



Short- and Long-term Effects of Dialysate Calcium Concentrations on Mineral and Bone Metabolism in Hemodialysis Patients: The K4 Study

Teppei Sakoh, Masatomo Taniguchi, Shunsuke Yamada, Shotaro Ohnaka, Hokuto Arase, Masanori Tokumoto, Taihei Yanagida, Koji Mitsuiki, Hideki Hirakata, Toshiaki Nakano, Takanari Kitazono, and Kazuhiko Tsuruya

Rationale & Objective: The short- and long-term impact of conversion of dialysate calcium concentration from either 2.5 or 3.0 mEq/L to 2.75 mEq/L on mineral and bone metabolism remains unknown in hemodialysis patients.

Study Design: Nonrandomized intervention study.

Setting & Population: 12 hemodialysis patients treated at baseline with a 2.5-mEq/L dialysate calcium concentration and another 12 hemodialysis patients treated with a 3.0-mEq/L dialysate calcium concentration.

Intervention: Use of 2.75-mEq/L dialysate calcium concentration.

Outcomes: Changes in intradialytic calcium and phosphate clearance and changes in predialysis and intradialytic serum and ionized mineral and biochemical parameters over the 24 weeks following dialysate calcium conversion.

Results: Conversion of dialysate calcium concentration from 2.5 to 2.75 mEq/L increased intradialytic calcium loading and serum total and ionized calcium levels, whereas conversion of dialysate calcium from 3.0 to 2.75 mEq/L decreased intradialytic calcium loading and serum total and ionized

calcium levels. Dialysate calcium concentration conversion did not affect intradialytic serum parathyroid hormone level, intradialytic phosphate elimination, or predialysis serum calcium, phosphate, parathyroid hormone, and fibroblast growth factor 23 levels. Intradialytic calcium influx was determined by dialysate calcium concentration and predialysis serum calcium levels, whereas intradialytic phosphate elimination was determined by predialysis serum phosphate levels.

Limitations: Small sample size and no control groups treated with 2.5- and 3.0-mEq/L dialysate calcium concentrations during the 24 weeks of the observation period.

Conclusions: Conversion of dialysate calcium concentration from either 3.0 or 2.5 to 2.75 mEq/L results in expected changes in calcium loading based on predialysis calcium concentration. The dialysate calcium concentration should be personalized based on clinical factors.

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Correspondence to M. Taniguchi (macha1214@gmail.com)

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Selection of the optimal dialysate calcium concentration is important in the management of mineral and bone disorder in hemodialysis patients. Historically, a variety of dialysate calcium concentrations have been introduced and compared based on the viewpoint of their

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impacts on serum mineral and bone markers, intradialytic flux of calcium and phosphorus, parathyroid function, bone metabolism, and cardiovascular function and structure.¹ In 2003, the Kidney Disease Outcomes Quality Initiative (K/DOQI) guideline recommended use of 2.5-mEq/L dialysate calcium concentration,² and since 2009, the KDIGO (Kidney Disease: Improving Global Outcomes) guideline has recommended that a dialysate calcium concentration between 2.5 and 3.0 mEq/L be used.³ These recommendations are based on the general concept that a higher dialysate calcium concentration increases intradialytic calcium loading and possibly promotes vascular calcification. It is unclear which dialysate

calcium concentration within this range may be optimal for hemodialysis patients.⁴⁻⁶

Generally, a 2.5-mEq/L dialysate calcium concentration enables calcium-based phosphate binders and vitamin D receptor activators to be used because there is less intradialytic calcium loading and a lower prevalence of hypercalcemia compared with a higher dialysate calcium concentration. However, controlling secondary hyperparathyroidism may be challenging in patients treated with a 2.5-mEq/L dialysate calcium concentration due to potential negative calcium balance and subsequent hypocalcemia, with resultant intradialytic serum parathyroid hormone (PTH) level elevation.⁵⁻⁷ Additionally, a lower dialysate calcium concentration may increase the risk for hypotension and arrhythmia.^{8,9} Conversely, a 3.0-mEq/L dialysate calcium concentration enables easier control of serum PTH level and reduces intradialytic hypotension. However, the daily net calcium balance may be positive, especially when calcium-based phosphate binders and vitamin D receptor activators are used.^{9,10,11} This may be associated with more vascular calcification.^{12,13}

A 2.75-mEq/L dialysate calcium concentration may be more useful than a 2.5- or 3.0-mEq/L dialysate calcium concentration to manage mass hemodialysis patients, especially when selecting a dialysate calcium concentration for an entire facility, with a previous report demonstrating that net intradialytic calcium balance is neutral in hemodialysis patients treated with a 2.75-mEq/L dialysate calcium concentration.¹⁰ In the current study, we determined the effect of a 2.75-mEq/L dialysate calcium concentration on chronic kidney disease–mineral bone disorder by prospectively examining serial changes in intradialytic calcium and phosphorus elimination and serum mineral and bone turnover markers over 24 weeks after dialysate calcium conversion from either 2.5- or 3.0-mEq/L to 2.75-mEq/L dialysate calcium concentration in patients receiving maintenance hemodialysis.

METHODS

Study Design and Informed Consent

This prospective interventional study was conducted at Japanese Red Cross Fukuoka Hospital and Steel Memorial Yawata Hospital. The study protocol was approved by the Local Ethics Committee of Japanese Red Cross Fukuoka Hospital (2012-181) and Steel Memorial Yawata Hospital (12-01) and was registered at the University Hospital Medical Information Network (R000040105, UMIN000035184). This study was performed in accordance with the Ethics of Clinical Research (Declaration of Helsinki). Written informed consent was obtained from each patient before participating in the study.

Until December 2012, a 2.5-mEq/L dialysate calcium concentration had been used at the Steel Memorial Yawata Hospital Dialysis Center and a 3.0-mEq/L dialysate calcium concentration had been used at the Japanese Red Cross Fukuoka Hospital Dialysis Center for hemodialysis patients. Since December 2012, a 2.75-mEq/L dialysate calcium concentration was used in both facilities. When converting the dialysate calcium of the central dialysate supplier at each hospital from 2.5- to 2.75-mEq/L and from 3.0 to 2.75-mEq/L dialysate calcium concentration, predialysis blood tests and a clinical survey were performed at the start and at 1, 4, and 24 weeks thereafter. To examine intradialytic changes in levels of blood markers, blood and dialysate were collected during dialysis sessions at the beginning of this study and at 24 weeks. Kindaly No. 2, No. 3, and No. 4 solutions (Fuso Pharmaceutical Industry, Ltd) were used for the 3.0-, 2.75-, and 2.5-mEq/L dialysate calcium concentrations. The summary of the study protocol is shown in [Figure S1](#).

Blood Samples

Blood sampling was performed at the start of a midweek dialysis session (Wednesday or Thursday) before dialysate calcium concentration conversion and at 1, 4, and 24 weeks. Blood tests included serum albumin, calcium, phosphate, bone-type alkaline phosphatase, tartrate-resistant

acid phosphatase 5b, fibroblast growth factor 23 (FGF-23), and ionized calcium levels. These parameters, except for serum calcium ion, were measured at Bio Medical Laboratory Inc (Tokyo, Japan). Serum bone-type alkaline phosphatase, tartrate-resistant acid phosphatase 5b, and FGF-23 were measured using commercially available enzyme-linked immunosorbent assay kits. Ionized calcium measurement was performed using i-STAT (Abbott) immediately after blood collection.

Before and at 24 weeks after dialysate calcium conversion, blood was drawn through the dialysate circuit at the start of a hemodialysis session and after 1 hour, 3 hours, and at the end of dialysis (4 or 5 hours depending on the patients' hemodialysis schedule; [Fig S1A](#)). To reduce the influence of interchange between blood and dialysate, blood collection was conducted after stopping dialysate circulation for about 5 minutes.

Dialysate Samples

Samples were collected at the inlet and outlet of the dialysate circuit located just before and after the hemodialyzer, soon after starting hemodialysis, and at 1 hour, 3 hours, and the end of the dialysis procedure ([Fig S2A](#)). The concentration gradient at each time point was calculated as described next using the dialysate concentration of “mineral X” before the dialyzer (“D-in” [mg/dL]) and the dialysate concentration after the dialyzer (“D-out” [mg/dL]; [Fig S2B](#)). When dialysate flow rate was 500 mL/min, dialysate flow rate per hour was 30 L/h (or 300 dL/h). We determined the estimated amount of mineral X removed during each hemodialysis session using the area under the curve ([Fig S2C and D](#)). The estimated removal of mineral X was done by calculating the area under the curve using the following trapezoidal rule: the amount of mineral X removed during a hemodialysis session = $(a + b) \times 300 \times 1/2 + (b + c) \times 300 \times 2/2 + (c + d) \times 300 \times 2/2$.^{10,14} Mineral X included total calcium, calcium ion, and phosphorus. The a, b, c, and d denote the concentration gradient of mineral X in dialysate at the start, 1 hour, 3 hours, and end of the dialysis procedure. Concentration gradient was “D-out – D-in,” and this gradient can be negative, null, or positive depending on the predialysis serum calcium level, dialysate calcium concentration, and other factors, including serum albumin level. Mineral X removal during a hemodialysis session was expressed as a negative number, whereas mineral X gain was expressed as a positive number. In our estimation of the amount of intradialytic mineral X transfer, ultrafiltration volume was not included in our calculation.

Blood Pressure Measurement and Medications

Predialysis systolic and diastolic blood pressures were measured at the time of dialysate calcium concentration conversion, 4 weeks, and 24 weeks. Information for dose of phosphate binders and vitamin D receptor activators was collected during the study period.

Statistical Analysis

Data are presented as mean \pm standard deviation, median and interquartile range, or number and percentage. All statistical analyses were conducted using R, version 3.5.1 (<http://cran.rproject.org>) and JMP Pro 13 (SAS Institute). We used Mann-Whitney *U* test for unpaired nonparametric data and Wilcoxon signed rank test for continuous variables, as appropriate. Dunnett test and Steel test were also used for multiple comparisons. Mixed-effect models were also applied to some of the comparisons, setting patients as random effect and time as fixed effect. $P < 0.05$ was considered statistically significant.

RESULTS

Dialysate Calcium Conversion and Intradialytic Changes in Serum Mineral and Bone Turnover Markers

Baseline clinical backgrounds of the 2 groups before dialysate calcium concentration conversion are shown in Table 1. In the 2.5- to 2.75-mEq/L dialysate calcium concentration group, before dialysate calcium conversion, both serum total calcium and calcium ion levels were stable during a hemodialysis session (Fig 1A and B). However, at 24 weeks after dialysate calcium concentration conversion, both serum total calcium and calcium ion

levels significantly increased at the end of a hemodialysis session: serum total calcium, 9.53 ± 0.56 to 9.88 ± 0.37 mg/dL; serum total calcium ion, 1.28 ± 0.07 to 1.33 ± 0.04 mmol/L (Fig 1A and B). Before dialysate calcium concentration conversion, intradialytic serum whole PTH levels remained stable during a hemodialysis session (Fig 1C). At 24 weeks after dialysate calcium concentration conversion, there was no statistically significant change in PTH levels (Fig 1C).

In the 3.0- to 2.75-mEq/L dialysate calcium concentration group, before dialysate calcium concentration conversion, serum total calcium and calcium ion levels significantly increased during a hemodialysis session: serum total calcium, 9.41 ± 0.34 to 10.23 ± 0.33 mg/dL; serum total calcium ion, 1.25 ± 0.03 to 1.34 ± 0.04 mmol/L (Fig 1D and E). Even after dialysate calcium concentration conversion, although serum total calcium and calcium ion levels significantly increased during a hemodialysis session, the magnitude of an intradialytic increase in serum total calcium and calcium ion levels was significantly less than when treated with 3.0-mEq/L dialysate calcium concentration: serum total calcium, 9.24 ± 0.42 to 9.59 ± 0.29 mg/dL; serum total calcium ion, 1.22 ± 0.06 to 1.28 ± 0.03 mmol/L (Fig 1D and E). At 24 weeks after dialysate calcium concentration conversion, there was no statistically significant change in PTH levels (Fig 1F).

Table 1. Baseline Clinical Characteristics in the 2 Groups Stratified by Dialysate Calcium Concentration Before Conversion

Variables	Dialysate Conversion	
	2.5 DCa \rightarrow 2.75 DCa (2.5 DCa group) (n = 12)	3.0 DCa \rightarrow 2.75 DCa (3.0 DCa group) (n = 12)
Demographic data		
Age, y	69 \pm 7	69 \pm 7
Men	12 (100%)	12 (100%)
Dialysis vintage, mo	129 \pm 87	118 \pm 94
Primary kidney disease (DM)	6 (50%)	6 (50%)
Hemodialysis time per session		
4 h	5 (42%)	0 (0%)
5 h	7 (58%)	12 (100%)
Serum biochemistries		
Albumin, g/dL	3.5 \pm 0.3	3.7 \pm 0.3
Corrected calcium, mg/dL	9.5 \pm 0.5	9.3 \pm 0.5
Phosphorus, mg/dL	4.0 \pm 0.7	3.9 \pm 0.8
Whole PTH, pg/mL	117 \pm 96	70 \pm 45
Bone-type alkaline phosphatase, μ g/L	20.8 \pm 12.6	11.6 \pm 4.0
Tartrate -resistant acid phosphatase 5b, mU/dL	655 \pm 467	340 \pm 191
FGF-23, pg/mL	4,210 \pm 8,059	3,514 \pm 5,335
Medication		
Calcium carbonate, mg/d	1,250 \pm 1,031	1,909 \pm 1,663
Sevelamer hydrochloride, mg/d	1,313 \pm 1,987	205 \pm 462
Lanthanum carbonate, mg/d	313 \pm 480	432 \pm 512
Alfacalcidol (oral), μ g/d	0.15 \pm 0.1	0.09 \pm 0.12
Maxacalcitol (intravenous), μ g/wk	8.8 \pm 6.8	4.1 \pm 6.7
Cinacalcet hydrochloride, mg/d	18.8 \pm 27.2	4.5 \pm 14.4

Note: Data are shown as mean \pm standard deviation or number (percentage).

Abbreviations: DCa, dialysate calcium concentration; DM, diabetes mellitus; FGF-23, fibroblast growth factor 23; PTH, parathyroid hormone.

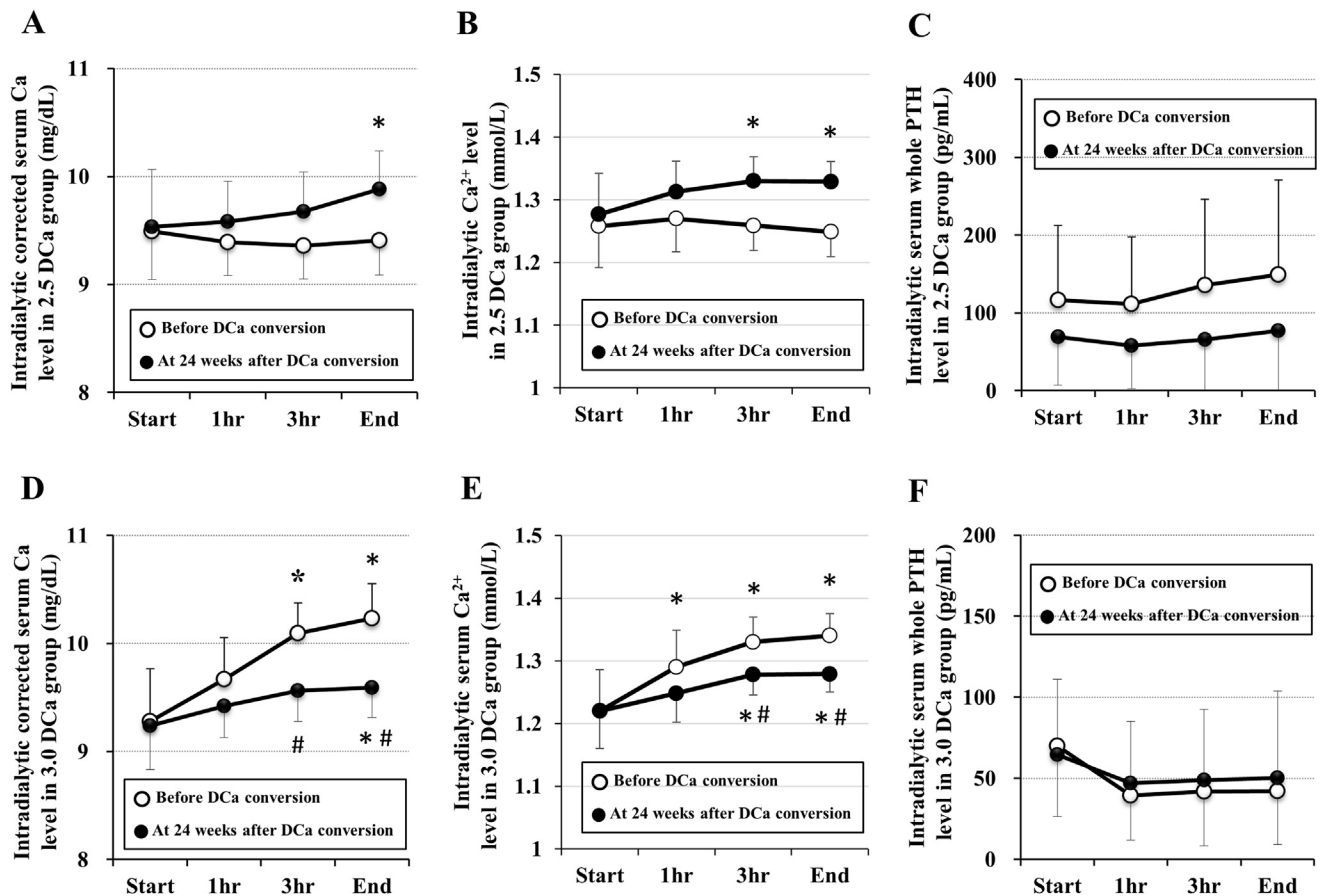


Figure 1. Changes in serum mineral markers during a single hemodialysis session before dialysate calcium (DCa) concentration conversion (open circle: ○) and at 24 weeks after DCa conversion (closed circle: ●) in the 2.5 DCa→2.75 DCa and 3.0 DCa→2.75 DCa groups. Serum levels of (A) corrected calcium, (B) calcium ion (Ca²⁺), and (C) whole parathyroid hormone (PTH) in the DCa 2.5-mEq/L group. Serum levels of (D) calcium, (E) Ca²⁺, and (F) whole PTH in the DCa 3.0-mEq/L group. Serum PTH values were determined using whole PTH assays, which exclusively detects the 1-84 PTH fragment and not the 7-84 PTH fragment. Data are presented as mean ± standard deviation. **P* < 0.05 versus “values at the start.” # *P* < 0.05 versus “before DCa conversion at the same blood sampling timing.”

Conversion of Dialysate Calcium and Predialysis Serum Calcium and Bone Turnover Marker Levels

During the 24-week observation period, serum calcium, whole PTH, bone-type alkaline phosphatase, and tartrate-resistant acid phosphatase 5b levels did not change significantly in either the 2.5- to 2.75-mEq/L or 3.0- to 2.75-mEq/L dialysate calcium concentration groups, although serum whole PTH and bone-type alkaline phosphatase levels appeared to decrease at the end of the observation period in the 2.5- to 2.75-mEq/L dialysate calcium concentration group (Fig 2A to D).

Conversion of Dialysate Calcium and Intradialytic Total Calcium Loading

In the 2.5- to 2.75-mEq/L dialysate calcium group, before dialysate calcium concentration conversion, median total calcium influx was 60 (interquartile range [IQR]. -45, 195) mg per hemodialysis session. After dialysate calcium concentration conversion, mean total calcium influx value

gradually increased, reaching 0 (IQR, -255, 210) mg per hemodialysis session after 24 weeks (Fig 3A). In the 3.0- to 2.75-mEq/L dialysate calcium concentration group, before dialysate calcium concentration conversion, the median of the intradialytic total calcium removal was -210 (IQR, -375, 0) mg per hemodialysis session. However, after dialysate calcium concentration conversion, the median of the intradialytic total calcium removal value gradually decreased, reaching -45 (IQR, -345, 225) mg per hemodialysis session at 24 weeks (Fig 3A).

When a mixed-effect model was applied, a significant change was observed in the intradialytic calcium transfer only at week 4 compared with that before dialysate calcium concentration conversion (2.5- to 2.75-mEq/L dialysate calcium concentration group). By contrast, in the 3.0- to 2.75-mEq/L dialysate calcium concentration group, there were significant changes in the intradialytic total calcium transfer at 1, 4, and 24 weeks compared with before conversion.

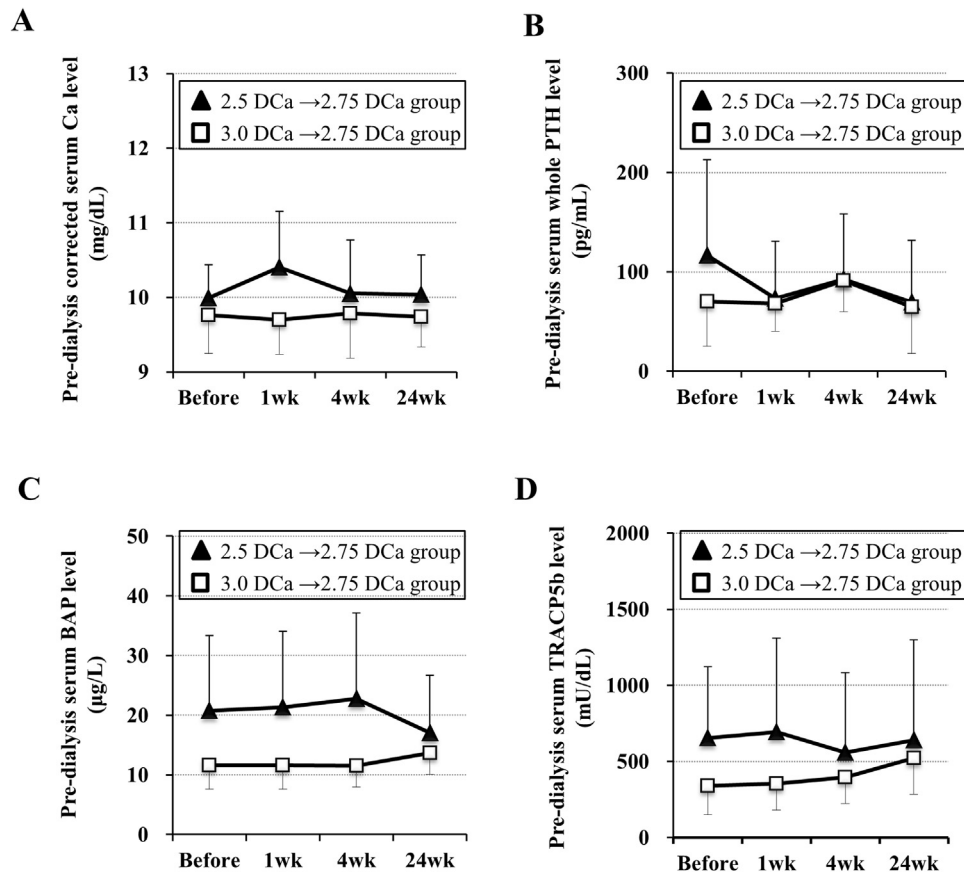


Figure 2. Changes in serum mineral and bone turnover markers before and after dialysate calcium (DCa) concentration conversion in the 2.5 DCa → 2.75 DCa group (closed triangle: ▲) and 3.0 DCa → 2.75 DCa group (open square: □). Serum levels of (A) corrected calcium (Ca), (B) whole parathyroid hormone (PTH), (C) bone-specific alkaline phosphatase (BAP), and (D) tartrate-resistant acid phosphatase-5b (TRACP-5b). Data are presented as mean ± standard deviation.

When intradialytic influx of total calcium was compared among the 3 dialysate calcium concentrations, the median of the intradialytic inflow of total calcium was negative for 2.5 mEq/L of dialysate calcium (−60 [IQR, −195, 45] mg/session), almost zero for 2.75 mEq/L of dialysate calcium (15 [IQR, −225, 340] mg/session), and positive for 3.0 mEq/L of dialysate calcium (210 [IQR, 0, 375] mg/session; Fig 3B). In addition, intradialytic total calcium influx was negatively correlated with predialysis serum calcium level in the 2.75- and 3.0-mEq/L dialysate calcium concentration groups ($R^2 = 0.303$; $P < 0.05$ and $R^2 = 0.772$; $P < 0.05$, respectively), but not in the 2.5-mEq/L dialysate calcium concentration group ($R^2 = 0.140$; $P = 0.23$; Fig 3C-E).

When the same analyses were conducted using calcium ion, results obtained using a mixed-effect model showed that intradialytic calcium ion influx significantly increased at all time points in the 2.5- to 2.75-mEq/L dialysate calcium concentration group, whereas intradialytic calcium ion influx decreased at all timepoints in the 3.0- to 2.75-mEq/L dialysate calcium concentration group (Fig S3A). When the amount of intradialytic calcium ion influx was compared across dialysate calcium

concentrations, 2.5 mEq/L of dialysate calcium was significantly lower, whereas 3.0 mEq/L of dialysate calcium was significantly higher than 2.75 mEq/L of dialysate calcium (Fig S3B). Intradialytic calcium ion influx was negatively correlated with predialysis serum calcium ion level in all dialysate calcium concentration groups (Fig S3C-E).

Conversion of Dialysate Calcium, Intradialytic Phosphate Elimination, and Predialysis Serum Phosphate Levels

In the 2.5- to 2.75-mEq/L dialysate calcium concentration group, serum phosphate levels significantly decreased during a hemodialysis session before dialysate calcium concentration conversion and at 24 weeks after dialysate calcium concentration conversion: before dialysate calcium conversion, 4.0 ± 0.7 mg/dL (at start) versus 2.1 ± 0.3 mg/dL (at end), $P < 0.05$; 24 weeks after dialysate calcium conversion, 3.7 ± 0.7 (at start) versus 2.0 ± 0.3 mg/dL (at end), $P < 0.05$ (Fig 4A).

In the 3.0- to 2.75-mEq/L dialysate calcium concentration group, serum phosphate levels significantly decreased during a hemodialysis session before dialysate

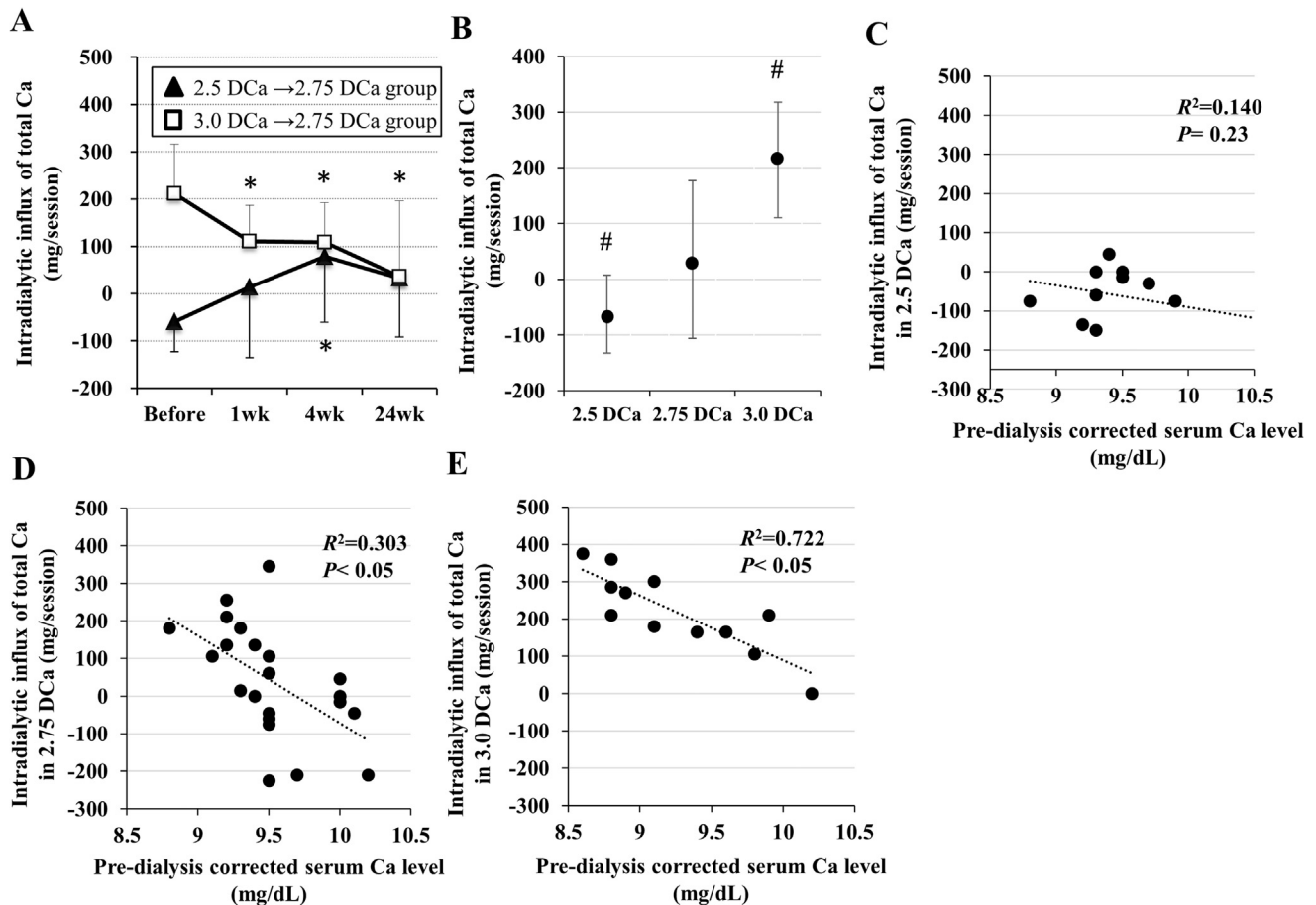


Figure 3. Changes in intradialytic total calcium influx in 2 hemodialysis sessions in the 2.5 dialysis calcium (DCa) → 2.75 DCa group (closed triangle: ▲) and 3.0 DCa → 2.75 DCa group (open square: □). (A) Intradialytic total calcium influx in 1 hemodialysis session. (B) The impact of DCa type on intradialytic total calcium influx in 1 hemodialysis session. Correlation between predialysis corrected serum calcium level and intradialytic total calcium influx in the (C) 2.5-mEq/L DCa (n = 12), (D) 2.75-mEq/L DCa (n = 24), and (E) 3.0-mEq/L DCa (n = 12) groups. Data are presented as mean ± standard deviation. Pearson correlation coefficients were used for the analyses. Dunnett test was used for multiple comparisons. $P < 0.05$ was considered statistically significant. * $P < 0.05$ versus “before DCa conversion”; # $P < 0.05$ versus “2.75 DCa.”

calcium concentration conversion and at 24 weeks after dialysate calcium concentration conversion: before dialysate calcium concentration conversion; 4.2 ± 1.1 (at start) versus 2.0 ± 0.5 mg/dL (at end), $P < 0.05$; 24 weeks after dialysate calcium conversion, 4.1 ± 0.8 (at start) versus 1.9 ± 0.4 mg/dL (at end), $P < 0.05$ (Fig 4B). Predialysis serum phosphate level did not change during the 24-week observation period except for at 4 weeks in the 2.5- to 2.75-mEq/L dialysate calcium concentration group: 4.0 ± 0.7 (before) versus 3.3 ± 0.7 mg/dL (at 4 week), $P < 0.05$ (Fig 4C).

Next, we calculated the amount of intradialytic phosphate elimination. Total intradialytic phosphate elimination did not change over 24 weeks in the 3.0- to 2.75-mEq/L dialysate calcium concentration group (Fig 4D). In the 2.5- to 2.75-mEq/L dialysate calcium concentration group, intradialytic phosphate elimination appeared to gradually decrease, and the change was statistically significant at 4 weeks: 840 (IQR, 575, 1,170) mg/session (before

dialysate calcium conversion) versus 683 (IQR, 510, 855) mg/session (at 4 weeks), $P < 0.05$ (Fig 4D). When a mixed-effect model was applied by setting patients as random effects and time as a fixed effect, there was a significant change in intradialytic phosphate elimination at weeks 4 and 24 compared with before conversion (2.5- to 2.75-mEq/L dialysate calcium concentration group). By contrast, in the 3.0- to 2.75-mEq/L dialysate calcium concentration group, there were no significant changes in the intradialytic total phosphate elimination at any timepoint compared with that measured before dialysate calcium concentration conversion (Fig 4D).

Notably, these trends for intradialytic phosphate elimination during the 24-week observation period were consistent with trends in the predialysis serum phosphate level. Furthermore, the amount of intradialytic phosphate elimination was closely correlated with predialysis serum phosphate level ($R^2 = 0.561$; $P < 0.01$; Fig 4E). There was no statistically significant difference when mean

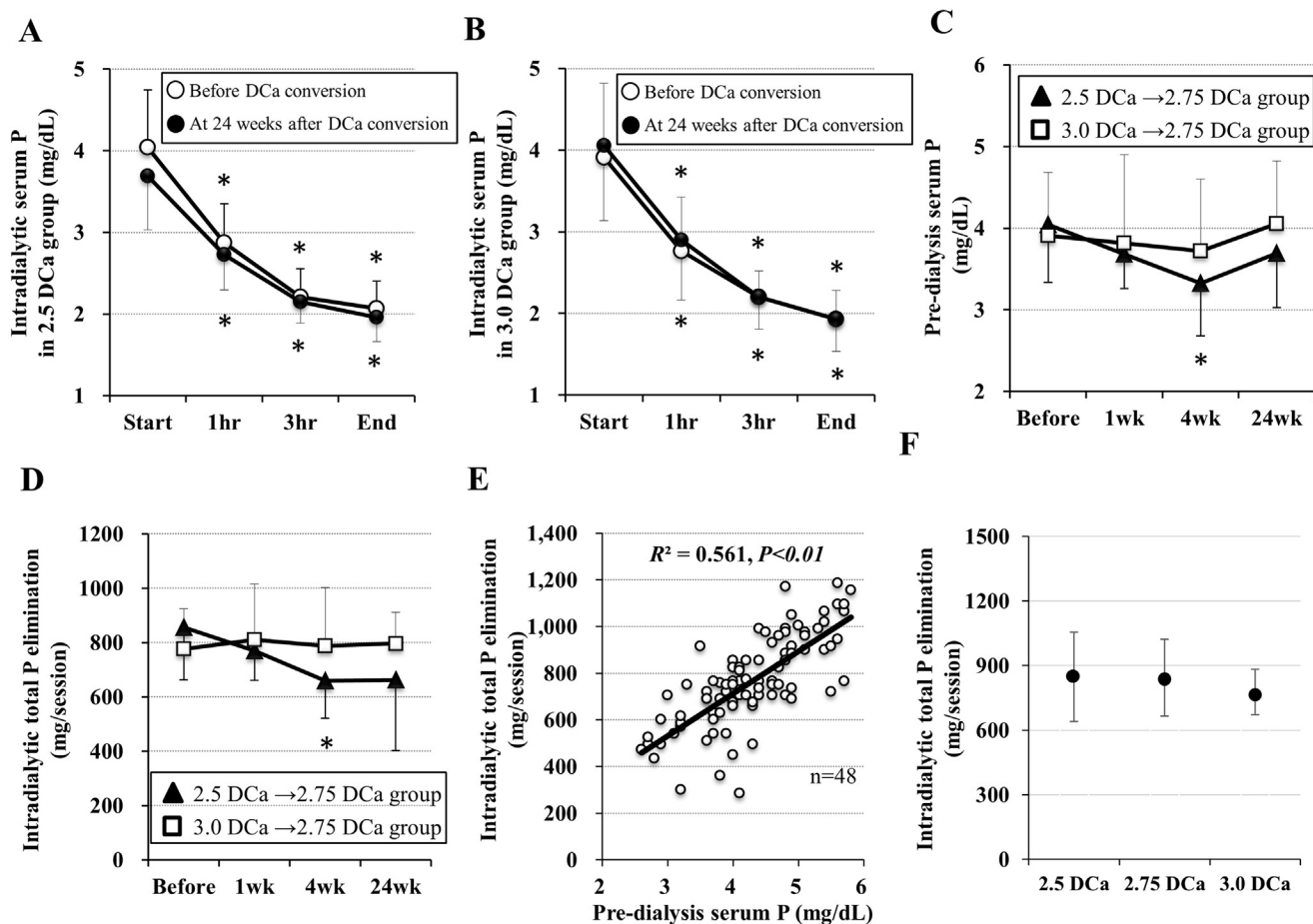


Figure 4. Changes in serum phosphorus (P) level and total phosphorus elimination during a hemodialysis session before and after dialysate calcium (DCa) concentration conversion. Intradialytic serum phosphorus level in the (A) 2.5 DCa → 2.75 DCa group and (B) 3.0 DCa → 2.75 DCa group before DCa concentration conversion (open circle: ○) and at 24 weeks after DCa concentration conversion (closed circle: ●). (C) Predialysis serum phosphorus level during the observation period in the 2.5-mEq/L DCa group (closed triangle: ▲) and 3.0 DCa group (open square: □). (D) Intradialytic total phosphorus elimination in the 2.5-mEq/L DCa group (closed triangle: ▲) and 3.0-mEq/L DCa group (open square: □) during the observation period. (E) Correlation between serum phosphorus level and intradialytic total phosphorus elimination in the 2.5-mEq/L DCa group (closed triangle: ▲) and 3.0-mEq/L DCa group (open square: □). (F) Comparison of intradialytic total phosphorus elimination among 3 different DCAs. Data are presented as mean ± standard deviation. Pearson correlation coefficient was used for correlation analysis. $P < 0.05$ was considered to be statistically significant. * $P < 0.05$ versus “at start.”

intradialytic phosphate elimination value was compared across 3 types of dialysate calcium concentrations (Fig 4F).

Dialysate Calcium Conversion and Serum FGF-23 Levels

When intradialytic serum FGF-23 level at baseline was monitored during a hemodialysis session, no significant changes were observed in the 2.5- to 2.75-mEq/L and 3.0- to 2.75-mEq/L dialysate calcium concentration groups (Fig 5A). There was no significant difference in predialysis serum FGF-23 levels during the 24-week observation period in the 2.5- to 2.75-mEq/L and 3.0- to 2.75-mEq/L dialysate calcium concentration groups (Fig 5B).

To identify the determinants of predialysis serum FGF-23 level, correlations between log serum FGF-23 and corrected serum calcium, serum phosphate, and corrected

serum calcium-phosphate product levels were assessed. The log serum FGF-23 level was significantly ($P < 0.05$) and positively correlated with predialysis serum phosphate ($R^2 = 0.405$; $P < 0.05$) and predialysis corrected serum calcium-phosphate product levels ($R^2 = 0.425$; $P < 0.05$; Fig 5C and D), but not with predialysis serum calcium level.

Dialysate Calcium Conversion and Chronic Kidney Disease–Mineral and Bone–Related Medications

We also investigated whether there were changes in doses of phosphate binders, vitamin D receptor activators, and cinacalcet hydrochloride after dialysate calcium concentration conversion. Although the dose of some of the drugs was decreased in response to dialysate calcium concentration conversion, no significant changes were observed in doses of drugs in the 2.5- to 2.75-mEq/L and 3.0- to

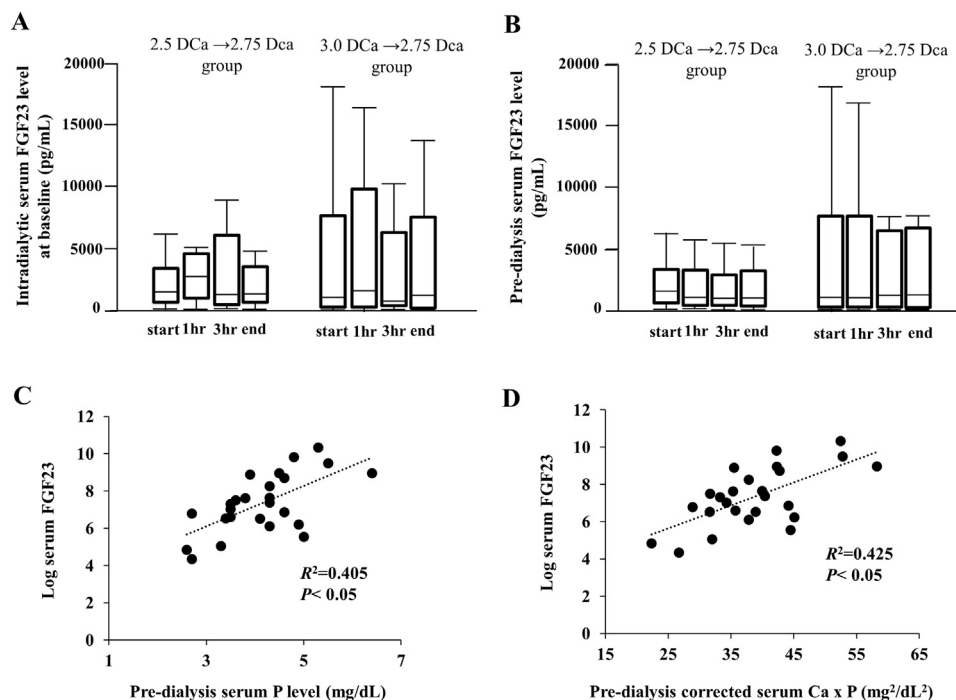


Figure 5. Serial changes in intradialytic serum fibroblast growth factor 23 (FGF-23) levels and predialysis serum FGF-23 levels. (A) Changes in the intradialytic serum FGF-23 level in the 2.5 dialysate calcium concentration (DCa) → 2.75 DCa group (closed triangle: ▲) and 3.0 DCa → 2.75 DCa group (open square: □) before DCa conversion. (B) Changes in predialysis serum FGF-23 level in the 2.5 DCa → 2.75 DCa group (closed triangle: ▲) and 3.0 DCa → 2.75 DCa group (open square: □) before DCa conversion at 24 weeks after DCa conversion. (C) Correlation between predialysis serum phosphorus (P) level and log serum FGF-23 level ($n = 24$). (D) Correlation between corrected serum calcium level or serum phosphorus level and log serum FGF-23 level ($n = 24$). Data are presented as mean \pm standard deviation. Pearson correlation coefficients were used for the correlation analyses. $P < 0.05$ was considered statistically significant.

2.75-mEq/L dialysate calcium concentration groups (Fig S4; Table S1).

Dialysate Calcium Conversion and Predialysis Blood Pressure Levels

No significant changes were observed in predialysis systolic and diastolic blood pressure levels in the 2.5- to 2.75-mEq/L and 3.0- to 2.75-mEq/L dialysate calcium concentration groups (Fig S5).

DISCUSSION

In the present study, during 6 months of study period, intradialytic calcium loading increased as dialysate calcium concentration increased. On average, intradialytic calcium had a negative balance when using 2.5-mEq/L dialysate calcium concentration, a near-even balance in 2.75-mEq/L dialysate calcium concentration, and positive balance in 3.0-mEq/L dialysate calcium concentration. Intradialytic calcium transfer was affected by predialysis serum calcium level. The amount of intradialytic phosphate elimination was comparable among 3 different dialysate calcium concentrations. Dialysate calcium concentration conversion did not induce statistically significant changes in

intradialytic serum PTH level, intradialytic phosphate elimination, and predialysis serum calcium, phosphate, PTH, and FGF-23 levels.

Because the intradialytic total calcium flux in patients treated with 2.75 mEq/L of dialysate calcium was almost zero, this dialysate concentration may be useful for managing hemodialysis patients who are treated with the central dialysate supply system. However, as shown in Figure 3D, when we examined intradialytic calcium balance in patients treated with 2.75 mEq/L of dialysate calcium, a predialysis serum total calcium level of 9.5 mg/dL was the critical threshold that determined intradialytic calcium balance.

These results suggest that even when dialysate calcium concentration of 2.75 mEq/L is used, patients with a predialysis serum total calcium level < 9.5 mg/dL may be at increased risk for an intradialytic negative calcium balance and bone loss, and conversely, patients with a predialysis serum total calcium level > 9.5 mg/dL may be placed at increased risk for an intradialytic positive calcium balance and vascular calcification. Thus, when choosing 2.75 mEq/L of dialysate calcium, a predialysis serum total level should be used as an indicator for each patient's intradialytic calcium balance, and the dose and type of

phosphate binders, vitamin D receptor activators, and calcimimetics should be optimized to minimize the risk for bone loss and vascular calcification.

Generally, conversion from a lower to a higher dialysate calcium concentration suppressed the intradialytic serum PTH level increase, which occasionally led to reduced bone turnover.¹⁵ These changes are likely achieved because of a positive intradialytic calcium influx and an increase in serum calcium levels. Thus, selecting a higher dialysate calcium concentration usually provides the benefit of better secondary hyperparathyroidism control, but places hemodialysis patients at increased risk for low bone turnover and adynamic bone, followed by heightened risk for vascular calcification. Although predialysis serum whole PTH and bone turnover marker levels seemed to decrease during the 24 weeks when dialysate calcium concentration was increased from 2.5 to 2.75 mEq/L, those changes were not statistically significant. This might be caused by the relatively small sample size.

In patients initially treated with 3.0 mEq/L of dialysate calcium, intradialytic total calcium influx into the body significantly decreased after dialysate calcium concentration conversion to 2.75 mEq/L of dialysate calcium. However, there was no statistically significant change in intradialytic serum PTH level. Predialysis serum PTH levels remained suppressed during the 24-week treatment even after dialysate calcium conversion. Additionally, there was no significant change in corrected serum calcium level after dialysate calcium conversion in the 3.0-mEq/L dialysate calcium group, potentially reflecting the relatively small sample size of our study.

As we and others have reported previously, conversion from 3.0 to 2.75 mEq/L of dialysate calcium increased the predialysis serum PTH level and required an increased dose of vitamin D receptor activators.^{16,17} Thus, it is important to be aware of the potential aggravation of secondary hyperparathyroidism when dialysate calcium concentration is lowered, although other interventions, including vitamin D receptor activators and calcimimetics, may be available to manage this.¹⁸

It is an ongoing subject of debate whether intradialytic calcium transfer should be positive, negative, or even.^{4,5,19} Although the mean or median value of intradialytic total calcium transfer was near zero in patients treated with 2.75 mEq/L of dialysate calcium in our study, the amount of intradialytic total calcium transfer was positive or negative depending on the predialysis serum calcium level. Because predialysis serum calcium level is influenced by the status of parathyroid and bone and comedications, although mean intradialytic total calcium transfer was near zero, our data suggest that 2.75 mEq/L of dialysate calcium could provide either a positive or negative balance and the optimal dialysate calcium concentration should be personalized depending on patients' medical conditions.

Accumulating evidence suggests that FGF-23 causes various adverse outcomes, including cardiac hypertrophy, and FGF-23 level elevation increases the risk for death in

hemodialysis patients.²⁰⁻²⁶ Calcium metabolism is an important factor among the variety of potential factors that regulate FGF-23 synthesis and secretion.²⁷ However, serum FGF-23 levels did not change after dialysate calcium concentration conversion. Log serum FGF-23 level was not correlated with serum calcium levels, but it was associated with predialysis serum phosphate level and intradialytic phosphate elimination. These results suggest that neither the intradialytic calcium change nor the dialysate calcium concentration affect serum FGF-23 level, and the predialytic serum phosphate level mainly determines intradialytic phosphate elimination, as shown in Figure 4.

The major limitation in the current study is small sample size. The reason for the relatively small sample size was that the current study design required frequent collection of dialysate and blood samples, which were time-consuming and required extra cost and human resources. Additionally, conducting this research at 1 facility for the purpose of matching the background for treatment would have been ideal. However, because most dialysis facilities in Japan use a central dialysate supply system, it is particularly challenging to provide different dialysate calcium concentrations to different patients. Thus, when we designed the current study, we selected 2 dialysis facilities from the dialysis facilities that intended to change dialysate calcium concentrations. Another potential limitation is that the current study did not have control groups because patients who remained treated with 2.5 and 3.0 mEq/L of dialysate calcium for 24 weeks were not included. Thus, we cannot truly confirm that the changes observed in the present study were induced by dialysate calcium concentration conversion.

Third, it is plausible to think that the differences in baseline backgrounds between the 2 dialysate calcium concentration groups may have influenced the results, especially when we compared the 2 different dialysate calcium concentration groups. However, our main purpose was to examine serial changes within groups. Hence, our observation should be cautiously interpreted when we focus on the differences between groups.

Fourth, in the current study, we did not take ultrafiltration volume into account when we calculated the amount of intradialytic calcium transfer. In this regard, we inaccurately estimated the amount of intradialytic calcium transfer. However, as we described, the contribution of intradialytic ultrafiltration volume was as high as 5%, and the estimation of intradialytic calcium transfer in the current study would not have been greatly affected.

Fifth, in the current study, we had no data for the impact of dialysate calcium concentration conversion on vascular function, including endothelial function.

Sixth, dialysate calcium concentration conversion from a lower to a higher level enables the use of a lower dose of vitamin D receptor activators and calcium-based phosphate binders to maintain calcium balance. However, not all patients in the 3.0- to 2.75-mEq/L dialysate calcium concentration group had a decreased dose of calcium-

based phosphate binders. This was because our study protocol did not force physicians to lower the doses of calcium-based phosphate binders and vitamin D receptor activators in response to dialysate calcium concentration conversion. In this regard, our data should be interpreted as such and show some aspect of the real-world practice after dialysate calcium concentration conversion.

In conclusion, intradialytic calcium transfer after dialysate calcium concentration conversion to 2.75-mEq/L was almost zero. From the viewpoint of prevention of vascular calcification, conversion of dialysate calcium concentration from 3.0 to 2.75 mEq/L may be a better therapeutic option to manage hemodialysis patients receiving a 3.0-mEq/L dialysate calcium concentration, whereas for patients treated with 2.5 dialysate calcium, conversion to 2.75-mEq/L dialysate calcium concentration may not be beneficial. However, we cannot conclude that a 2.75-mEq/L dialysate calcium concentration is the optimal dialysate calcium concentration for hemodialysis patients. This is because the impact of dialysate calcium concentration is diverse and intradialytic calcium transfer is not the sole purpose of dialysate calcium concentration selection. Furthermore, intradialytic calcium transfer is affected by a variety of factors and calcium mass balance in hemodialysis patients is very complex. In this regard, any dialysate calcium concentration can be wisely used as long as medications and medical conditions are coordinated. However, the optimal dialysate calcium concentration should be personalized depending on the patients' clinical background, including comedications, serum levels of bone and mineral metabolism markers, parathyroid function, bone turnover, and history of cardiovascular diseases. Further studies are necessary to determine the multifaceted impact of 2.75 mEq/L of dialysate calcium on bone and mineral metabolism in hemodialysis patients.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1: Study protocol.

Figure S2: Location and timing of collection of fresh dialysate and waste dialysate.

Figure S3: Changes in intradialytic Ca^{2+} influx in one hemodialysis session in the 2.5 DCa \rightarrow 2.75 DCa group and the 2.5 DCa \rightarrow 2.75 DCa group.

Figure S4: Changes in medication before and after DCa conversion.

Figure S5: Serial changes in predialysis SBP and DBP levels after DCa conversion.

Table S1: The number of patients who showed an increase, no change, or decrease in the dose of each drug at 24 weeks after DCa conversion.

ARTICLE INFORMATION

Authors' Full Names and Academic Degrees: Tepei Sakoh, MD, Masatomo Taniguchi, MD, PhD, Shunsuke Yamada, MD, PhD, Shotaro Ohnaka, MD, Hokuto Arase, MD, Masanori Tokumoto, MD, PhD, Taihei Yanagida, MD, PhD, Koji Mitsuiki, MD, PhD,

Hideki Hirakata, MD, PhD, Toshiaki Nakano, MD, PhD, Takanao Kitazono, MD, PhD, and Kazuhiko Tsuruya, MD, PhD.

Authors' Affiliations: Division of Nephrology, Japanese Red Cross Fukuoka Hospital (TS, KM, HH); Fukuoka Renal Clinic (MTaniguchi, HH); Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University (SY, HA, TN, TK, KT); Division of Nephrology, Steel Memorial Yawata Hospital (SO, TY); Department of Internal Medicine, Fukuoka Dental College (MTokumoto); Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, Fukuoka (KT); and Department of Nephrology, Nara Medical University, Nara, Japan (KT).

Address for Correspondence: Masatomo Taniguchi, MD, PhD, Fukuoka Renal Clinic, 4-6-20 Watanabe-dori, Chuo-ku, Fukuoka 810-0004, Japan. E-mail: macha1214@gmail.com

Authors' Contributions: Research idea and study design: TS, MTaniguchi, SO; data acquisition: TS, MTaniguchi, TY, KM; data analysis interpretation: TS, MTaniguchi, SY, SO, HA, MTokumoto; statistical analysis: TS, MTaniguchi, SY, SO; supervision or mentorship: HH, TN, TK, KT. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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REFERENCES

- Hou SH, Zhao J, Ellman CF, et al. Calcium and phosphorus fluxes during hemodialysis with low calcium dialysate. *Am J Kidney Dis.* 1991;18:217-224.
- National Kidney Foundation. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis.* 2003;42(4)(suppl 3):S1-S201.
- KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int Suppl.* 2009;113: S1-S130.
- Gotch FA. Pro/con debate: the calculation on calcium balance in dialysis lowers the dialysate calcium concentrations (pro part). *Nephrol Dial Transplant.* 2009;24:2994-2996.
- Drüeke TB, Touam M. Calcium balance in haemodialysis—do not lower the dialysate calcium concentration too much (con part). *Nephrol Dial Transplant.* 2009;24:2990-2993.
- Johnson WJ. Optimum dialysate calcium concentration during maintenance hemodialysis. *Nephron.* 1976;17:241-258.
- Bielez BO, Hecking M, Plischke M, et al. Correlations and time course of FGF23 and markers of bone metabolism in maintenance hemodialysis patients. *Clin Biochem.* 2014;47:1316-1319.
- Näppi SE, Virtanen VK, Saha HH, et al. QTc dispersion increases during hemodialysis with low-calcium dialysate. *Kidney Int.* 2002;57:2117-2122.

9. Sherman RA, Bialy GB, Gazinski B, et al. The effect of dialysate calcium levels on blood pressure during hemodialysis. *Am J Kidney Dis.* 1986;8:244-247.
10. Basile C, Libutti P, Di Turo AL, et al. Effect of dialysate calcium concentrations on parathyroid hormone and calcium balance during a single dialysis session using bicarbonate hemodialysis: a crossover clinical trial. *Am J Kidney Dis.* 2012;59:92-101.
11. Karohl C, de Paiva Paschoal J, de Castro MC, et al. Effects of bone remodelling on calcium mass transfer during haemodialysis. *Nephrol Dial Transplant.* 2010;25:1244-1251.
12. Yamada K, Fujimoto S, Nishiura R, et al. Risk factors of the progression of abdominal aortic calcification in patients on chronic haemodialysis. *Nephrol Dial Transplant.* 2007;22:2032-2037.
13. LeBeouf A, Mac-Way F, Utescu MS, et al. Effects of acute variation of dialysate calcium concentrations on arterial stiffness and aortic pressure waveform. *Nephrol Dial Transplant.* 2009;24:3788-3794.
14. Yeh KC, Kwan KC. A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximation. *J Pharmacokinet Biopharm.* 1978;6:79-98.
15. Jean G, Mayor B, Hurot JM, et al. Biological impact of targeted dialysate calcium changes in haemodialysis patients: the key role of parathyroid hormone. *Nephrol Dial Transplant.* 2013;28:176-182.
16. Sakai Y, Otsuka T, Ohno D, et al. Clinical benefit of the change of dialysate calcium concentration from 3.0 to 2.75 mEq/L. *Ther Apher Dial.* 2014;18:181-184.
17. Yamada S, Ueki K, Tokumoto M, et al. Effects of lowering dialysate calcium concentration on mineral and bone disorders in chronic hemodialysis patients: conversion from 3.0 mEq/L to 2.75 mEq/L. *Ther Apher Dial.* 2016;20:31-39.
18. Nakai K, Komaba H, Fukagawa M. Management of mineral and bone disorder in chronic kidney disease: quo vadis? *Ther Apher Dial.* 2009;13(suppl 1):S2-S6.
19. van der Sande FM, Ter Meulen KJA, Kotanko P, Kooman JP. Dialysate calcium levels: do they matter? *Blood Purif.* 2019;47:230-235.
20. Gutiérrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med.* 2008;359:584-592.
21. Isakova T, Xie H, Yang W, et al; Chronic Renal Insufficiency Cohort (CRIC) Study Group. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA.* 2011;305:2432-2439.
22. Kendrick J, Cheung AK, Kaufman JS, et al. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. *J Am Soc Nephrol.* 2011;22:1913-1922.
23. Ix JH, Katz R, Kestenbaum BR, et al. Fibroblast growth factor-23 and death, heart failure, and cardiovascular events in community-living individuals: CHS (Cardiovascular Health Study). *J Am Coll Cardiol.* 2012;60:200-207.
24. Faul C, Amaral AP, Oskouei B, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest.* 2011;121:4393-4408.
25. Koizumi M, Komaba H, Nakanishi S, Fujimori A, Fukagawa M. Cinacalcet treatment and serum FGF23 levels in haemodialysis patients with secondary hyperparathyroidism. *Nephrol Dial Transplant.* 2012;27:784-790.
26. Kuro-o M. Klotho, phosphate and FGF-23 in ageing and disturbed mineral metabolism. *Nat Rev Nephrol.* 2013;(9):650-660.
27. Shikida Y, Mizobuchi M, Inoue T, et al. Effect of continuous intravenous calcium loading on fibroblast growth factor 23 in normal and uremic rats. *Calcif Tissue Int.* 2018;103:455-464.