|---|---|
| Age (years) | 65.7 ± 12.2 | 68.4 ± 11.0 | 63 ± 12.8 | 0.04 |
| Male sex | 54 (48%) | 25 (44%) | 29 (52%) | 0.6 |
| Race | | | | 0.1 |
| White | 87 (78%) | 47 (84%) | 40 (71%) | |
| Black | 13 (12%) | 3 (5%) | 10 (18%) | |
| Other | 12 (10%) | 6 (11%) | 5 (11%) | |
| Diabetes | 63 (56%) | 35 (63%) | 28 (50%) | 0.3 |
| Hypertension | 110 (98%) | 54 (96%) | 56 (100%) | 0.5 |
| Coronary Artery Disease | 25 (22%) | 16 (29%) | 9 (16%) | 0.2 |
| Hyperlipidemia | 100 (89%) | 51 (91%) | 49 (88%) | 0.8 |
| BMI | 31.5 ± 7.3 | 32.4 ± 7.4 | 30.7 ± 7.0 | 0.2 |
| eGFR | 31.5 ± 8.7 | 31.7 ± 7.5 | 31.3 ± 9.6 | 0.5 |
| Mineral Metabolites | | | | |
| Calcium (mg/dL) | 9.3 ± 0.4 | 9.3 ± 0.4 | 9.3 ± 0.4 | 0.7 |
| Phosphate (mg/dL) | 4.2 ± 0.4 | 4.1 ± 0.4 | 4.3 ± 0.4 | 0.04 |
| $1,25(OH)_2D$ (pg/mL) | 26.3 ± 10.9 | 25.7 ± 10.4 | 27.0 ± 11.5 | 0.7 |
| PTH (pg/mL) | 79.6 ± 54.1 | 75.3 ± 54.7 | 83.8 ± 53.6 | 0.3 |
| FGF-23 (pg/mL) | 215.0 [132.0–307.9] | 241.3 [149.6–320.6] | 202.5 [128.4–280.5] | 0.5 |
| CAC volume score | 246.0 [43.0–743.0] | 474.0 [126.5–1017.5] | 143.0 [0.0–525.0] | 0.004 |
| Log CAC volume score | 2.1 ± 1.1 | 2.5 ± 0.9 | 1.7 ± 1.2 | <0.001 |
| BMD ($g/cm^2$) | 113.1 ± 40.4 | 99.6 ± 33.7 | 125.5 ± 42.4 | 0.007 |
| DCA (ng/mL) | 58.4 [29.2–111.8] | 111.8 [80.3–168.2] | 28.7 [16.5–41.7] | <0.001 |
| Log DCA | 1.7 ± 0.5 | 2.1 ± 0.3 | 1.4 ± 0.3 | 0.005 |
| Treatment assignment | | | | 0.3 |
| Active | 72 | 39 | 33 | |
| Placebo | 40 | 17 | 23 | |

Note: Values for categorical variables are given as number (percentage); values for continuous variables, as mean ± standard deviation or median [interquartile range]. Conversion factors for units: calcium in mg/dL to mmol/L, ×0.2495; $1,25(OH)_2D$ in pg/mL to pmol/L, ×2.6;

Abbreviations: BMD, bone mineral density; BMI, body mass index; eGFR, estimated glomerular filtration rate; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; FGF-23, fibroblast growth factor 23; CAC, coronary artery calcification; DCA, deoxycholic acid

**Table 2**

Association of baseline deoxycholic acid >58 ng/mL with log-transformed CAC volume score

| | β estimate (95% CI) | P |
|---|---|---|
| Unadjusted | 0.74 (0.29–1.19) | 0.002 |
| Model 1 | 0.55 (0.12–0.98) | 0.02 |
| Model 2 | 0.63 (0.24–1.02) | 0.002 |
| Model 3 | 0.62 (0.23–1.01) | 0.003 |
| Model 4 | 0.68 (0.29–1.07) | 0.001 |
| Model 5 | 0.71 (0.26–1.16) | 0.003 |

Note: n=112. 58 ng/mL is median level of deoxycholic acid. Model 1: unadjusted model + age; Model 2: model 1 + sex, race, body mass index, diabetes mellitus, hypertension, coronary artery disease; Model 3: model 2 + estimated glomerular filtration rate; Model 4: model 3 + calcium, phosphate; and Model 5: model 4 + intact parathyroid hormone, fibroblast growth factor 23

CAC, coronary artery calcification; CI, confidence interval

## Table 3

Association of baseline deoxycholic acid > 58 ng/mL with baseline BMD (n = 112)

| | β estimate (95% CI) | P |
|---|---|---|
| Unadjusted | −26.0 (−43.1 to −8.9) | 0.004 |
| Model 1 | −19.0 (−35.1 to −2.9) | 0.02 |
| Model 2 | −16.4 (−31.9 to −0.9) | 0.04 |
| Model 3 | −16.1 (−31.6 to −0.6) | 0.05 |
| Model 4 | −17.0 (−33.1 to −0.9) | 0.04 |
| Model 5 | −20.3 (−39.1 to −1.5) | 0.04 |

Note: n=112. 58 ng/mL is median level of deoxycholic acid. Model 1: unadjusted model + age; Model 2: model 1 + sex, race, body mass index, diabetes mellitus, hypertension, coronary artery disease; Model 3: model 2 + estimated glomerular filtration rate; Model 4: model 3 + calcium, phosphate; and Model 5: model 4 + intact parathyroid hormone, fibroblast growth factor 23.

BMD, bone mineral density; CI, confidence interval

**Table 4**

Association of baseline deoxycholic acid > 58 ng/mL with change in log-transformed CAC volume score after 9 months

| | β estimate (95% CI) | P |
|---|---|---|
| Unadjusted | 0.04 (−0.07 to 0.15) | 0.8 |
| Model 1 | 0.03 (−0.09 to 0.15) | 0.6 |
| Model 2 | 0.04 (−0.09 to 0.17) | 0.5 |
| Model 3 | 0.05 (−0.08 to 0.18) | 0.4 |
| Model 4 | 0.04 (−0.08 to 0.16) | 0.5 |
| Model 5 | 0.06 (−0.09 to 0.21) | 0.4 |

Note: n=75. 58 ng/mL is median level of deoxycholic acid. Model 1: unadjusted model + age; Model 2: model 1 + sex, race, body mass index, diabetes mellitus, hypertension, coronary artery disease; Model 3: model 2 + estimated glomerular filtration rate; Model 4: model 3 + calcium, phosphate; and Model 5: model 4 + intact parathyroid hormone, fibroblast growth factor 23, treatment group (active vs placebo)

CAC, coronary artery calcification; CI, confidence interval