Association of hypercalciuria with vitamin D supplementation in patients undergoing ketogenic dietary therapy

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Background: Ketogenic dietary therapy (KDT) is used as an effective treatment for epilepsy. However, KDT carries the risk of bone health deterioration; therefore, vitamin D supplementation is required. Vitamin D replacement therapy in KDT has not been established because it may be related to hypercalciuria/urolithiasis, which are common adverse effects of KDT. Hence, this study aimed to evaluate the dose-dependent association between vitamin D₃ and hypercalciuria/urolithiasis in patients undergoing KDT and dose optimization for renal complications.

Materials and methods: Overall, 140 patients with intractable childhood epilepsy started 3:1 KDT (lipid to non-lipid ratio) at the Severance Children’s Hospital from January 2016 to December 2019. Regular visits were recommended after KDT initiation. Participants were assessed for height, weight, serum 25-hydroxyvitamin D (25-OH-D₃) level, parathyroid hormone level, and ratio of urinary excretion of calcium and creatinine (Uca/Ucr). Kidney sonography was conducted annually. Patients who already had urolithiasis and were taking hydrochlorothiazide before KDT, failed to maintain KDT for 3 months, did not visit the pediatric endocrine department regularly, did not take prescribed calcium and vitamin D₃ properly, or needed hospitalization for > 1 month because of serious medical illness were excluded. Data from patients who started diuretic agents, e.g., hydrochlorothiazide, were excluded from that point because the excretion of calcium in the urine may be altered in these patients.

Result: In total, 49 patients were included in this study. Uca/Ucr ratio significantly decreased with increasing levels of 25-OH-D₃ (p = 0.027). The odds ratio for hypercalciuria was 0.945 (95% confidence interval, 0.912–0.979; p = 0.002) per 1.0 ng/mL increment in 25-OH-D₃ level. Based on findings
of receiver operating characteristic curve analysis and Youden’s J statistic, the cut-off 25-OH-D$_3$ level for preventing hypercalciuria was $> 39.1$ ng/mL at 6 months. Furthermore, the vitamin D$_3$ supplementation dose cut-off was $> 49.5$ IU/kg for hypercalciuria prevention.

**Conclusion:** An inverse relationship between Uca/Ucr ratio and 25-OH-D$_3$ level was noted, which means that vitamin D supplementation is helpful for preventing hypercalciuria related to KDT. We suggest that the recommended 25-OH-D$_3$ level is $> 40$ ng/mL for hypercalciuria prevention and that KDT for children with epilepsy can be optimized by vitamin D$_3$ supplementation at 50 IU/kg.

**KEYWORDS**

vitamin D, vitamin D deficiency, ketogenic diet, hypercalciuria, urolithiasis

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**Introduction**

Since 1921, ketogenic dietary therapy (KDT) has been considered a well-known non-pharmacologic anti-convulsant treatment for both children and adults with multi-drug resistant epilepsy (1). KDT is based on the fact that lipophilic compounds known as ketone bodies, such as acetocacetate, acetone, and beta-hydroxybutyrate, can cross the blood-brain barrier and act as direct anticonvulsants (2). It is also known that intermediate chain triglycerides, such as decanoic acid, can directly inhibit $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and that KDT increases adenosine level and inhibits DNA methylation, which are the known key mechanisms of KDT for treating epilepsy (3). However, KDT without careful management may accompany various side effects such as gastrointestinal symptoms, hepatic dysfunction, dyslipidemia, growth retardation, urolithiasis, pancreatitis, and cardiac abnormalities, especially when it is used together with high-dose anti-epileptic drugs (AEDs) (1).

Bone health deterioration is one of the most common clinical issues in patients undergoing KDT. Patients with intractable epilepsy usually have prolonged exposure to high-dose AEDs and are at risk of vitamin D deficiency (4). Furthermore, KDT is a diet prone to being deficient in essential nutrients, including calcium, phosphorus, magnesium, vitamin K, and vitamin D. Additionally, ketone bodies produced by KDT also induce acidification, which converts active vitamin D into an inactive form (1). Therefore, KDT can lead to calcium and vitamin D deficiencies and worsen bone vulnerability, causing osteoporosis; hence, calcium and vitamin D supplementation are needed. Given the possible risks and complications of KDT, patients on KDT are advised to visit an endocrinologist regularly to ensure adequate calcium and vitamin D supplementation.

Meanwhile, hypercalciuria and urolithiasis are also common complications of KDT, which are caused by increased bone demineralization due to acidosis. Acidosis induces hypocitraturia, which in turn increases free calcium, and increases the less soluble uric acid levels (5). As calcium is the most frequent component of urinary calculi and the major constituent of approximately 75% of the stones, an increase in the urinary excretion of calcium is the most common risk factor for urolithiasis (6, 7). Low urine volume and hypercalciuria increase Randall’s plaque formation, which is specific to calcium oxalate stone formation (8, 9). Consequently, urolithiasis may develop in children undergoing KDT (10, 11). Although hypercalciuria and urolithiasis are not absolute contraindications for KDT or indications for cessation of KDT (1), they may cause poor compliance and treatment failure, as the presence of kidney stones may lead to severe abdominal pain or dysuria, which may reduce the patient’s quality of life.

Calcium intake and vitamin D supplementation have been thought to be risk factors for hypercalciuria because they can increase intestinal absorption of calcium and cause hypercalcemia, even without KDT (12). However, as mentioned above, calcium and vitamin D supplementation are required for patients undergoing KDT. In addition, as vitamin D levels in patients with urolithiasis are lower than those in the normal population, vitamin D deficiency is thought to increase the occurrence of kidney stone formation (13). Therefore, whether calcium intake and vitamin D supplementation worsen hypercalciuria and promote kidney stone formation as well as the optimal level of 25-hydroxyvitamin D (25-OH-D$_3$) and appropriate use of vitamin D supplementation in patients with KDT remain controversial.

Considering the above-mentioned arguments, in this study, we aimed to establish the correlation between vitamin D$_3$ dose and the occurrence of hypercalciuria/urolithiasis in patients undergoing KDT. In addition, we evaluated the optimal dose to minimize renal complications.
Materials and methods

Participants

Overall, 140 patients with intractable childhood epilepsy were started on KDT at a 3:1 lipid to non-lipid ratio in the pediatric neurology department of Severance Children’s Hospital, Seoul, South Korea from January 2016 to December 2019. All patients were referred to the pediatric endocrine department for monitoring the endocrinologic adverse effects of KDT, such as growth retardation, dyslipidemia, multivitamin deficiency, and hypothyroidism. Among these patients, those who maintained KDT for > 3 months and regularly visited the pediatric endocrine department for monitoring were included in this study. Regular outpatient visits were recommended at 1, 3, 6, and 12 months, unless there were medical issues. Patients who already had urolithiasis and received hydrochlorothiazide before KDT, failed to maintain KDT for 3 months, did not take the prescribed calcium and vitamin D$_3$ supplementation properly, or did not undergo endocrinological follow-up studies including biochemical laboratory tests, were excluded. Additionally, we excluded patients requiring prolonged hospitalization due to serious illness after KDT initiation, since the changes in their systemic condition and changes in treatment such as AEDs and KDT could significantly alter their clinical aspects. Further, data from patients who started diuretic agents, such as hydrochlorothiazide, were eliminated from that point in time because the excretion of calcium in the urine may be altered in these patients. Finally, 49 patients were included in this study. Among those who continued KDT, 6-month data were obtained from 38 patients and 1-year data were obtained from 22 patients.

The type and dosage of AEDs were not changed significantly during KDT, and the formulations were modified to contain as little carbohydrates as possible. Patients on KDT were supplemented with multivitamins, L-carnitine, calcium, and vitamin D$_3$ (cholecalciferol). We used a combination tablet containing 100 mg of calcium and 1,000 IU of vitamin D$_3$ per pill for calcium and vitamin D supplementation. Daily doses of calcium and vitamin D$_3$ were approximated based on weight: 0.5 tablets (elemental 50 mg of calcium and 300 IU of vitamin D$_3$) for a bodyweight up to 10 kg, 1.0 tablet (100 mg of elemental calcium and 1,000 IU of vitamin D$_3$) for a bodyweight of 10–20 kg, 1.5 tablets (150 mg of elemental calcium and 1,500 IU of vitamin D$_3$) for a bodyweight of 20–40 kg, and 2 tablets (200 mg of elemental calcium and 2,000 IU of vitamin D$_3$) for a bodyweight of > 40 kg. If the serum 25-OH-D$_3$ level was < 20 ng/mL or > 50 ng/mL, the doses of the calcium and vitamin D$_3$ complex were increased or decreased by 0.25 tablets (25 mg of elemental calcium and 250 IU of vitamin D$_3$).

The Institutional Review Board of Severance Hospital Clinical Trial Center (subject no. 4-2020-0549) approved this study. Because this was a retrospective study that analyzed only the results obtained during the general course of medical treatment, the need for informed consent was waived. We complied with the Declaration of Helsinki to protect participant rights and personal information.

Data collection

Patients’ heights and weights were measured at each visit. Further, levels of serum calcium (mg/dL), phosphorus (mg/dL), alkaline phosphatase (mg/dL), 25-OH-D$_3$ (ng/mL), parathyroid hormone (PTH, pg/mL), urinary excretion of calcium (Uca, mg/dL), and creatinine (Ucr, mg/dL) in spot urine samples, and urine osmolality were assessed at each visit to identify whether hypercalciuria or any side effects of vitamin D$_3$ supplementation had occurred. Serum calcium, phosphate, and alkaline phosphatase levels were measured using Hitachi chemistry autoanalyzer 7600-110 (Hitachi Ltd., Tokyo, Japan) at the central laboratory of Severance Hospital. Serum 25-OH-D$_3$ level was determined using a radioimmunoassay (DiaSorin, Inc., Stillwater, MN, United States; intraassay CV < 4.1%, inter-assay CV < 7.0%). The serum PTH concentration was measured at our hospital using a second-generation PTH assay (Elecsys PTH; Roche Diagnostics, Mannheim, Germany) on the Cobas e801 immunoassay analyzer (Roche Diagnostics). Serum osteocalcin level was measured using an electrochemiluminescence immunoassay (Elecsys N-MID Osteocalcin; Roche Diagnostics; intraassay CV < 1.8%, inter-assay CV < 3.3%), and the urinary N-terminal telopeptide was calculated by competitive immunoassay (Vitros$^\text{TM}$ NTx reagent pack; Ortho-clinical Diagnostics, Inc., Rochester, NY, United States). Urinary calcium excretion (calculated as the ratio of urine calcium level to creatinine level) of the patients was measured by random urine tests using an automated urine chemistry analyzer AUS5800 (Beckman Coulter, Fullerton, CA, United States) and LIAISON system (DiaSorin, Saluggia, Italy) at every admission before the initiation of each cycle. The criteria for hypercalciuria were applied differently by age according to the Uca/Ucr ratio (≥ 0.86 for up to 7 months old, ≥ 0.60 for 7–18 months old, ≥ 0.42 for 19 months to 6 years old, and ≥ 0.20 for > 6 years old) (14). The status of the serum 25-OH-D$_3$ level was classified as deficient (< 20 ng/mL), insufficient (20–30 ng/mL), and sufficient (> 30 ng/mL) (15).

Statistical analysis

All statistical analyses were performed using SAS version 9.4 (SAS Inc., Cary, NC, United States) and R package version 3.6.3. Continuous variables are presented as means and standard deviation (SD). Linear regression analysis was performed to investigate the factors that affect the Uca/Ucr ratio at each

1 http://www.R-project.org
visit. To consider the longitudinal structure of data (i.e., four assessment time points), linear mixed-effect models were used to investigate the factors affecting the Uca/Ucr ratio. An autoregressive model (1) correlation structure was assumed among repeated measures of all longitudinal analyses. A logistic regression model was used to investigate factors affecting the occurrence of hypercalciuria at each visit. Moreover, since the occurrence of hypercalcemia was measured at each visit, data were analyzed using the generalized estimating equation model to identify factors affecting the occurrence of hypercalcemia throughout the study period. The results are indicated by odds ratios and confidence intervals. The analysis results of the entire period were adjusted by time effect. To obtain the cut-off value, receiver operating characteristic curve analysis and Youden’s J statistic were used. Statistical significance was set at \( p < 0.05 \).

**Results**

Of the 49 participants enrolled in this study, 31 (63.3%) were boys. All participants were diagnosed with intractable epilepsy, and their seizures began at a mean age of 2.1 ± 2.4 years (range, 0.0–11.7 years). At KDT initiation, the mean age was 4.3 ± 3.2 years (range, 0.3–14.1 years), which was an average of 2.2 ± 2.1 years (0.1–9.5 years) after the onset of seizures (Table 1). The participants received an average of 2.4 ± 1.1 AEDs (range, 0–5 AEDs). The average level of 25-OH-D_3_ was 22.4 ng/mL, with 21 (42.9%) patients being deficient in 25-OH-D_3_, whereas 19 (38.8%) had insufficient levels, and nine (18.4%) had sufficient levels before KDT initiation. One patient had hyperparathyroidism and 25-OH-D_3_ deficiency, and among five patients with hypoparathyroidism, four had insufficient 25-OH-D_3_ levels and one had deficient levels. Although 11 (22.4%) patients already met the definition of hyperparathyroidism before KDT initiation, they were enrolled in this study because none of them had taken hydrochlorothiazide in accordance with the judgment of the pediatric nephrologists. The patients took an average of 49.9 IU/kg (range, 19.6–102.6 IU/kg) vitamin D_3_ supplementation (Table 1).

Three months after KDT initiation with an average vitamin D_3_ supplementation of 50.8 IU/kg, only one patient had hypercalcemia (serum calcium, 11.4 mg/dL; normal range, 8.5–10.5 mg/dL). The patient was administered 55.6 IU/kg of vitamin D_3_ and 100 mg of elemental calcium, and their 25-OH-D_3_ level increased from 30.84 to 46.7 ng/mL and PTH level was low at 7.88 pg/mL. In addition, he had hypercalcemia, with a Uca/Ucr ratio of 2.56. Therefore, we reduced the supplemental doses of vitamin D_3_ and calcium by half (vitamin D_3_, 27.8 IU/kg; elemental calcium, 50 mg/kg). Although his 25-OH-D_3_ level remained similar (43.4 ng/mL) during the follow-up observation, hypercalcemia, and hypercalcuria resolved (Uca/Ucr ratio = 0.19) without any medication. Hyperparathyroidism was not observed in any patient, whereas hypoparathyroidism was identified in 17 (34.7%) patients; however, there was no association between PTH level and Uca/Ucr ratio (Table 2). Hypercalcemia was observed in 27 (55.5%) patients; however, no factors affected Uca/Ucr ratio (Table 2). Moreover, the risk of hypercalcemia decreased as the dose of vitamin D_3_ supplementation increased (odds ratio = 0.950; \( p = 0.014 \)) (Table 3).

Six months after KDT initiation with an average vitamin D_3_ supplementation dose of 44.5 IU/kg, calcium and phosphorus levels of all participants were within the normal range. There were 13 (34.0%) patients with hypoparathyroidism and none with hyperparathyroidism. Hypercalcemia was observed in 19 (50.0%) patients. Age at KDT initiation, height, weight, and 25-OH-D_3_ levels were negatively associated with Uca/Ucr ratio (Figure 1 and Table 2). Additionally, an increased dose of vitamin D_3_ supplementation (odds ratio = 0.956; \( p = 0.028 \)) and a sufficient 25-OH-D_3_ level (odds ratio = 0.888; \( p = 0.010 \)) decreased the risk of hypercalcemia (Figure 2 and Table 3). The optimal level of 25-OH-D_3_, which minimizes the occurrence of hypercalcemia with maximum sensitivity and specificity, was 39.14 ng/mL (Figure 3A), and the optimal dose of vitamin D_3_ supplementation was 49.47 IU/kg (Figure 3B).

One year after KDT initiation with an average vitamin D_3_ supplementation dose of 35.3 IU/kg, calcium and phosphorus levels of all participants were within the normal range, and six (27.3%) patients had hypoparathyroidism. Further, nine patients (40.9%) had hypercalcemia. The dose of vitamin D_3_ supplementation and 25-OH-D_3_ levels did not affect the Uca/Ucr ratio, whereas height and 25-OH-D_3_ levels were negatively associated with Uca/Ucr ratio (Table 2). However, none of these factors increased the risk of hypercalcuria occurrence (Table 3).

In the analysis of the overall follow-up period in which the time variable was corrected through the linear mixed model, the increased dose of vitamin D_3_ supplementation (odds ratio = 0.976; \( p = 0.043 \)) and increased 25-OH-D_3_ level (odds ratio = 0.945; \( p = 0.002 \)) decreased the risk of hypercalcemia, consistent with the trend shown at 3 and 6 months.

Urolithiasis developed in three patients (6.1%): two boys and one girl. Patient 1 was a 3-month-old boy for whom vitamin D_3_ (60.98 IU/kg) was prescribed and discontinued 6 months after KDT initiation because of kidney stone formation. Patient 2 was a 7-year-old girl for whom 45.66 IU/kg of vitamin D_3_ was prescribed. She was diagnosed as having urolithiasis 6 months after KDT initiation; however, vitamin D_3_ supplementation was continued. This was because (1) her 25-OH-D_3_ level was only 21.39 ng/mL, which was only slightly higher than the lower recommended limit of vitamin D_3_, and (2) up to 40–60% of kidney stones in children are reported to be non-calcium-based; considering the lower vitamin D levels, it was unlikely that her urolithiasis was calcium-based (14). Patient 3 was a 19-month-old boy who was diagnosed with urolithiasis 9 months after KDT initiation and vitamin
### TABLE 1  Characteristics of children with intractable epilepsy at ketogenic dietary therapy initiation.

|                | Baseline (N = 49) | At 3 months (N = 47) | At 6 months (N = 38) | At 12 months (N = 21) |
|----------------|-------------------|----------------------|----------------------|-----------------------|
| Sex M. F. (n)  | 31:18             | 30:17                | 25:13                | 14:7                  |
| Age (years)    | 4.3 ± 3.2 (0.3–14.1) | 4.6 ± 3.2 (0.6–14.4) | 4.7 ± 3.3 (0.8–14.6) | 4.4 ± 2.4 (1.3–10.4)  |
| Current AED (n)| 2.4 ± 1.1 (0.0–5.0) | 2.1 ± 1.2 (0.0–5.0)  | 2.2 ± 1.1 (0.1–9.5)  | 1.7 ± 2.8 (0.7–7.5)   |
| Age at first seizure (year) | 2.1 ± 0.0 (0.0–11.7) | 2.1 ± 0.4 (0.0–11.7) | 2.1 ± 2.5 (0.0–11.7) | 2.7 ± 1.5 (1.1–7.5)   |
| Duration of seizure (year)   | 2.2 ± 0.9 (0.1–9.5)  | 2.2 ± 1.0 (0.1–9.5)  | 2.6 ± 2.1 (0.6–10.0) | 2.7 ± 1.5 (0.7–10.5)  |
| Height (cm)    | 100.9 ± 22.2 (64.0–170.0) | 103.1 ± 21.9 (65.7–170.0) | 105.8 ± 21.7 (71.0–171.0) | 103.3 ± 16.8 (78.5–142.5) |
| Height SDS     | −0.03 ± 1.15 (−3.15–1.98) | −0.32 ± 0.91 (−2.44–1.46) | −0.24 ± 0.96 (−1.98–1.66) | −0.42 ± 0.97 (−2.43–1.51) |
| Weight (kg)    | 17.5 ± 9.2 (7.1–51.0) | 18.2 ± 9.4 (6.9–57.8) | 18.9 ± 10.3 (8.7–61.2) | 16.8 ± 5.8 (9.0–33.5)  |
| Weight SDS     | −0.09 ± 1.31 (−3.20–2.95) | −0.27 ± 1.31 (−3.15–3.47) | −0.36 ± 1.22 (−2.63–2.09) | −0.14 ± 1.02 (−1.90–2.32) |
| BMI            | 16.4 ± 2.3 (12.3–24.2) | 16.4 ± 2.4 (12.3–22.9) | 16.0 ± 2.0 (13.3–20.9) | 16.3 ± 1.7 (12.6–20.0) |

#### Serum variables

|                | Baseline (N = 49) | At 3 months (N = 47) | At 6 months (N = 38) | At 12 months (N = 21) |
|----------------|-------------------|----------------------|----------------------|-----------------------|
| Calcium (mg/dL)  | 9.7 ± 0.5 (8.9–10.5) | 9.6 ± 0.6 (8.3–11.4) | 9.6 ± 0.5 (8.8–10.6) | 9.5 ± 0.5 (8.8–10.4)  |
| Phosphorus (mg/dL)| 5.3 ± 0.6 (4.1–6.8) | 4.7 ± 0.5 (3.1–6.1)  | 4.8 ± 0.5 (3.8–6.0)  | 4.8 ± 0.8 (3.7–5.8)   |
| ALP (mg/dL)      | 231.7 ± 89.2 (68.0–499.0) | 186.6 ± 57.6 (66.0–321.0) | 196.7 ± 80.6 (52.0–441.0) | 205.2 ± 85.4 (97.0–433.0) |
| PTH (pg/mL)      | 26.4 ± 12.0 (8.0–75.7) | 17.8 ± 7.0 (6.8–33.6) | 20.1 ± 8.8 (6.1–42.2) | 19.6 ± 5.3 (9.0–28.1)  |
| 25-OH-D$_3$ (ng/mL) | 22.4 ± 9.0 (9.8–49.1) | 35.5 ± 9.9 (10.1–58.8) | 33.9 ± 9.9 (11.8–55.0) | 29.9 ± 8.5 (12.3–48.8) |
| Deficiency, n (%)| 21 (42.9%) | 3 (6.1%) | 2 (5.4%) | 1 (4.5%) |
| Insufficiency, n (%)| 19 (38.8%) | 8 (16.3%) | 12 (25.2%) | 10 (25%) |
| Sufficient, n (%) | 9 (18.4%) | 38 (77.6%) | 23 (62.2%) | 9 (25%) |
| Not checked      | 1                 | 1                    | 1                    | 1                     |

#### Urinary excretion

|                | Baseline (N = 49) | At 3 months (N = 47) | At 6 months (N = 38) | At 12 months (N = 21) |
|----------------|-------------------|----------------------|----------------------|-----------------------|
| Calcium        | 8.9 ± 9.2 (0.0–39.8) | 31.1 ± 22.8 (2.2–95.6) | 25.3 ± 21.1 (3.3–83.4) | 26.3 ± 17.4 (0.5–59.5) |
| Creatinine     | 57.8 ± 38.3 (3.6–178.9) | 72.0 ± 52.8 (3.6–264.5) | 79.5 ± 69.6 (3.8–399.0) | 83.5 ± 57.7 (12.2–244.0) |
| Uca/Ucr        | 0.26 ± 0.38 (0.00–1.63) | 0.6 ± 0.6 (0.0–2.8) | 0.5 ± 0.4 (0.0–2.1) | 0.4 ± 0.3 (0.0–1.4) |
| Hypercalcuria, n (%) | 11, 22.4% | 27, 57.4% | 19, 50.0% | 9, 42.9% |

#### Vitamin D$_3$ supplementation (IU/kg)

|                | Baseline (N = 49) | At 3 months (N = 47) | At 6 months (N = 38) | At 12 months (N = 21) |
|----------------|-------------------|----------------------|----------------------|-----------------------|
| 25-OH-D$_3$ | 50.8 ± 18.3 (15.4–102.6) | 44.5 ± 20.4 (0.0–81.3) | 35.1 ± 17.4 (0.0–66.1) |

#### TABLE 2  Factors affecting the ratio of urinary excretion of calcium (Uca) to urinary excretion of creatinine ratio (Ucr) using longitudinal mixed-effect models.

|                | Month 3 | Month 6 | Month 12 | Overall |
|----------------|---------|---------|----------|---------|
| Sex (ref = M)  | 0.088   | 0.188   | 0.159    | 0.291   |
| Age at KDT initiation | −0.038 | 0.028 | 0.180 | −0.034 |
| Seizure onset age | −0.036 | 0.037 | 0.333 | −0.039 |
| Height          | −0.006  | 0.004  | 0.142    | −0.006  |
| Weight (kg)     | −0.015  | 0.009  | 0.128    | −0.012  |
| Vitamin D (IU/kg)| −0.002 | 0.005 | 0.655 | −0.002 |
| 25-OH-D$_3$ (ng/mL) | −0.007 | 0.010 | 0.486 | −0.011 |
| PTH             | −0.020  | 0.011  | 0.077    | −0.004  |
| Osteocalcin     | −0.006  | 0.0015 | 0.7057   | −0.0003 |
| NTx             | 0.0003  | 0.0002 | 0.1123   | 0.00004 |

25-OH-D$_3$, 25-hydroxyvitamin D; β, beta coefficient; KDT, ketogenic dietary therapy; SE, standard error; PTH, parathyroid hormone; NTx, N-telopeptide; Uca, urinary excretion of calcium; Ucr, urinary excretion of creatinine; ref, reference; M, male.

Statistically meaningful data are shown in bold.
### TABLE 3 Odds ratio of factors related to the occurrence of hypercalciuria.

|               | Month 3 OR (95% CI) | P-value | Month 6 OR (95% CI) | P-value | Month 12 OR (95% CI) | P-value | Overall OR (95% CI) | P-value |
|---------------|---------------------|---------|---------------------|---------|-----------------------|---------|---------------------|---------|
| Sex (ref = M) | 1.008 (0.299, 3.403) | 0.989   | 1.745 (0.437, 6.972) | 0.431   | 0.429 (0.057, 3.223)  | 0.410   | 1.213 (0.515, 2.857) | 0.658   |
| Age at beginning KDT | 1.047 (0.869, 1.261) | 0.629   | 0.896 (0.727, 1.105) | 0.303   | 1.187 (0.814, 1.733)  | 0.374   | 1.030 (0.869, 1.221) | 0.733   |
| Seizure onset age | 1.072 (0.831, 1.382) | 0.594   | 0.861 (0.645, 1.148) | 0.307   | 1.318 (0.800, 2.172)  | 0.279   | 1.028 (0.814, 1.300) | 0.814   |
| Height (cm)   | 1.012 (0.985, 1.041) | 0.379   | 0.980 (0.950, 1.011) | 0.208   | 1.010 (0.962, 1.060)  | 0.691   | 1.004 (0.981, 1.027) | 0.756   |
| Weight (kg)   | 1.009 (0.947, 1.076) | 0.779   | 0.953 (0.878, 1.034) | 0.249   | 1.042 (0.898, 1.209)  | 0.587   | 0.998 (0.942, 1.058) | 0.952   |
| Vitamin D (IU/kg) | 0.950 (0.911, 0.990) | **0.014** | 0.956 (0.918, 0.995) | **0.028** | 1.005 (0.956, 1.056)  | 0.857   | 0.976 (0.954, 0.999) | **0.043** |
| 25-OH-D₃ (ng/mL) | 0.963 (0.901, 1.029) | 0.267   | 0.888 (0.812, 0.971) | **0.010** | 0.955 (0.851, 1.072)  | 0.436   | 0.945 (0.912, 0.979) | **0.002** |

25-OH-D₃, 25-hydroxyvitamin D; KDT, ketogenic dietary therapy; OR, odds ratio; CI, confidence interval; ref, reference; M, male. Statistically meaningful data are shown in bold.

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**FIGURE 5**
Scatter plot between the urinary excretion of calcium (Uca) to urinary excretion of creatinine (Ucr) ratio and serum vitamin D level (ng/mL). Uca, urinary excretion of calcium; Ucr, urinary excretion of creatinine.

**D₃ supplementation (51.02 IU/kg), even though he did not develop hypercalciuria during the follow-up period. Before KDT initiation, these three patients had neither hypercalciuria nor urolithiasis, and all children with documented stones were first managed medically with increased fluids and urine alkalization using oral potassium citrate to yield a urine pH of 6.5. All three patients reached remission within 2 years with the aid of medical treatment and did not require lithotripsy for their kidney stones. For patients 2 and 3, vitamin D₃ supplementation was continued for the remission of urolithiasis.**

**Discussion**

To our best knowledge, this is the first study to assess the relationship between several clinical variables, including vitamin D₃ dose, serum 25-OH-D₃ level, and occurrence of hypercalciuria/urolithiasis in pediatric KDT patients. We found that serum 25-OH-D₃ level and hypercalciuria have an inverse correlation, and as 25-OH-D₃ level rises by 1.0 ng/mL, Uca/Ucr ratio decreases by 0.011. The optimal serum 25-OH-D₃ level for preventing hypercalciuria was > 39.1 ng/mL, and the cut-off vitamin D₃ supplementation dose was > 49.5 IU/kg.
Kidney stone formation is a complex process, which includes urine supersaturation and nucleation, growth, aggregation, and retention of crystals in the kidney (13). KDT can cause kidney stone formation, and the incidence of urolithiasis in children undergoing KDT is 1.4–7% (5, 10, 16). This might be due to hyperuricemia, which increases calcium excretion related to metabolic acidosis, or urine acidification, which results in uric acid supersaturation and decreased urinary citrate concentration (5). According to the “free-particle theory” and “fixed-particle theory,” supersaturated urine is the key process involved in kidney stone formation because the formation and growth of crystals occurs within highly saturated urine (11, 17).

It is unclear whether vitamin D supplementation or high serum 25-OH-D$_3$ level increases the risk of hypercalciuria or kidney stone formation. Many physicians are hesitant to treat vitamin D deficiency in patients with kidney stones because of concerns that vitamin D$_3$ supplementation increases
urinary calcium excretion. This hesitation might be because the most prevalent type of kidney stone is calcium based, and vitamin D increases intestinal calcium absorption and then urinary calcium excretion (18). Calcitriol binds to vitamin D receptors in enterocytes and increases calcium absorption (19). In addition, intestinal calcium absorption is increased in absorptive hypercalciuria (20), and calcitriol serum levels are also correlated with urinary calcium excretion (21). According to a systematic review and meta-analysis, increased circulating calcitriol was associated with kidney stones, and among patients with urolithiasis, circulating 25-OH-D₃ levels were markedly higher in hypercalciuria than in normocalciuria (22). Therefore, vitamin D is often cited as a risk factor for hypercalciuria and kidney stones (19).

However, several studies have shown that vitamin D supplementation is not associated with urolithiasis. In a prospective study, despite supplementation with high-dose vitamin D₃ (mean daily dose, 3,440 IU) in healthy controls to maintain 25-OH-D₃ levels within 30–88 ng/mL for 6 months, no hypercalcaemia or hypercalciuria was noted (23). Another study on patients with urolithiasis showed that hypercalcemia or significant changes in urinary calcium excretion did not occur when 50,000 IU of vitamin D₃ was administered each week, and there was no relation between 25-OH-D₃ level change and urinary calcium excretion (24). A large systematic review and meta-analysis study found that vitamin D supplementation may increase the risk of hypercalcemia and hypercalciuria, but did not increase the risk of kidney stone formation, regardless of the duration of supplementation, dosage, co-supplementation with calcium, and baseline 25-OH-D₃ level (12). In a large cohort study, there was also no association found between vitamin D₃ intake and incidence of kidney stones (25).

Furthermore, despite being controversial, vitamin D deficiency may be a predisposing factor for kidney stone formation. Several studies have shown that vitamin D deficiency is more prevalent in patients with kidney stone formation than in those without (26, 27). There are several hypotheses, as follows: first, secondary hyperparathyroidism caused by vitamin D deficiency can lead to urolithiasis. Second, there are several risk factors shared between vitamin D deficiency and urolithiasis, including obesity and decreased dietary calcium intake. Third, vitamin D deficiency might be responsible for inducing oxidative stress and inflammation in the kidney, which can cause urolithiasis (13).

Vitamin D deficiency causes a decrease in the absorption of dietary calcium, resulting in secondary hyperparathyroidism, which attempts to maintain serum calcium by mobilizing calcium from the bones by increasing osteoclastic activity. These processes decrease bone mineral density. Moreover, hyperparathyroidism increases phosphorus wasting in the kidneys, which results in a low normal or low serum phosphorus level. This results in an inadequate calcium-phosphorus product, causing a mineralization defect in the bones. Consequently, vitamin D deficiency results in osteopenia and osteoporosis (15).

In addition to bone health, vitamin D has various health benefits, as vitamin D receptors exist in most tissues and cells and active vitamin D influences the expression levels of more than 200 genes (28). Vitamin D deficiency causes muscle weakness, whereas increased 25-OH-D₃ level markedly improves performance speed and proximal muscle strength (29). Further, vitamin D has recently been found to be a key factor in the immune system, as (1) it induces the production of antimicrobial peptides and cytokines, (2) it simulates autophagy for controlling intracellular infections, and (3) vitamin D signaling promotes innate immune response. Thus, vitamin D deficiency is associated with susceptibility toward infections (30). In addition, active vitamin D has biological actions, including angiogenesis, renin production, insulin stimulation, macrophage cathelicidin production, and cellular proliferation inhibition (31). In chronic inflammatory diseases, such as type 2 diabetes and autoimmune diseases, vitamin D is supposed to play an important role in gene regulation (32), and supraphysiological doses of the active form of vitamin D may reduce excessive cell proliferation, even in cancer (31). Furthermore, vitamin D supplementation has been suggested to be potentially preventative against cardiovascular diseases through several mechanisms including upregulation of the renin-angiotensin-aldosterone system, blood pressure increase, and ventricular musculo-hypertrophy (33). In a meta-analysis of eight prospective cohort study, the group with the lower 20% of serum vitamin D levels was associated with increased cardiovascular mortality and all-cause mortality (34). Vitamin D deficiency is also correlated with dyslipidemia (35) and is thought to be more influential in high-fat diets such as KDT.

Given the known benefits of vitamin D in maintaining bone health and its potential benefits for cardiovascular, autoimmune, and neoplastic diseases, and given findings suggesting its safety, active vitamin D supplementation is required in patients undergoing KDT. In addition, we found out that maintaining adequate levels of vitamin D is helpful for hypercalciuria and urolithiasis. Serum 25-OH-D₃ level and Uca/Ucr ratio showed an inverse correlation during KDT, and although not statistically significant, Uca/Ucr ratio decreased with an increase in the dose of vitamin D₃ supplementation per weight. In addition, results at 6 months of KDT showed that 25-OH-D₃ level of < 39.1 ng/mL and inadequate vitamin D supplementation of < 49.5 IU/kg could also increase the risk of hypercalciuria. Therefore, it might be helpful to maintain sufficient serum levels of vitamin D (almost 40 ng/mL) and implement vitamin D supplementation (50 IU/kg) to prevent hypercalciuria.

Although there is no consensus on the optimal serum levels of 25-OH-D₃, vitamin D deficiency is defined with a 25-OH-D₃ level of < 20 ng/mL, relative insufficiency with levels between 20 and 29 ng/mL, and sufficient level is ≥ 30 ng/mL. Further, vitamin D poisoning is defined by 25-OH-D₃ level > 150 ng/mL.
In a normal population. In this regard, our study has the advantage that it was conducted under the same, controlled dietary conditions. In addition, the results will be applicable to children undergoing KDT.

In conclusion, we recommend that all children on KDT receive 50 IU/kg of daily vitamin D supplementation and maintain a serum 25-OH-D$_3$ level of 40 ng/mL to minimize the incidence of hypercalciuria. Further studies with larger numbers of multicenter patients over a longer period of follow-up are required for more evidence and better recommendations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Severance Hospital Clinical Trial Center. Written informed consent from the participants’ legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

ML and AK: conceptualization, methodology, and writing—original draft preparation. HL, KS, HSC, JS, and SK: formal analysis, investigation, resources, and data curation. HWC, H-CK, JL, HK, H-SK, and AK: writing—review and editing and supervision. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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13. Tavasoli S, Taheri M. Vitamin D and calcium kidney stones: a review and a

14. Hernandez JD, Ellison JS, Lendvay TS. Current trends, evaluation, and

15. Holick MF. Vitamin D deficiency. *N Engl J Med.* (2007) 357:266–81. doi: 10.1056/NEJMra0700750

16. Cai QY, Zhou ZJ, Luo R, Gan J, Li SP, Mu DZ, et al. Safety and tolerability of the ketogenic diet used for the treatment of refractory childhood epilepsy: a systematic review of published prospective studies. *World J Pediatr.* (2017) 13:528–36. doi: 10.1007/s12519-017-0553-2

17. Sakhaei K. Recent advances in the pathophysiology of nephrolithiasis. *Kidney Int.* (2009) 75:585–95. doi: 10.1038/ki.2008.626

18. Worcester EM, Cox FL. Clinical practice. Calcium kidney stones. *N Engl J Med.* (2010) 363:954–63. doi: 10.1056/NEJMcp1001101

19. Hoenderop JG, Niïls B, Bindels RJ. Calcium absorption across epithelia. *Physiol Rev.* (2005) 85:373–422. doi: 10.1152/physrev.00003.2004

20. Pak CY, East DA, Sanzenbacher LJ, Delea CS, Barter FC. Gastrointestinal calcium absorption in nephrolithiasis. *J Clin Endocrinol Metab.* (1972) 35:261–70. doi: 10.1210/jcem-35-2-261

21. Shakhlasim N, Gilani KR, Parvin M, Torbati PM, Kashi AH, Azadvari M, et al. An assessment of parathyroid hormone, calcitomin, 1,25 (OH)2 vitamin D3, estradiol and testosterone in men with active calcium stone disease and evaluation of its biochemical risk factors. *Urol Res.* (2011) 39:1–7. doi: 10.1007/s00240-010-0276-3

22. Hu H, Zhang J, Lu Y, Zhang Z, Qin B, Gao H, et al. Association between circulating Vitamin D level and uric acid: a systematic review and meta-analysis. *Nutrients.* (2017) 9:301. doi: 10.3390/nu9030301

23. Aloia JF, Patel M, Dimanno R, Li-Ng M, Talwar SA, Mikhail M, et al. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr.* (2008) 87:1952–8. doi: 10.3945/ajcn/87.8.1952

24. Leaf DE, Koerts R, Taylor EN, Tang J, Asplin JR, Goldfarb DS, et al. Effect of vitamin D repletion on urinary calcium excretion among kidney stone formers. *Clin J Am Soc Nephrol.* (2012) 7:829–34. doi: 10.2215/CJN.11311111

25. Ferrari PM, Taylor EN, Gambaro G, Curhan GC. Vitamin D intake and the risk of incident kidney stones. *J Urol.* (2017) 197:405–10. doi: 10.1016/j.juro.2016.08.084

26. Ticinesi A, Nouwenne A, Ferrari PM, Folesani G, Laurenzi F, Allegri F, et al. Idiopathic calcium nephrolithiasis and Hypovitaminosis D: a case-control study. *Urology.* (2016) 87:40–5. doi: 10.1016/j.urology.2015.10.009

27. Girón-Prieto MS, Del Carmen Cano-García M, Arrabal-Polo M, Poyatos-Andojar A, Quesada-Charneco M, de Haro-Muñoz T. Analysis of vitamin D deficiency in calcium stone-forming patients. *Int Urol Nephrol.* (2014) 46:1243–6. doi: 10.1007/s11255-014-1290-3

28. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* (2011) 96:1911–30. doi: 10.1210/jc.2011-0385

29. Bischoff-Ferrari HA, Giovanucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr.* (2006) 84:18–28. doi: 10.1093/ajcn/84.1.18

30. Ismailova A, White JH. Vitamin D: infections and immunity. *Rev Endocr Metab Disord.* (2022) 23:265–77. doi: 10.1007/s11154-021-09679-5

31. Leyssens C, Verlinden L, Verstuyf A. The future of vitamin D analogs. *Front Physiol.* (2014) 5:244. doi: 10.3389/fphys.2014.00244

32. Li YC, Kong J, Wei M, Chen ZZ, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D3 (D(3)) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* (2002) 110:2104–13. doi: 10.1172/JCI15219

33. Li YC, Kong J, Wei M, Chen ZZ, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D3 (D(3)) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* (2002) 110:2104–13. doi: 10.1172/JCI15219

34. Schöttker B, Jorde R, Peasey A, Thordarson B, Jansen EH, Groot LD, et al. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ.* (2014) 348:g3656. doi: 10.1136/bmj.g3656

35. Song K, Park G, Choi Y, Oh JS, Choi HS, Sub J, et al. Association of vitamin D status and physical activity with lipid profile in Korean children and adolescents: a population-based study. *Children.* (2020) 7:241. doi: 10.3390/children7110241

36. Letavernier E, Daudon M. Vitamin D, hypercalciuria and kidney stones. *Nutrients.* (2018) 10.366. doi: 10.3390/nu10030366