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# Effects of on-line hemodiafiltration regimens and dialysate composition on serum concentrations of magnesium and calcium ions

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## Abstract

**Background:** Low-ionized magnesium and high-ionized calcium levels are associated with increased cardiovascular mortality in patients undergoing dialysis. We examined the effects of the dilution method, substitution volume, and dialysate of on-line hemodiafiltration on the total and ionized magnesium and calcium levels.

**Methods:** Eighteen patients were randomly assigned to three dialysate groups: two acetic acid dialysate groups and one citrate dialysate group. Five treatment conditions were applied: pre-diluted on-line hemodiafiltration, post-diluted on-line hemodiafiltration, and hemodialysis.

**Results:** The total and ionized serum levels of magnesium and calcium were evaluated and found to be unaffected by the dilution methods and substitution volumes. The albumin leakage was approximately 3 g/session under the pre-dilution and hemodiafiltration conditions, and approximately 4–5 g/session under the post-dilution condition. The ionized magnesium concentration decreased in the citrate dialysate group.

**Conclusion:** The on-line hemodiafiltration parameters had a negligible effect on ionized magnesium and calcium; however, the use of citrate dialysate decreased the ionized magnesium levels, probably because of chelation.

**Trial registration:** 000028172. The study was registered on July 11 2017.

**Keywords:** Citrate dialysate, Ionized calcium, Ionized magnesium, On-line hemodiafiltration, Standard dialysate

## Background

Excretion of magnesium tends to decline when renal function deteriorates, resulting in an elevated risk of hypermagnesemia [1]. Hemodialysis (HD) treatment typically results in reduced post-dialysis serum magnesium concentration because of the low magnesium concentration in the dialysate (0.5 mmol/L) [2]. Recent reports have suggested that magnesium in serum prevents

vascular calcification, thus reducing the risk of death from cardiovascular diseases [3, 4]. These findings indicate that it may be important to maintain pre-dialysis serum magnesium concentrations at 2.7–3.0 mg/dL in patients undergoing HD [3, 4]. These studies have led researchers to reconsider the magnesium concentration during dialysis.

Other research studies have suggested that elevated serum calcium levels are correlated with increased risk of cardiovascular mortality [5, 6]. Therefore, calcium and magnesium may be important factors affecting prognosis. In Japan, the following four dialysate calcium

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**Table 1** Demographic characteristics of subjects

Parameter	StDi-1 (n = 6)	StDi-2 (n = 6)	CiDi (n = 6)	P
Age (years)	68.8 ± 11.7	73.9 ± 8.2	62.8 ± 8.0	0.154
Female, N (%)	2 (33.3)	1 (16.7)	2 (33.3)	0.676
HD dur (years)	19.2 ± 12.3	14.5 ± 9.1	19.5 ± 4.8	0.579
DW (Kg)	55.9 ± 15.3	50.8 ± 8.1	56.1 ± 8.8	0.644
BMI (kg/m <sup>2</sup> )	21.8 ± 2.6	22.1 ± 3.2	22.4 ± 2.9	0.418
Ultrafiltration (L)	1.2 ± 0.3	1.2 ± 0.2	1.1 ± 0.3	0.734
WBC count (10 <sup>3</sup> /μL)	5.4 ± 1.1	5.3 ± 1.3	5.8 ± 1.2	0.761
RBC count (10 <sup>6</sup> /μL)	3.5 ± 0.3	3.4 ± 0.3	3.5 ± 0.2	0.667
Hemoglobin (g/dL)	12.1 ± 0.9	11.0 ± 1.2	10.8 ± 1.9	0.381
PLT count (10 <sup>3</sup> /μL)	178 ± 48	185 ± 14	197 ± 21	0.591
Albumin (g/dL)	3.4 ± 0.2	3.5 ± 0.2	3.3 ± 0.4	0.296
Total calcium	9.1 ± 0.7	9.1 ± 0.5	9.3 ± 0.7	0.599
Total magnesium	2.5 ± 0.2	2.3 ± 0.3	2.3 ± 0.3	0.751

StDi standard dialysate, CiDi citrate dialysate, HD dur hemodialysis duration, DW dry weight, WBC white blood cell, RBC red blood cell, PLT platelet

concentrations are used more commonly: 1.25, 1.375, 1.5, and 1.75 mmol/L. It is assumed that the serum ionized calcium concentration eventually converges to the dialysate calcium concentration. Monitoring the post-dialysis calcium concentrations may help clinicians choose and adjust the dialysate conditions to provide optimal outcomes.

Although many reports on the magnesium and calcium levels in patients undergoing HD have been published, only few have assessed the magnesium and calcium levels in on-line hemodiafiltration (OL-HDF). To the best of our knowledge, this is the first report of evaluation of magnesium and calcium levels using

dialysate with a calcium concentration of 1.375 mmol/L in OL-HDF [7, 8]. This procedure is also novel in Japan, where the dialysate is prepared in a central dialysate delivery system (CDDS), and therefore, the same concentrations are administered to the patients. In contrast, in Europe, individual use is the norm and the composition of the dialysis solution administered to each patient may vary [7–10]. Moreover, the specifications of the membrane employed and albumin leakage are presented here.

Ionized magnesium and calcium comprise approximately 60% of the total serum magnesium and calcium levels [11] and indicate biological availability [12]. Here, we present the first report focused on the change of

**Table 2** Dialysis prescription

Parameter	StDi-1 (n = 6)	StDi-2 (n = 6)	CiDi (n = 6)
Blood flow rate (mL/min)	280	280	280
Dialysate flow rate (mL/min)	500	500	500
Effective treatment time (min)	240	240	240
Filter	FIX-250S eco	FIX-250S eco	FIX-250S eco
Dialysis solutions			
Acetate (mmol/L)	8	10.2	-
Citrate (mmol/L)	-	-	0.67
Bicarbonate (mmol/L)	27.5	25	35
Chloride (mmol/L)	112.25	113	111
Glucose (mg/dL)	125	100	150
Potassium (K) (mmol/L)	2	2	2
Sodium (Na) (mmol/L)	140	140	140
Total calcium (mmol/L)	1.375	1.5	1.5
Total magnesium (mmol/L)	0.5	0.5	0.5

ionized magnesium and calcium in OL-HDF under various conditions. In this study, we aimed to clarify the influence of the dilution methods, substitution volume, and dialysate on magnesium and calcium levels in OL-HDF.

## Methods

This prospective study was conducted in a specialized dialysis facility in Japan (Kawashima Dialysis Clinic, Tokushima). The study was approved by the Kawashima Hospital Ethics Review Committee (No. 0302) and was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. Written informed consent was obtained from every patient. The study was enrolled in the University Hospital Medical Information Network. We selected 18 stable maintenance HD patients who would be treated with OL-HDF. All vascular access was through an arteriovenous fistula

and no recirculation was allowed. The patients were aged > 20 years and had normal serum magnesium and calcium levels (1.8–2.4 and 8.4–10.0 mg/dL, respectively) prior to study initiation. During the study period, the doses of current medications containing magnesium and calcium remained unchanged. Patients with serious inflammatory symptoms and severe dysfunction of the liver, heart, and lungs were excluded. They were randomly assigned to three different dialysate groups ( $n = 6$  in each group). No significant differences were found among the patients' characteristics in the three dialysate groups (Table 1). The dialysate compositions for each group are presented in Table 2: two dialysate groups containing acetic acid (standard dialysate [StDi]) and one dialysate group containing citrate (citrate dialysate [CiDi]). The dialysate magnesium concentration was 0.5 mmol/L, and the dialysate calcium concentrations were 1.375 and 1.5 mmol/L for the StDi-1 and StDi-2 subgroups, respectively. There were five treatment

### Maintenance hemodialysis patients in our group

( $n=515$ )

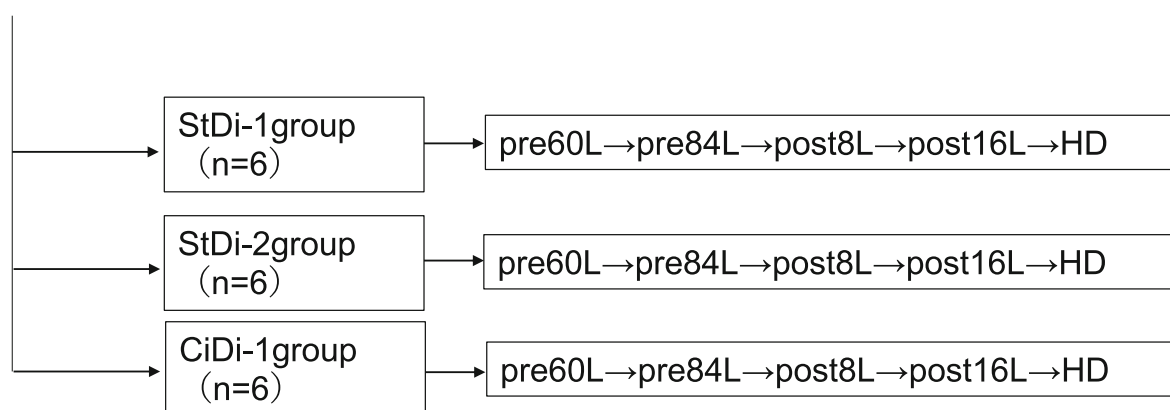
#### Exclusion ( $n=497$ )

- Patients who do not agree to change treatment conditions for 5 weeks.
- Patients whose serum total magnesium and total calcium are outside the standard values.
- Patients who are unable to maintain a stable blood flow rate of 280 mL/min.
- Patients who are likely to change their magnesium or calcium medication during the evaluation period.

Patients with serious inflammatory symptoms and severe dysfunction of the liver, heart, and lungs were excluded.

### Final target patients

( $n=18$ )



**Fig. 1** Schematic flow chart depicting the study schedule. The eligible patients were divided into three groups. Each group comprised six patients (StDi-1, StDi-2, and CiDi). The evaluation days were the second and third dialysis days of each week, and five treatment conditions were applied once in each group. On evaluation days, blood samples were collected to assess the total and ionized magnesium and calcium levels in the serum before and after dialysis. HD was performed on treatment days apart from the evaluation day. StDi, standard dialysate; CiDi, citrate dialysate; HD, hemodialysis

conditions for each dialysate group: pre-dilution OL-HDF (substitution volume 60 and 84 L/session), post-dilution OL-HDF (substitution volume 8 and 16 L/session), and HD. In total, 30 and 18 treatments in each dialysate and prescription group, respectively, were performed. A flow diagram of this study is presented in Fig. 1. The evaluations were conducted once for each condition, immediately after specimen collection. The first day of treatment was excluded from the evaluation because of the potential effects of water removal volume. The treatment conditions were as follows: duration, 4 h; frequency, three times per week; blood flow rate, 280 mL/min; and total dialysate flow rate, 500 mL/min. Blood was collected before and after dialysis under each condition in front of the membrane. Pre-dialysis blood collection was performed 5 min after treatment initiation, and post-dialysis blood collection was performed 5 min before treatment completion. The ionized magnesium and calcium levels were immediately measured by the ion selective electrode method (Stat Profile Fox Ultra, Nova Biomedical, Waltham, MA, USA). In addition, the total magnesium and calcium levels were

measured by the colorimetric method. When the serum albumin concentration was < 4.0 g/dL, we calculated the corrected calcium concentration as follows:

corrected calcium (mg/dL) = measured total calcium (mg/dL) + 0.8 (4.0 – serum albumin (g/dL)).

The change from pre-dialysis to post-dialysis (%) was calculated as follows: (post-dialysis concentration – pre-dialysis concentration)/pre-dialysis concentration × 100 + 100.

We also evaluated the removed amount of magnesium and calcium as well as the albumin leakage using the partial storage method for the spent dialysate. In this method, the drained dialysate was extracted at a rate of 0.9 L/h using a multi-roller pump (MF-01; JMS Corp., Hiroshima, Japan) and was stored for 4 h. At our hospital, we observed that there was no significant difference between the measurement errors after performing the total and partial storage methods.

All data were expressed as means ± standard deviations (SDs). Statistical analyses were performed using a repeated-measures analysis of variance, one-way analysis

**Table 3** Total and ionized concentrations of magnesium and calcium according to treatment conditions in group StDi-1

Substitution volume [L/ session]	Pre-dilution		Post-dilution		HD (n = 6)	P (5 groups)
	60 (n = 6)	84 (n = 6)	8 (n = 6)	16 (n = 6)		
Pre-dialysis values						
Total magnesium (mg/dL)	2.60 ± 0.12	2.50 ± 0.10	2.58 ± 0.13	2.50 ± 0.20	2.54 ± 0.23	0.817
Ionized magnesium (mmol/L)	0.64 ± 0.03	0.59 ± 0.03	0.62 ± 0.04	0.60 ± 0.04	0.59 ± 0.03	0.146
Total calcium (mg/dL)	8.96 ± 0.76	9.22 ± 0.78	9.16 ± 0.62	9.18 ± 0.76	9.02 ± 0.79	0.981
Ionized calcium (mmol/L)	1.16 ± 0.09	1.15 ± 0.08	1.17 ± 0.06	1.14 ± 0.07	1.12 ± 0.11	0.926
pH	7.38 ± 0.03	7.40 ± 0.03	7.40 ± 0.02	7.38 ± 0.03	7.39 ± 0.04	0.861
Post-dialysis values						
Total magnesium (mg/dL)	1.98 ± 0.11	2.04 ± 0.13	1.98 ± 0.13	1.88 ± 0.11	1.98 ± 0.04	0.283
Ionized magnesium (mmol/L)	0.48 ± 0.06	0.47 ± 0.06	0.45 ± 0.06	0.47 ± 0.04	0.48 ± 0.05	0.229
Total calcium (mg/dL)	9.08 ± 0.28	8.96 ± 0.25	9.18 ± 0.17	9.10 ± 0.22	9.12 ± 0.28	0.705
Ionized calcium (mmol/L)	1.20 ± 0.02	1.19 ± 0.03	1.16 ± 0.05	1.18 ± 0.03	1.19 ± 0.03	0.524
pH	7.42 ± 0.01	7.41 ± 0.03	7.42 ± 0.03	7.41 ± 0.02	7.41 ± 0.02	0.951
Pre-dialysis to post-dialysis						
Total magnesium (%)	76.3 ± 6.3	81.6 ± 4.5	76.9 ± 7.0	75.5 ± 5.4	78.4 ± 7.0	0.551
Ionized magnesium (%)	75.3 ± 7.6	80.6 ± 10.2	72.6 ± 8.3	80.0 ± 9.1	81.9 ± 11.5	0.496
Total calcium (%)	101.5 ± 6.1	97.6 ± 5.9	100.5 ± 5.4	99.6 ± 7.5	101.5 ± 6.2	0.843
Ionized calcium (%)	103.8 ± 6.3	104.0 ± 5.2	99.5 ± 6.5	104.5 ± 5.2	106.4 ± 9.2	0.586
pH	100.4 ± 0.3	100.2 ± 0.5	100.2 ± 0.3	100.3 ± 0.4	100.3 ± 0.3	0.827
Removal amount						
Magnesium (mg)	129 ± 97	126 ± 57	102 ± 72	128 ± 107	129 ± 55	0.979
Calcium (mg)	472 ± 176	385 ± 183	362 ± 126	343 ± 152	470 ± 181	0.615
Albumin leakage (g/session)	3.5 ± 0.6	3.0 ± 0.3	4.3 ± 0.7	5.5 ± 0.7	3.0 ± 0.4	< 0.001

of variance, and a paired t-test using SPSS statistics ver. 23 (IBM Corp., Armonk, NY, USA). The chi-square and Fisher's exact tests were used to compare the categorical variables. Values of  $p < 0.05$  were considered statistically significant.

## Results

### Effect of dialysis parameters on magnesium and calcium serum concentration

To determine the influence of the five dialysis treatment parameters on the magnesium and calcium levels, we compared the post-dialysis levels of ionized and total serum levels of each group ( $n = 6$  in each group). Tables 3, 4, and 5 summarize the magnesium and calcium levels for the StDi and CiDi dialysate groups. The data indicated that the dilution methods and the substitution volume did not affect the total or ionized magnesium and calcium serum concentrations after dialysis. Furthermore, pH did not differ between the five dialysis treatments.

Although the albumin leakage was approximately 3 g/session in the pre-dilution and HD methods and tended

to increase to approximately 4–5 g/session in the post-dilution method, the magnesium and calcium removal levels did not differ between the five dialysis treatments.

### Effect of dialysate on magnesium serum concentration

Figures 2 and 3 present the total and ionized magnesium pre- and post-dialysis concentrations during the five treatment conditions, respectively ( $n = 6$  in each group). The obtained data showed that the pre-dialysis ionized magnesium levels in the CiDi group were significantly lower than those of the StDi-1 group ( $p < 0.001$ ). There was no significant difference in the total magnesium concentrations at post-dialysis, but the ionized magnesium concentrations at post-dialysis in the CiDi group were lower than those of the StDi-1 and StDi-2 groups ( $p < 0.001$ ). The total and ionized magnesium levels were significantly decreased by dialysis after all three dialysate treatments ( $p < 0.001$ ).

There was no difference in the magnesium removal levels after the three dialysate treatments (Fig. 4).

**Table 4** Total and ionized concentrations of magnesium and calcium according to treatment conditions in group StDi-2

Substitution volume [L/ session]	Pre-dilution		Post-dilution		HD (n = 6)	P (5 groups)
	60 (n = 6)	84 (n = 6)	8 (n = 6)	16 (n = 6)		
Pre-dialysis values						
Total magnesium (mg/dL)	2.30 ± 0.35	2.27 ± 0.30	2.33 ± 0.32	2.22 ± 0.30	2.30 ± 0.28	0.983
Ionized magnesium (mmol/L)	0.61 ± 0.10	0.56 ± 0.04	0.55 ± 0.06	0.53 ± 0.05	0.57 ± 0.05	0.545
Total calcium (mg/dL)	9.08 ± 0.72	8.98 ± 0.65	8.95 ± 0.45	9.12 ± 0.47	9.13 ± 0.41	0.969
Ionized calcium (mmol/L)	1.16 ± 0.08	1.16 ± 0.11	1.14 ± 0.07	1.15 ± 0.07	1.16 ± 0.05	0.992
pH	7.38 ± 0.03	7.38 ± 0.01	7.38 ± 0.03	7.38 ± 0.02	7.38 ± 0.02	0.591
Post-dialysis values						
Total magnesium (mg/dL)	1.93 ± 0.14	1.95 ± 0.16	2.00 ± 0.15	1.95 ± 0.10	1.93 ± 0.12	0.911
Ionized magnesium (mmol/L)	0.45 ± 0.05	0.44 ± 0.03	0.49 ± 0.02	0.43 ± 0.04	0.46 ± 0.02	0.166
Total calcium (mg/dL)	9.92 ± 0.23	9.70 ± 0.18	10.0 ± 0.22	10.0 ± 0.25	9.95 ± 0.20	0.113
Ionized calcium (mmol/L)	1.27 ± 0.06	1.21 ± 0.07	1.28 ± 0.02	1.27 ± 0.07	1.25 ± 0.04	0.207
pH	7.41 ± 0.03	7.41 ± 0.03	7.42 ± 0.03	7.41 ± 0.02	7.41 ± 0.02	0.790
Pre-dialysis to post-dialysis						
Total magnesium (%)	85.1 ± 9.2	86.6 ± 6.2	86.4 ± 6.7	87.2 ± 7.9	84.6 ± 5.6	0.967
Ionized magnesium (%)	73.9 ± 7.3	79.1 ± 11.8	79.3 ± 7.9	78.4 ± 8.3	81.3 ± 9.5	0.373
Total calcium (%)	109.0 ± 7.9	108.4 ± 6.9	111.9 ± 4.0	110.1 ± 5.8	109.1 ± 4.1	0.878
Ionized calcium (%)	109.8 ± 6.7	105.8 ± 4.4	112.8 ± 7.0	109.2 ± 2.5	107.5 ± 6.7	0.672
pH	100.3 ± 0.4	100.5 ± 0.4	100.6 ± 0.5	100.4 ± 0.3	100.4 ± 0.3	0.750
Removal amount						
Magnesium (mg)	90 ± 95	67 ± 62	43 ± 72	128 ± 91	87 ± 94	0.513
Calcium (mg)	196 ± 154	81 ± 85	187 ± 160	204 ± 90	202 ± 113	0.397
Albumin leakage (g/session)	3.1 ± 1.2	3.0 ± 0.4	4.3 ± 0.9	5.2 ± 0.9	2.8 ± 0.5	< 0.001

**Table 5** Total and ionized concentrations of magnesium and calcium according to treatment conditions in group CiDi

Substitution volume [L/ session]	Pre-dilution		Post-dilution		HD (n = 6)	P (5 groups)
	60 (n = 6)	84 (n = 6)	8 (n = 6)	16 (n = 6)		
Pre-dialysis values						
Total magnesium (mg/dL)	2.32 ± 0.37	2.32 ± 0.35	2.27 ± 0.33	2.25 ± 0.32	2.32 ± 0.39	0.995
Ionized magnesium (mmol/L)	0.53 ± 0.05	0.52 ± 0.06	0.52 ± 0.04	0.53 ± 0.05	0.52 ± 0.06	0.994
Total calcium (mg/dL)	9.33 ± 0.76	9.27 ± 0.83	9.23 ± 0.54	9.35 ± 0.71	9.12 ± 0.59	0.979
Ionized calcium (mmol/L)	1.13 ± 0.05	1.15 ± 0.06	1.11 ± 0.07	1.17 ± 0.06	1.14 ± 0.05	0.588
pH	7.40 ± 0.02	7.39 ± 0.02	7.39 ± 0.03	7.40 ± 0.02	7.40 ± 0.02	7.848
Post-dialysis values						
Total magnesium (mg/dL)	1.92 ± 0.10	1.97 ± 0.12	1.85 ± 0.14	1.92 ± 0.12	1.92 ± 0.19	0.488
Ionized magnesium (mmol/L)	0.39 ± 0.05	0.40 ± 0.04	0.38 ± 0.05	0.39 ± 0.04	0.40 ± 0.04	0.956
Total calcium (mg/dL)	9.60 ± 0.20	9.62 ± 0.36	9.65 ± 0.19	9.67 ± 0.31	9.45 ± 0.19	0.437
Ionized calcium (mmol/L)	1.14 ± 0.02	1.14 ± 0.01	1.13 ± 0.03	1.14 ± 0.03	1.13 ± 0.03	0.868
pH	7.46 ± 0.01	7.46 ± 0.01	7.45 ± 0.01	7.47 ± 0.02	7.47 ± 0.02	0.557
Pre-dialysis to post-dialysis						
Total magnesium (%)	83.8 ± 8.4	86.0 ± 9.4	82.4 ± 6.9	86.1 ± 8.1	83.8 ± 9.1	0.928
Ionized magnesium (%)	74.9 ± 7.7	75.6 ± 9.0	73.8 ± 10.3	73.3 ± 9.8	78.3 ± 10.2	0.904
Total calcium (%)	103.3 ± 6.1	104.2 ± 5.7	104.7 ± 5.0	103.7 ± 4.6	103.9 ± 5.1	0.999
Ionized calcium (%)	100.8 ± 4.6	99.4 ± 4.1	101.9 ± 4.4	98.1 ± 5.7	99.3 ± 5.6	0.717
pH	100.9 ± 0.2	100.9 ± 0.2	100.9 ± 0.3	100.9 ± 0.2	101.0 ± 0.5	0.988
Removal amount						
Magnesium (mg)	104 ± 80	113 ± 87	37 ± 68	120 ± 50	141 ± 63	0.192
Calcium (mg)	294 ± 210	222 ± 107	267 ± 134	294 ± 199	217 ± 115	0.941
Albumin leakage (g/session)	3.1 ± 0.3	3.3 ± 0.3	4.9 ± 0.7	5.1 ± 0.9	3.2 ± 0.5	0.003

### Effect of dialysate on calcium serum concentration

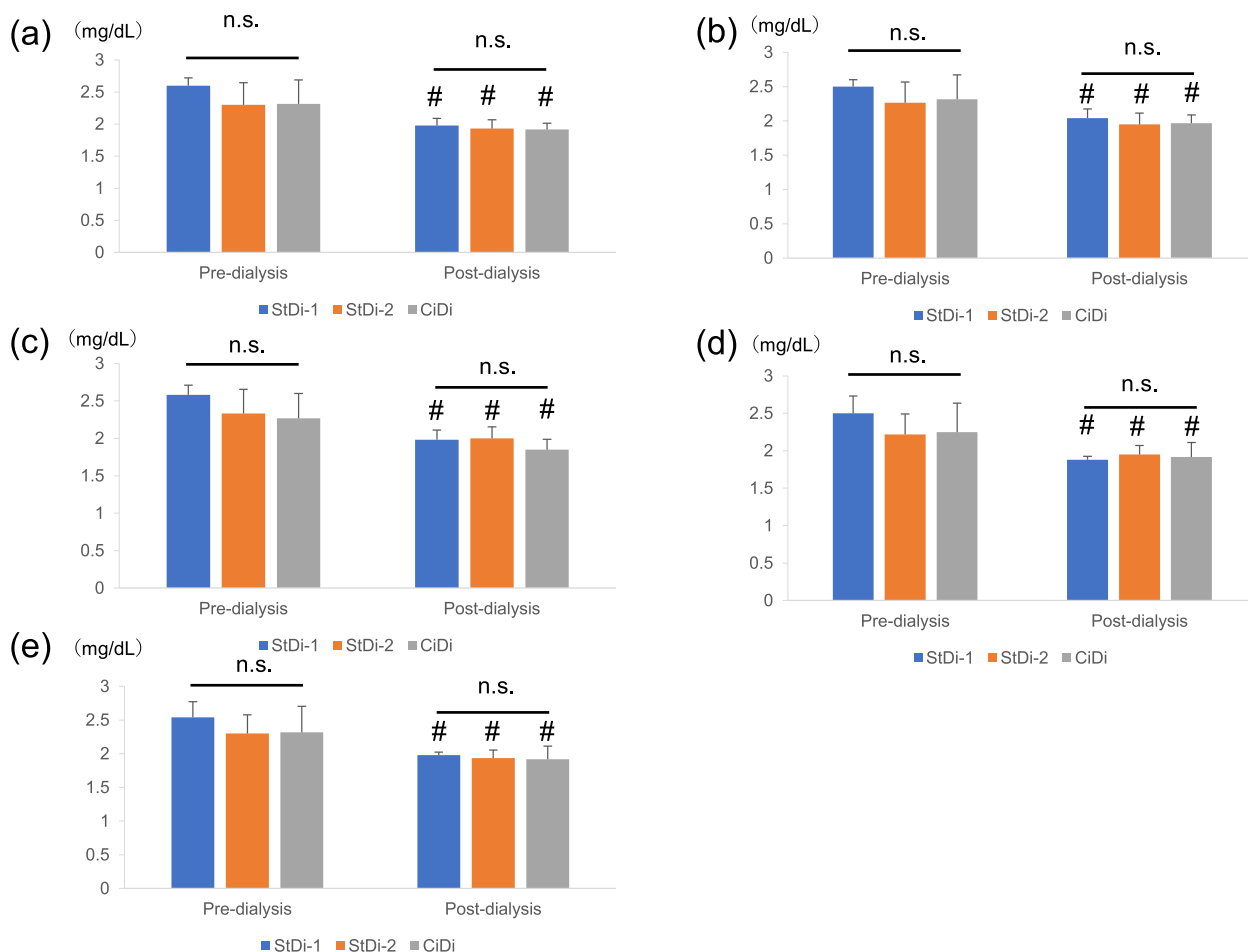
Figures 5 and 6 present the total and ionized calcium pre- and post-dialysis concentrations during the five treatment conditions, respectively (n = 6 in each group). There was no significant difference in the total and ionized calcium concentrations at pre-dialysis. The total and ionized calcium concentrations at post-dialysis in the StDi-2 group were higher than those of the StDi-1 and CiDi groups ( $p < 0.001$ ). The total and ionized calcium concentrations were significantly increased by dialysis in the StDi-2 group after treatment ( $p < 0.001$ ).

The calcium removal amount was higher in the StDi-1 than in the StDi-2 group ( $p < 0.05$ ) (Fig. 4).

### Discussion

To the best of our knowledge, this is the first report showing that three different dialysis solutions, each with a different calcium concentration, were used in CDDS and that the method of dilution and the amounts of the replacement solution have little effect on the ionized magnesium and calcium concentrations. A previous study showed that there was no correlation between the

total and ionized magnesium serum concentrations [13]. Therefore, we investigated the changes in the total and ionized magnesium levels. OL-HDF is a treatment method that utilizes a large volume of replacement fluid, leading to concerns about the possibility of significant electrolyte fluctuation. Particularly, in post-dilution OL-HDF, which is the mainstream treatment method in Europe, a large volume of replacement fluid flows directly into the body after passing through the membrane [14–16]. We hypothesized that with increased albumin leakage, albumin-bound magnesium and calcium are removed in post-dilution OL-HDF. Albumin leakage is analyzed by a combination of the membrane used, the dilution method, and the volume of replacement fluid. However, European OL-HDF assessments do not specify the membrane pore size or the requirement of solute removal [14–16]. Similarly, existing reports do not provide detailed specifications of the membrane or solute removal; thus, the effect of albumin leakage is unknown [7–10]. Regarding the effect of albumin leakage into the dialysate on calcium delivery, each gram of leaking albumin is considered to remove approximately 1 mg of

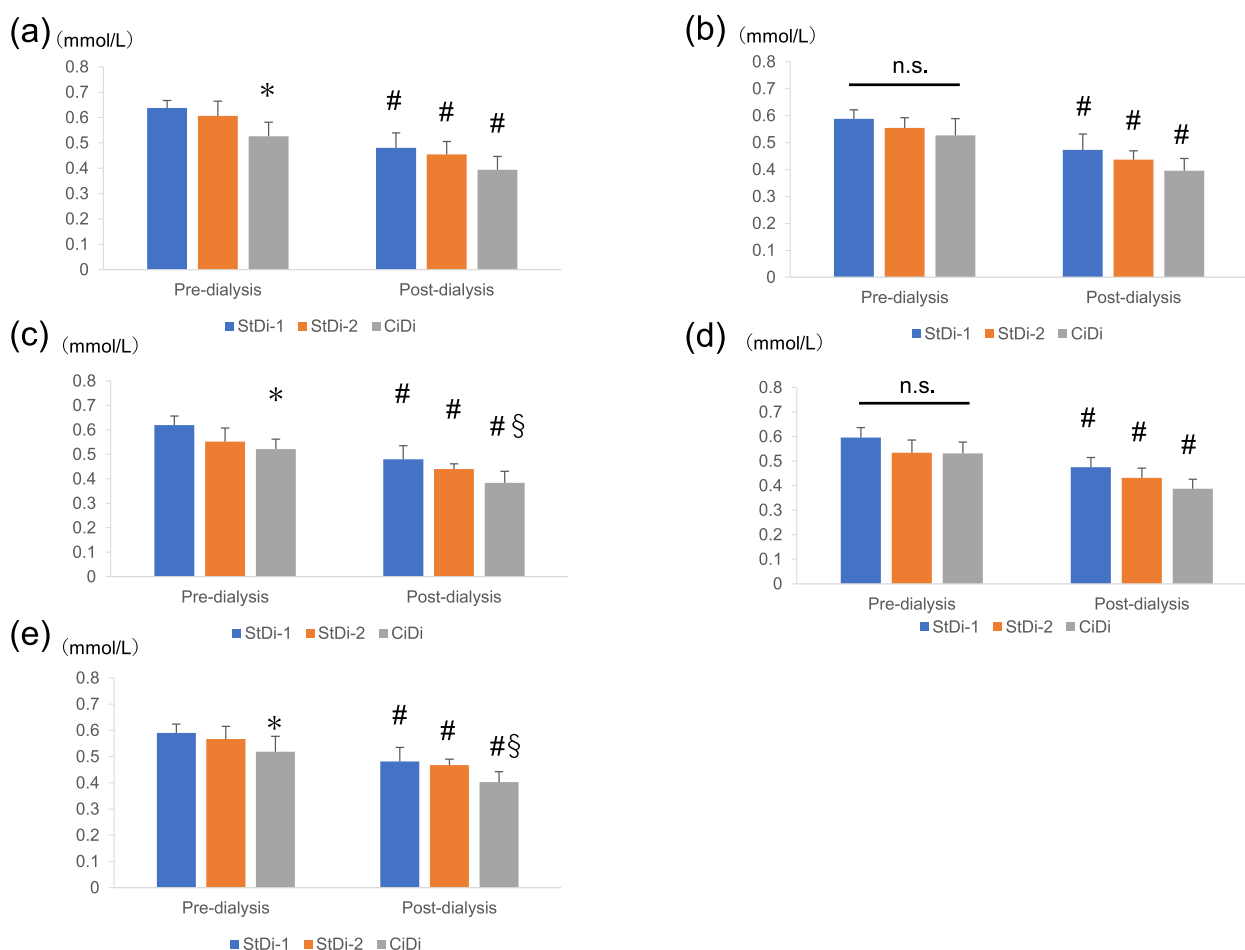


**Fig. 2** Pre-dialysis and post-dialysis comparison of total magnesium concentrations among three dialysates by five modalities: **a** pre-dilution 60 L, **b** pre-dilution 84 L, **c** post-dilution 8 L, **d** post-dilution 16 L, and **e** HD. # $p < 0.001$  versus StDi-1, StDi-2, and CiDi at pre-dialysis. StDi, standard dialysate; CiDi, citrate dialysate; HD, hemodialysis

calcium [17]. Therefore, the effect of albumin leakage on calcium delivery is considered to be small; nevertheless, the effect on magnesium delivery remains unknown. Although they are both divalent cations, the effect of albumin leakage on magnesium and calcium may be different; therefore, we examined their relationships with albumin leakage. Interestingly, we found no difference in the amount of magnesium and calcium removed or in the values of ionized magnesium and calcium in the blood when albumin leakage increased with OL-HDF after dilution. Furthermore, the albumin leakage in the membrane we employed was well defined. Given that the membrane used in this study did not cause excessive transmembrane pressure or significant albumin leakage [18], our results indicated that post-dilution OL-HDF does not significantly impact the concentration of ionized magnesium and calcium when albumin leakage is limited to approximately 5 g/session.

We further investigated the magnesium and calcium concentrations with different dialysates. Although the post-dialysis total magnesium concentration did not differ between dialysates, the ionized magnesium levels were significantly reduced in the CiDi group compared to those in the StDi-1 and StDi-2 groups. As ionized magnesium is removed by diffusion, the post-dialysis levels should be consistent with the dialysate concentration (0.5 mmol/L). The amount of removed magnesium was not significantly different but tended to be lower in the StDi-2 group. However, the value of ionized magnesium was lower in the StDi-2 than in the StDi-1 group. The lack of association between the results of removal amount and blood values differed from that of calcium, suggesting that magnesium and calcium may have different dynamics in the body. Particularly, some patients receiving CiDi had ionized magnesium concentrations < 0.45 mmol/L, which may be attributed to chelation of





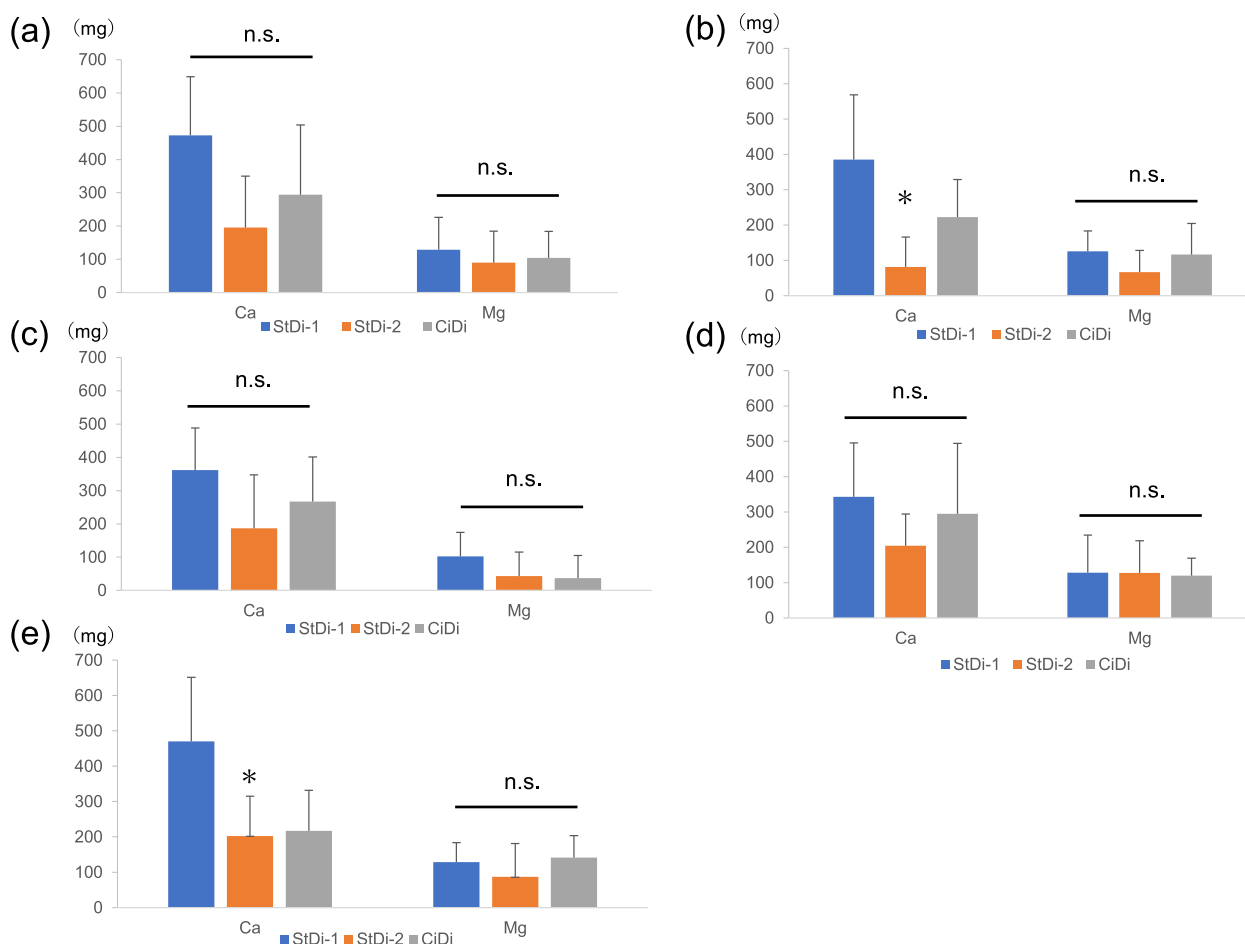
**Fig. 3** Pre-dialysis and post-dialysis comparison of the ionized magnesium concentrations among three dialysates by five modalities: **a** pre-dilution 60 L, **b** pre-dilution 84 L, **c** post-dilution 8 L, **d** post-dilution 16 L, and **e** HD. \* $p < 0.001$  versus StDi-1 at pre-dialysis. § $p < 0.001$  versus StDi-1 at post-dialysis. # $p < 0.001$  versus StDi-1, StDi-2, and CiDi at pre-dialysis. StDi, standard dialysate; CiDi, citrate dialysate; HD, hemodialysis

citric acid and  $Mg^{2+}$  in the dialysate [19], thus resulting in lower than theoretical ionized magnesium levels in the dialysate, which in turn results in the removal of ionized magnesium from the blood. However, the amount of magnesium removal was not significantly higher in the CiDi group. It is also important to note that we cannot predict the decrease in ionized magnesium from the removed amount. This finding suggested that we should be cautious when using CiDi to prevent increased risks of lethal arrhythmia and cardiovascular mortality from hypomagnesemia. In addition, since the total magnesium levels in all the pre-dialysates were  $< 2.7$  mg/dL, we should reconsider the magnesium concentration in the dialysate.

Regarding calcium, the results seemed to be related to the removal amount: as the dialysate calcium concentration in the StDi-2 group was high (1.5 mmol/L), the total

and ionized calcium concentrations after treatment were higher in the StDi-2 group, and the removal amount at that time was also low. However, the patients in the CiDi group, whose dialysate calcium concentration was also 1.5 mmol/L, showed a tendency different from those in the StDi-2 group. Although the total calcium concentration increased in the CiDi group, the ionized calcium concentration remained unchanged before and after dialysis and was significantly lower than the StDi-2 ionized calcium concentration. In line with the aforementioned results for ionized magnesium in the CiDi treatment group, citric acid likely formed a chelate complex with ionized calcium, thus leading to a reduced number of free calcium ions in the serum. In addition, in bicarbonate HD, calcium mass balance tests showed positive calcium transfer from dialysate to blood at a concentration of 1.5 mmol/L, whereas at 1.25 mmol/L, most patients





**Fig. 4** Removal amount of magnesium and calcium levels among three dialysates by five modalities: **a** pre-dilution 60 L, **b** pre-dilution 84 L, **c** post-dilution 8 L, **d** post-dilution 16 L, and **e** HD. \* $p < 0.001$  versus StDi-1. StDi, standard dialysate; CiDi, citrate dialysate; HD, hemodialysis

displayed an almost neutral or slightly negative calcium transfer [20]. Therefore, the evaluation at the intermediate value of 1.375 mmol/L is critically important.

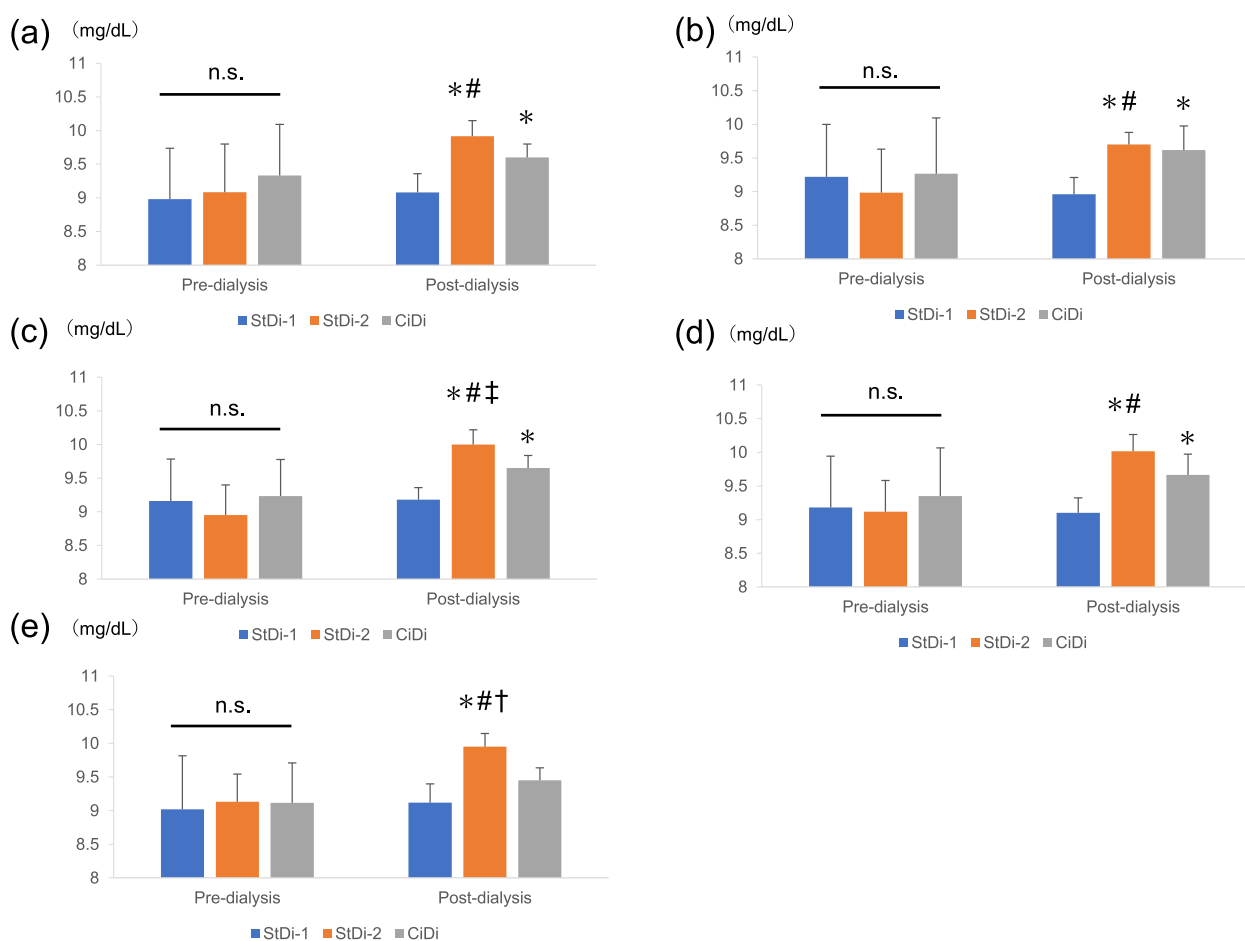
Although magnesium and calcium are divalent cations, the rates of change of the concentrations of ionized magnesium and calcium between dialysates were distinct. A previous study indicated that the ionized magnesium and calcium concentrations are affected by several factors, including the pH and the bicarbonate ion concentration [11]. However, as this was a crossover study in the same patients, we hypothesized that the effects of pH and bicarbonate ions on the ionized magnesium and calcium concentrations should be equivalent. Therefore, we considered that the chelation kinetics in the body may be significantly different for  $Mg^{2+}$  compared to  $Ca^{2+}$  [21].

Our study had several potential limitations. First, the ionized magnesium and calcium levels were measured before the membrane only. It is possible that homeostasis in the body overcomes the dilution methods and the

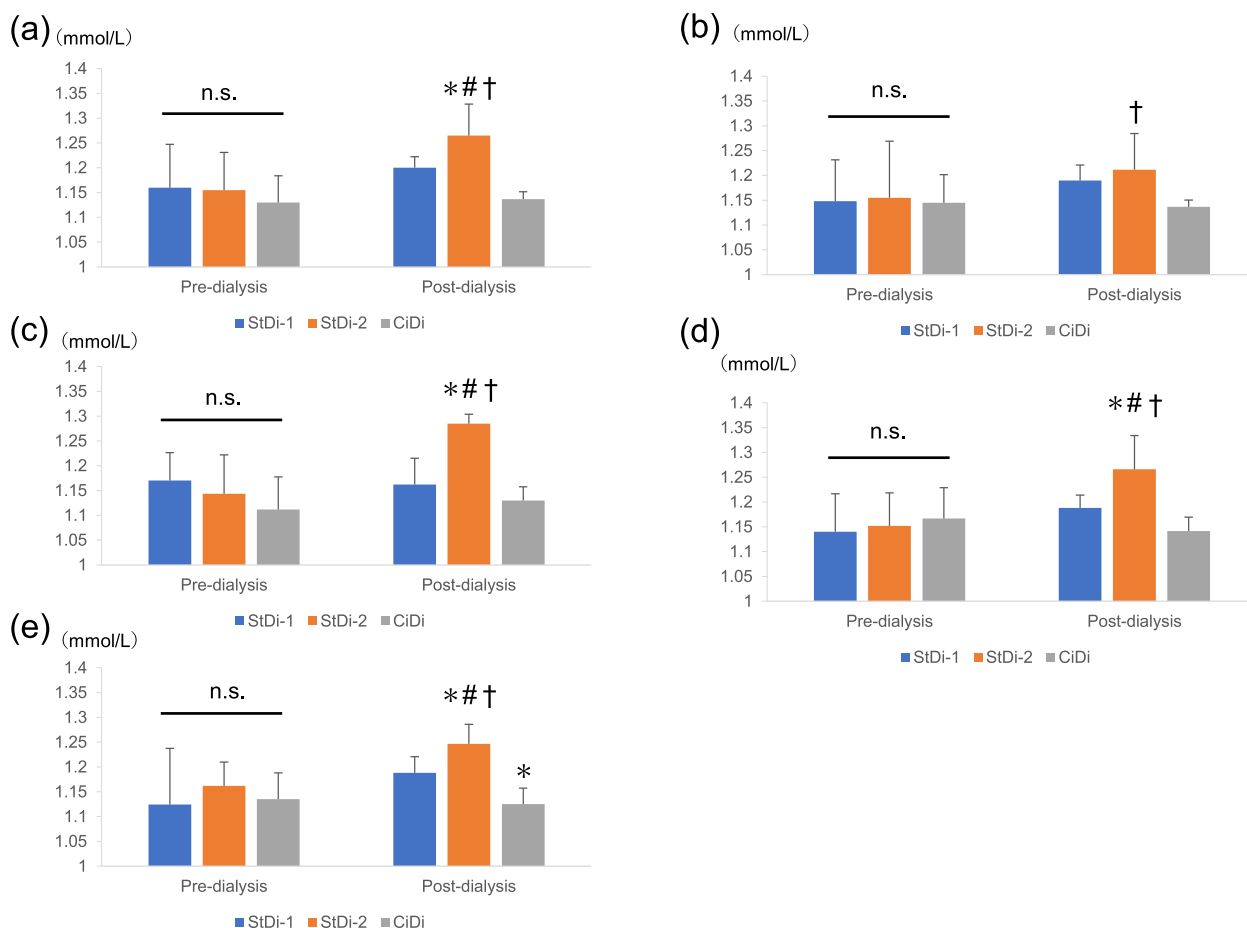
replacement volume, resulting in a lack of differences between the methods. Future studies should also measure the ionized magnesium and calcium concentrations after the membrane to investigate changes in either side of the membrane. Second, our study collected short-term data only. Further studies are needed to investigate the effects of the same dialysate on the concentrations of magnesium and calcium for a longer period of time. Third, the sample size was considerably small; therefore, the detection power of the analysis would not produce sufficient statistically relevant values. However, from a practical point of view, it is difficult to further increase the sample size. Other parameters examined herein that did not show substantial variations may appear as significant statistical differences when a larger sample size is used.

## Conclusions

In this study, when using acetate-based dialysate, we observed no difference among the five investigated



**Fig. 5** Pre-dialysis and post-dialysis comparison of total calcium concentrations among three dialysates by five modalities: **a** pre-dilution 60 L, **b** pre-dilution 84 L, **c** post-dilution 8 L, **d** post-dilution 16 L, and **e** HD. \* $p < 0.001$  versus StDi-1 at post-dialysis,  $^{\dagger}p < 0.05$  versus CiDi at post-dialysis,  $^{\ddagger}p < 0.001$  versus CiDi at post-dialysis. # $p < 0.001$  versus StDi-2 at pre-dialysis. StDi, standard dialysate; CiDi, citrate dialysate; HD, hemodialysis



**Fig. 6** Pre-dialysis and post-dialysis comparison of ionized calcium concentrations at among three dialysates by five modalities: **a** pre-dilution 60 L, **b** pre-dilution 84 L, **c** post-dilution 8 L, **d** post-dilution 16 L, and **e** HD. \* $p < 0.001$  versus StDi-1 at post-dialysis, † $p < 0.001$  versus CiDi at post-dialysis. # $p < 0.001$  versus StDi-2 at pre-dialysis. StDi, standard dialysate; CiDi, citrate dialysate; HD, hemodialysis

conditions of dialysis (i.e., hemodiafiltration with pre-or post-dilution, hemodiafiltration with higher or lower volume of substitution fluids, and HD) in post-dialysis ionized serum magnesium or calcium concentrations. However, when dialysate containing citric acid is used, the post-dialysis serum concentration of ionized magnesium may fall to a level below the corresponding concentration in the dialysate containing citric acid, possibly because of chelation of  $Mg^{2+}$  by citric acid. It is necessary to evaluate the relationship between the dialysate and mortality in the future.

#### Abbreviations

StDi: Standard dialysate; CiDi: Citrate dialysate; HD: Hemodialysis; OL-HDF: On-line hemodiafiltration; CDDS: Central dialysate delivery system

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#### Authors' contributions

YT designed the study, performed the data analysis, and wrote the manuscript. YT, RH, DO, and HM collected the clinical data. The authors read and approved the final manuscript.

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#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

#### Declarations

#### Ethics approval and consent to participate

This research was approved by the ethical committee of Kawashima Hospital (approval number, 0302). The original data were totally anonymized, and therefore, there were no risks to the privacy of patients. The data presented in the current manuscript did not contain any images, videos, or voice recording, which might have a risk for identifying an individual.

#### Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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