

Citric Acid-Based Bicarbonate Dialysate Attenuates Aortic Arch Calcification in Maintenance Haemodialysis Patients: An Observational Study

Ken-ichi Akiyama

Tokyo Women's Medical University

Norio Hanafusa (✉ hanafusa.norio@twmu.ac.jp)

Tokyo Women's Medical University

Yutaka Miura

Jichi Medical University

Momoko Seki

Tokyo Women's Medical University

Yoei Miyabe

Tokyo Women's Medical University

Yuko Iwabuchi

Tokyo Women's Medical University

Kazunori Karasawa

Tokyo Women's Medical University

Hidekazu Sugiura

Saiseikai Kurihashi Hospital

Keiko Uchida

Tokyo Women's Medical University

Hiroshi Kawaguchi

Joban Hospital

Ken Tsuchiya

Tokyo Women's Medical University

Makoto Kuro-o

Jichi Medical University

Kosaku Nitta

Tokyo Women's Medical University

Takahito Moriyama

Tokyo Women's Medical University

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Abstract

Introduction: The progression of aortic calcification is associated with mortality in haemodialysis patients. Blood calciprotein particle (CPP) levels are associated with coronary artery calcification, and was reported to be inhibited when using citric acid-based bicarbonate dialysate (CD). Therefore, we examined the effect of CD on the progression of the aortic arch calcification score (AoACS) and blood CPP levels in haemodialysis patients.

Methods: A 12-month retrospective observational study of 262 haemodialysis patients who met the eligibility criteria was conducted at Joban Hospital. Patients taking warfarin or bisphosphonates and those with baseline AoACS of 100% were excluded. Progression, defined as ΔAoACS (12 months – baseline) > 0%, was compared between the CD and acetic acid-based bicarbonate dialysate (AD) groups.

Results: The CD group had significantly lower AoACS progression than the AD group ($P = 0.037$). ΔCPP and ΔAoACS were not correlated in the AD group ($R^2 = 0.030$, $P = 0.098$), but were negatively correlated in the CD group ($R^2 = 0.065$, $P = 0.022$). Multivariate logistic analysis showed that the CD (odds ratio [OR] 0.51, 95% confidence interval [CI] 0.27–0.97, $P = 0.042$) was significantly associated with the AoACS progression.

Conclusion: CD may suppress the progression of vascular calcification in haemodialysis patients.

Introduction

The main cause of death in patients with chronic kidney disease (CKD), particularly in those undergoing maintenance haemodialysis, is heart failure and cardiovascular disease due to a high degree of systemic vascular calcification and atherosclerosis. In fact, the mortality rate in patients with CKD is extremely high compared to that for the general population.¹ The aortic arch calcification score (AoACS), based on a simple chest radiography, correlates well with the aortic arch calcification volume determined by a multidetector computed tomography ($R = 0.635$, $P < 0.001$).² Thus, this is a useful tool as it can be easily evaluated in the clinical setting. Moreover, the presence of aortic³ or coronary artery calcification⁴ and progression of aortic arch calcification^{5,6} are reported to be associated with all-cause and cardiovascular mortality. In contrast, clinical studies have reported an association of blood calciprotein particle (CPP) with coronary artery calcification,⁷ atherosclerosis, and markers of inflammation in blood.⁸ CPP in the blood has a physiological effect of transporting calcium-phosphorus, the raw material for teeth and bones; however, in patients with CKD, the amount of primary CPP in the blood is excessive or the dynamics of CPP are abnormally changed, resulting in the formation of secondary CPP, which has a pathological effect.^{9,10}

Our in vitro experiments show that citric acid inhibited CPP production as measured by the gel-filtration method.^{11,12}

We therefore hypothesised that the use of citric acid-based bicarbonate dialysate (CD) would inhibit the progression of vascular calcification by decreasing blood CPP levels in patients with CKD. Here, we aimed to examine the effect of CD on the progression of AoACS and blood CPP levels in patients on maintenance haemodialysis.

Results

Patient and baseline characteristics

Figure 1 shows the flow of participants through the study. Of the 262 patients who met the eligibility criteria, 19 patients in the AD group (bisphosphonate: 1, warfarin: 17, AoACS = 100%: 1) and 8 patients in the CD group (bisphosphonate: 1, warfarin: 7) were excluded, resulting in the analysis of 130 patients in the AD group and 105 patients in the CD group. Table 1 shows the characteristics at baseline along with the results of comparing the respective characteristics between the two groups.

Table 1
Baseline (0 month) characteristics of the AD and CD groups (n = 235).

Baseline characteristics				
	All	AD	CD	P-value
<i>n</i>	235	130	105	
Age (years)	69 (60–78)	70 (63.8–78.3)	67 (57.0–77.0)	0.132
Gender (female), %	84 (35.7)	44 (33.8)	40 (38.1)	0.499
Diabetes, %	107 (45.5)	60 (46.2)	47 (44.8)	0.831
Vintage (year)	5 (3.0–8.3)	5.0 (3.0–8.0)	5.3 (2.5–8.5)	0.942
Pi (mg/dL)	5.0 (4.1–6.1)	4.5 (3.9–5.5)	5.6 (4.5–6.7)	< 0.001
cCa (mg/dL)	9.0 (8.5–9.4)	9.0 (8.7–9.5)	8.9 (8.4–9.4)	0.036
Ca x Pi product (mg/dL)	44 (37–56)	40 (34–50)	50 (41–59)	< 0.001
iPTH (pg/mL)	131 (64–219)	101 (51–178)	171 (91–331)	< 0.001
ALP (U/L)	226 (172–280)	216 (175–272)	230 (171–289)	0.452
CRP (mg/dL)	0.12 (0.05–0.32)	0.09 (0.05–0.27)	0.15 (0.06–0.47)	0.051
Alb (g/dL)	3.8 (3.6–3.9)	3.8 (3.6–4.0)	3.7 (3.5–3.9)	0.008
nPCR (g/kg/day)	0.84 (0.74–0.99)	0.83 (0.73–0.96)	0.85 (0.74–1.02)	0.219
LDL-c (mg/dL)	86 (74–114)	86 (73–106)	90 (74–117)	0.260
HDL-c (mg/dL)	47 (39–59)	46 (39–56)	48 (40–62)	0.479
TG (mg/dL)	95 (66–135)	90 (66–133)	104 (67–136)	0.507
Ca-containing P binder, %	168 (71.5)	96 (73.8)	72 (68.6)	0.373
AoACS, %	19.8 (0–37.5)	25.0 (0–37.5)	18.8 (0–34.4)	0.126
Vitamin D, %	163 (69.4)	87 (66.9)	76 (72.4)	0.367
Calcimimetics, %	59 (25.1)	31 (23.8)	28 (26.7)	0.620
RAS inhibitors, %	34 (14.5)	24 (18.5)	10 (9.5)	0.053
MAP (mmHg)	106.2 ± 14.6	104.4 ± 15.2	108.5 ± 13.4	0.032

Data are presented as mean ± SD unless stated otherwise. The interquartile range (IQR) is the 25th–75th percentile. DM, diabetes mellitus; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; CRP, C-reactive protein; Alb, albumin; nPCR, normalised protein catabolic rate; LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; TG, triglycerides; AoACS, aortic arch calcification score; RAS inhibitors, renin–angiotensin system inhibitors; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; Cre, creatinine; BMI, body mass index; Hb, haemoglobin; FGF23, fibroblast growth factor-23; CPP, calciprotein particle.

Baseline characteristics				
Kt/Vurea (single-pool)	1.54 ± 0.30	1.57 (1.39–1.77)	1.45 (1.29–1.69)	0.015
Modality (HD, O, I), %	171, 26, 38 (73.0, 11.0, 16.0)	118, 11, 1 (90.8, 8.5, 0.8)	85, 16, 4 (81.0, 15.2, 3.8)	< 0.001
Cre (mg/dL)	6.53 (4.82–8.22)	6.71 (4.49–8.35)	6.40 (5.26–8.18)	0.360
BMI (kg/m ²)	21.9 (19.8–24.6)	21.5 (19.6–24.4)	22.0 (19.9–25.0)	0.368
Hb (g/dL)	11.1 (10.4–12.1)	11.0 (10.4–11.9)	11.1 (10.6–12.3)	0.109
FGF23 (pg/mL)	3,600 (931 – 12,834)	2,251 (587-7,895)	6,377 (1,669 – 17,687)	< 0.001
CPP (AU)	63,213 (37,364 – 123,667)	54,985 (36,825 – 101,573)	71,952 (39,582 – 148,760)	0.064
Data are presented as mean ± SD unless stated otherwise. The interquartile range (IQR) is the 25th–75th percentile. DM, diabetes mellitus; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; CRP, C-reactive protein; Alb, albumin; nPCR, normalised protein catabolic rate; LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; TG, triglycerides; AoACS, aortic arch calcification score; RAS inhibitors, renin–angiotensin system inhibitors; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; Cre, creatinine; BMI, body mass index; Hb, haemoglobin; FGF23, fibroblast growth factor-23; CPP, calciprotein particle.				

Outcomes

Table 2 shows the characteristics after 12 months, along with the results of comparing the respective characteristics between the two groups. At the end of the 12-month observation period, AoACS had progressed in 71 of the 130 patients in the AD group (54.6%) and 43 of the 105 patients in the CD group (41.0%) ($P= 0.037$) (Table 2). Multivariate logistic analysis showed that the use of CD (OR 0.51 [95% CI: 0.27–0.97], $P= 0.042$) and Age (10 years increase) (OR 2.15 [95% CI: 1.20–4.01], $P= 0.010$) were significantly associated with the progression of AoACS (Table 3).

Table 2
Characteristics of the AD and CD groups at 12 months (n = 235).

12 M characteristics				
	All	AD	CD	P-value
<i>n</i>	235	130	105	
Kt/V	1.59 ± 0.28	1.61 ± 0.28	1.56 ± 0.28	0.183
ERI	37.0 (22.1–54.4)	35.1 (20.5–53.4)	40.7 (25.2–55.1)	0.320
Pi (mg/dL)	5.1 (4.2–6.2)	4.8 (4.1–5.7)	5.6 (4.4–6.9)	< 0.001
cCa (mg/dL)	8.7 (8.3–9.2)	8.9 (8.5–9.3)	8.6 (8.1–9.0)	< 0.001
Ca x Pi product	45 (37–56)	43 (36–52)	48 (37–60)	0.015
iPTH (pg/mL)	107 (58–196)	88 (43–166)	143 (86–239)	< 0.001
Alb (g/dL)	3.8 (3.5–3.9)	3.8 (3.6–3.9)	3.7 (3.5–3.9)	0.065
nPCR (g/kg/day)	0.85 (0.72–0.97)	0.82 (0.71–0.95)	0.87 (0.73–1.02)	0.107
Cre (mg/dL)	6.82 (4.74–8.51)	6.93 (4.49–8.74)	6.63 (5.07–8.38)	0.905
CRP (mg/dL)	0.16 (0.06–0.46)	0.13 (0.04–0.32)	0.22 (0.07–0.70)	0.010
BMI (kg/m ²)	21.6 (19.5–24.5)	21.5 (19.3–24.1)	21.9 (20.0–25.0)	0.416
MAP (mmHg)	106.5 ± 16.2	104.9 ± 15.2	108.4 ± 17.2	0.099
Modality (HD, O, I), n (%)	5, 27, 203 (2.1, 11.5, 86.4)	1, 11, 118 (0.8, 8.5, 90.7)	4, 16, 85 (3.8, 15.2, 81.0)	0.642
FGF23 (pg/mL)	5,233 (1,275 – 15,086)	3,910 (887 – 11,793)	9,780 (3,144 – 22,940)	< 0.001
CPP (AU)	87,132 (34,537 – 151,811)	82,077 (34,239 – 143,315)	88,686 (35,243 – 166,513)	0.646
ΔAoACS	0 (0–12.5)	6.25 (0–12.5)	0 (0–12.5)	0.110
AoACS progression, n (%)	114 (49.5)	71 (54.6)	43 (41.0)	0.037
Data are presented as mean ± SD unless stated otherwise. The interquartile range (IQR) is the 25th–75th percentile. ERI, erythropoietin resistance index; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; Alb, albumin; nPCR, normalised protein catabolic rate; Cre, creatinine; CRP, C-reactive protein; BMI, body mass index; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; FGF23, fibroblast growth factor-23; CPP, calciprotein particle; AoACS, aortic arch calcification score.				

Table 3

Multivariate logistic regression analysis of univariate differences in background factors ^a.

	Univariate analysis			Multivariate analysis		
	OR	95% CI	P-value	OR	95% CI	P-value
Total n = 235						
Age (per 10 years increase)	2.12	1.34–3.44	0.017	2.15	1.20–4.01	0.01
Male (vs. Female)	1.06	0.62–1.81	0.838			
DM (vs. non DM)	1.15	0.69–1.93	0.503			
Vintage (per 3 years increase)	1.06	0.83–1.34	0.654			
Pi (per 2 mg/dL increase)	0.94	0.67–1.32	0.734			
cCa (per 0.5 mg/dL increase)	1.1	0.86–1.41	0.432			
CaxPi product (per 10 mg/dL increase)	0.88	0.70–1.09	0.237			
Intact PTH (per 60 pg/mL increase)	0.83	0.65–1.06	0.137			
ALP (per 50 U/L increase)	0.79	0.63–1.00	0.052			
CRP (per 0.12 mg/dL increase)	0.91	0.72–1.16	0.453			
Alb (per 0.2 mg/dL increase)	0.9	0.71–1.14	0.395			
nPCR (per 0.4 g/kg/day increase)	0.69	0.41–1.13	0.138			
LDL-c (per 20 mg/dL increase)	0.86	0.68–1.09	0.204			
HDL-c (per 10 mg/dL increase)	0.86	0.68–1.08	0.19			
TG (per 10 mg/dL increase)	0.7	0.51–0.94	0.02	0.73	0.51–1.04	0.082
Ca containing P binder use (vs. No)	0.75	0.42–1.32	0.312			
Dialysate : CD (vs. AD)	0.58	0.34–0.97	0.037	0.51	0.27–0.97	0.042
AoACS (per 10 % increase)	1.43	1.11–1.85	0.005	1.27	0.93–1.75	0.136
Vitamin D (+ vs. -)	0.99	0.57–1.73	0.983			
Calcimimetics (+ vs. -)	0.95	0.52–1.71	0.852			
RAS inhibitors (vs. non RAS)	1.07	0.52–2.23	0.851			
MAP (per 10 mmHg increase)	0.87	0.67–1.16	0.25			

^a Background factors: age (10 years increase), intact PTH (60 pg/mL increase), TG (10mg/dL increase), dialysate: CD (vs AD), and AoACS (10% increase), with the addition of FGF23 (Log) (pg/mL increase) and CPP (Log) (AU increase) to these factors, for progression of AoACS.

DM, diabetes mellitus; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; CRP, C-reactive protein; Alb, albumin; nPCR, normalised protein catabolic rate; LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; TG, triglycerides; CD, citric acid-containing dialysate; AD, acetic acid-containing dialysate; AoACS, aortic arch calcification score; RAS inhibitors, renin-angiotensin system inhibitors; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; Cre, creatinine; BMI, body mass index; Hb, haemoglobin; FGF23, fibroblast growth factor-23; CPP, calciprotein particle.

	Univariate analysis			Multivariate analysis		
Kt/V (per 0.3 increase)	1.17	0.84–1.64	0.362			
Modality (HD :1, O : 2, I :3)	0.87	0.62–1.23	0.438			
Cre (per 3 mg/dL increase)	1.13	0.87–1.49	0.352			
BMI (per 3 kg/m ² increase)	1.02	0.78–1.34	0.87			
Hb (per 1 g/dL increase)	0.83	0.65–1.07	0.155			
FGF23 (Log) (per pg/mL increase)	1.05	0.88–1.26	0.591	1.04	0.83–1.32	0.713
CPP (Log) (per AU increase)	1.29	0.91–1.85	0.158	1.57	0.99–2.52	0.056
^a Background factors: age (10 years increase), intact PTH (60 pg/mL increase), TG (10mg/dL increase), dialysate: CD (vs AD), and AoACS (10% increase), with the addition of FGF23 (Log) (pg/mL increase) and CPP (Log) (AU increase) to these factors, for progression of AoACS.						
DM, diabetes mellitus; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; CRP, C-reactive protein; Alb, albumin; nPCR, normalised protein catabolic rate; LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; TG, triglycerides; CD, citric acid-containing dialysate; AD, acetic acid-containing dialysate; AoACS, aortic arch calcification score; RAS inhibitors, renin–angiotensin system inhibitors; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; Cre, creatinine; BMI, body mass index; Hb, haemoglobin; FGF23, fibroblast growth factor-23; CPP, calciprotein particle.						

Subgroup analysis

Subgroup analysis showed that the factors CRP 0.1 mg/dL \geq 0.1 (vs. CRP $<$ 0.1 mg/dL), AoACS \geq 19% (vs. AoACS $<$ 19%), cCa \geq 9 mg/dL (vs. cCa $<$ 9 mg/dL), and iPTH \geq 132 pg/mL (vs. iPTH $<$ 132 pg/mL) were significantly different in the main effect of CD use. Interaction analysis revealed a significant difference only in the factor of AoACS ($P=0.045$) (Supplementary Fig.).

Propensity score matching

Subgroup analysis showed an interaction only in the comparison of the main effect between AoACS less than 19% and 19% or more. Based on previous report⁵ and our results, the patients were divided into the following groups according to their baseline AoACS: Grade 0; AoACS = 0%, grade 1; AoACS $>$ 0% and \leq 25%, grade 2; AoACS $>$ 25% and \leq 50%, grade 3; AoACS $>$ 50%. Of these, the mild to moderate calcification group with grade 2 or less was included in the study, and a comparison using propensity score matching between the two groups of AD and CD was performed. As a result, 15 patients in the AD group and 4 patients in the CD group with AoACS higher than 50% at baseline were excluded, and 115 patients in the AD group and 101 patients in the CD group were selected (Fig. 1). After 1:1 propensity score matching, 37 patients were extracted from each group (Fig. 1). We then compared the characteristics at baseline (Table 4) and after 12 months (Table 5). When examining the presence or absence of AoACS progression between the two groups of 115 patients in the AD group and 101 patients in the CD group, which consisted of the mild to moderate calcification group with AoACS of 50% or less, there was significantly less progression in the CD group, with 66 patients (57.4%) in the AD group and 42 patients (41.6%) in the CD group ($P=0.020$) (Table 5). Analysis of the presence of AoACS progression after propensity score matching between the two groups showed that AoACS progressed in 27 of the 37 patients in the AD group (73.0%) and 14 of the 37 patients in the CD group (37.8%) ($P=0.002$) (Table 5).

Table 4

Baseline (0 month) characteristics of the AD and CD groups before and after propensity score matching (n = 216).

Baseline characteristics	Before matching			After matching		
	AD	CD	P-value	AD	CD	P-value
Total n = 216						
<i>n</i>	115	101		37	37	
Age (years)	69 (62.0–78.0)	67 (56.5–77.0)	0.270	71 (59.5–81.5)	70 (58.5–80.5)	0.626
Gender (female), %	31.3	35.6	0.499	37.8	32.4	0.807
DM, %	44.3	44.6	0.975	45.9	48.6	0.815
Dialysis vintage (year)	5.0 (3–8.0)	5.3 (2.6–8.4)	0.747	5.0 (3–8.5)	5.3 (3.7–8.3)	0.943
Pi (mg/dL)	4.5 (3.9–5.5)	5.5 (4.5–6.7)	< 0.001	4.8 (4.1–5.4)	4.9 (4.0–6.0)	0.832
cCa (mg/dL)	9.0 (8.7–9.5)	8.9 (8.4–9.4)	0.054	8.9 (8.7–9.4)	9.1 (8.7–9.6)	0.357
Ca x Pi product (mg/dL)	41 (35–50)	50 (40–58)	< 0.001	42 (37–51)	43 (36–56)	0.709
iPTH (pg/mL)	102 (49–179)	169 (88–323)	< 0.001	145 (66–212)	94 (64–195)	0.372
ALP (U/L)	214 (171–267)	229 (167–286)	0.310	216 (172–269)	245 (186–289)	0.399
CRP (mg/dL)	0.09 (0.05–0.27)	0.15 (0.06–0.44)	0.092	0.13 (0.07–0.28)	0.20 (0.06–0.62)	0.337
Alb (g/dL)	3.8 (3.6–4.0)	3.7 (3.5–3.9)	0.005	3.8 (3.7–4.0)	3.7 (3.6–4.0)	0.377
nPCR (g/kg/day)	0.83 (0.73–0.96)	0.85 (0.73–1.00)	0.427	0.84 ± 0.35	0.84 ± 0.35	0.920
LDL-c (mg/dL)	85 (73–107)	90 (74–116)	0.316	85 (76–106)	98 (74–114)	0.713
HDL-c (mg/dL)	46 (39–56)	48 (40–62)	0.393	48 ± 28	49 ± 31	0.741
TG (mg/dL)	91 (69–137)	101 (65–136)	0.756	92 (73–136)	105 (72–143)	0.673
Ca-containing P binder, %	72.2	68.3	0.535	73	73	1
AoACS, %	18.8 (0–31.3)	18.8 (0–31.3)	0.270	25.0 (12.5–31.3)	25.0 (0–37.5)	0.960

Data are presented as mean ± SD unless stated otherwise. The interquartile range (IQR) is the 25th–75th percentile. DM, diabetes mellitus; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; CRP, C-reactive protein; Alb, albumin; nPCR, normalised protein catabolic rate; LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; TG, triglycerides; AoACS, aortic arch calcification score; RAS inhibitors, renin–angiotensin system inhibitors; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; Cre, creatinine; BMI, body mass index; Hb, haemoglobin; FGF23, fibroblast growth factor-23; CPP, calciprotein particle.

Baseline characteristics	Before matching			After matching		
Vitamin D, %	69.6	75.2	0.352	64.9	70.3	0.619
Calcimimetics, %	33	38.6	0.393	35.1	29.7	0.619
RAS inhibitors, %	18.3	8.9	0.047	8.1	10.8	0.691
MAP (mmHg)	105 ± 15	108 ± 13	0.074	102 ± 31	105 ± 23	0.390
Kt/Vurea (single-pool)	1.56 (1.36–1.79)	1.44 (1.27–1.66)	0.018	1.59 ± 0.48	1.54 ± 0.52	0.454
Intermittent/online/HD, %	7.8/9.6/82.6	25.7/13.9/60.4	< 0.001	13.5/16.2/70.3	2.4/13.5/62.2	0.492
Cre (mg/dL)	6.66 (4.67–8.41)	6.40 (5.26–8.18)	0.392	6.60 (4.45–8.43)	6.44 (4.82–8.17)	0.541
BMI (kg/m ²)	21.8 (19.6–24.6)	22.2 (19.9–25.1)	0.589	22.9 ± 8.3	23.0 ± 8.4	0.891
Hb (g/dL)	11.0 (10.4–11.9)	11.1 (10.6–12.3)	0.119	11.0 ± 2.6	11.0 ± 2.1	1
FGF23 (pg/mL)	2,016 (556–6,141)	6,377 (1,669 – 19,232)	< 0.001	3,496 (1,651 – 12,776)	3,600 (931 – 10,328)	0.611
CPP (AU)	54,410 (34,062–92,095)	71,952 (39,582 – 170,928)	0.032	70,013 (40,631 – 123,120)	70,491 (26,649 – 129,328)	0.854

Data are presented as mean ± SD unless stated otherwise. The interquartile range (IQR) is the 25th–75th percentile. DM, diabetes mellitus; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; CRP, C-reactive protein; Alb, albumin; nPCR, normalised protein catabolic rate; LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; TG, triglycerides; AoACS, aortic arch calcification score; RAS inhibitors, renin–angiotensin system inhibitors; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; Cre, creatinine; BMI, body mass index; Hb, haemoglobin; FGF23, fibroblast growth factor-23; CPP, calciprotein particle.

Table 5

Characteristics of the AD and CD groups at 12 months before and after propensity score matching for background factors at baseline (n = 216).

Before baseline matching			After baseline matching		
AD	CD	P-value	AD	CD	P-value
115	101		37	37	
1.61 ± 0.27	1.54 ± 0.27	0.097	1.60 ± 0.25	1.57 ± 0.23	0.455
33.6 (20.4–49.7)	40.8 (26.8–55.1)	0.099	33.2 (16.7–427)	38.8 (20.8–52.3)	0.279
4.8 (4.1–5.8)	5.6 (4.4–6.9)	0.001	5.0 ± 1.1	5.8 ± 1.8	0.030
9.1 (8.8–9.6)	9.0 (8.6–9.5)	0.042	9.3 (8.9–9.7)	9.0 (8.8–9.4)	0.141
43 (36–52)	48 (37–60)	0.024	45.0 ± 10.9	51.1 ± 17.2	0.074
86 (37–165)	146 (88–239)	< 0.001	103 (47–183)	126 (86–207)	0.224
3.8 (3.6–3.9)	3.7 (3.5–3.9)	0.023	3.7 ± 0.38	3.7 ± 0.32	0.551
0.84 ± 0.17	0.87 ± 0.20	0.161	0.84 ± 0.19	0.90 ± 0.19	0.209
6.94 (4.52–8.76)	6.63 (4.95–8.38)	0.880	6.41 (4.71–9.11)	6.99 (4.79–8.31)	0.918
0.13 (0.04–0.32)	0.25 (0.07–0.93)	0.003	0.23 (0.06–0.33)	0.33 (0.07–1.46)	0.116
21.6 (19.4–24.4)	22.0 (19.9–25.1)	0.638	22.6 ± 4.4	22.7 ± 4.3	0.958
104 (95–116)	110 (99–120)	0.070	107 ± 14	105 ± 19	0.569
103, 11, 1 (89.6, 9.6, 0.8)	83, 14, 4 (82.2, 13.9, 3.9)	0.181	30, 6, 1 (81, 16, 3)	31, 5, 1 (83.8, 13.5, 2.7)	0.947
4,260 (880 – 11,780)	9,780 (30,010–22,940)	0.001	5,091 (893 – 15,922)	4,515 (1,547 – 13,807)	0.948
8,9145 (36,918 – 151,811)	79,281 (32,571 – 166,513)	0.894	79,766 (31,599 – 158,128)	77,594 (38,146 – 136,009)	0.620
66 (57.4)	42 (41.6)	0.020	27 (73.0)	14 (37.8)	0.002
6.25 (0-12.5)	0 (0-12.5)	0.052	6.25 (0-12.5)	0 (0-12.5)	0.055
Data are presented as mean ± SD unless stated otherwise. The interquartile range (IQR) is the 25th–75th percentile. ERI, erythropoietin resistance index; Pi, inorganic phosphorus; cCa, corrected calcium; iPTH, intact parathyroid hormone; Alb, albumin; nPCR, normalised protein catabolic rate; Cre, creatinine; CRP, C-reactive protein; BMI, body mass index; MAP, mean arterial blood pressure; I, intermittent infusion hemodiafiltration; O, online hemodiafiltration; HD, haemodialysis; FGF23, fibroblast growth factor-23; CPP, calciprotein particle; AoACS, aortic arch calcification score.					

Although the conventional level of statistical significance was not reached, the simple subtraction 12-month AoACS – baseline [0-month] AoACS (%) tended to be lower in the CD group, both before (AD: 6.25 [0–12.5] % vs. CD: 0 [0–12.5] %, $P = 0.052$) and after (AD: 6.25 [0–12.5] % vs. CD: 0 [0–12.5] %, $P = 0.055$) propensity score matching (Table 5). Unexpectedly, the blood phosphorus levels were significantly higher in the CD group at 12 months, both before ($P = 0.001$) and after ($P = 0.030$) propensity score matching (Table 5).

Relationship between AoACS and blood CPP (total n = 235)

Correlation coefficients for Δ CPP and Δ AoACS were analyzed for 130 patients in the AD group and 105 patients in the CD group after excluding patients with AoACS = 100%, warfarin, and bisphosphonate use. The correlation between Δ CPP and Δ AoACS was examined in the AD ($R^2 = 0.030$, $P = 0.079$) and CD groups ($R^2 = 0.030$, $P = 0.079$), respectively, but there was no correlation in both groups (Fig. 2a, b).

Relationship between blood FGF23 and AoACS and blood CPP (total n = 235)

There was no correlation between Δ FGF23 and Δ AoACS in either the AD ($R^2 = 0.001$, $P = 0.932$) (Fig. 2c) or CD groups ($R^2 = 0.012$, $P = 0.321$) (Fig. 2d). In contrast, there was a weak but significant positive correlation between blood Δ FGF23 and Δ CPP in both the AD ($R^2 = 0.216$, $P = 0.001$) (Fig. 2e), and CD groups ($R^2 = 0.153$, $P = 0.001$) (Fig. 2f).

Relationship between AoACS and blood CPP (total n = 216)

Correlation coefficients for Δ CPP and Δ AoACS were analyzed for 115 patients in the AD group and 101 patients in the CD group after excluding patients with AoACS > 50%, warfarin, and bisphosphonate use. The AD group showed a non-significant but a weak positive correlation between Δ AoACS and Δ CPP ($R^2 = 0.030$, $P = 0.098$) (Fig. 3a), while the CD group showed a significant negative correlation between these factors ($R^2 = 0.065$, $P = 0.022$) (Fig. 3b).

Relationship between blood FGF23 and AoACS and blood CPP (total n = 216)

There was no significant association between Δ FGF23 and Δ AoACS in blood in either the AD ($R^2 < 0.001$, $P = 0.893$) (Fig. 3c) or CD groups ($R^2 = 0.001$, $P = 0.7315$) (Fig. 3d). In contrast, there was a significant positive correlation between blood Δ FGF23 and Δ CPP in both the AD ($R^2 = 0.174$, $P < 0.001$) (Fig. 3e), and CD groups ($R^2 = 0.115$, $P = 0.001$) (Fig. 3f).

Discussion

In this study, we examined the effect of CD on the progression of the AoACS (AoACS progression as defined by Δ AoACS > 0%) and blood CPP levels and showed that the use of CD attenuated blood CPP and suppressed the progression of vascular calcification in patients on maintenance haemodialysis.

The physiological role of primary CPP is to transport calcium-phosphorus, a raw material for teeth and bone, to these tissues, without it precipitating in soft tissue.^{9,10} The uremic environment may cause transformation of primary CPP to secondary CPP to be phagocytosed by the macrophages in the vessel wall, which may contribute to atherosclerosis¹³ and vascular calcification in the tunica media.¹⁴ In fact, it has been reported that CPP levels measured by the gel-filtration method were positively correlated with coronary plaque assessed by intravascular ultrasound, even after adjusting for a number of confounding factors.¹⁵ Due to this positive association between blood CPP and arterial calcification in CKD, the Ca-chelating effect of citric acid in CD may have inhibited the formation of blood secondary CPP, which in turn inhibited the progression of vascular calcification. In the present study, Δ CPP and Δ AoACS were negatively correlated in the CD group (Fig. 2b), suggesting that citric acid may have

inhibited the formation of primary to secondary CPP and promoted the incorporation of primary CPP into bone. There is a lack of consensus on whether FGF23 in blood is associated with vascular calcification in CKD patients.^{16,17} In our study, we did not find any significant association between blood FGF23 and AoACS (Fig. 2c, 2d). From the viewpoint that FGF23 is a phosphaturic hormone, in the early stage of CKD, when renal function is preserved, FGF23 acts on the kidney to produce Pi-diuretic effect and inhibition of vitamin D activation, and thus FGF23 is thought to have a protective effect against vascular calcification. However, in CKD patients undergoing maintenance hemodialysis, renal function is abolished, so the effects of Pi-diuresis and vitamin D inhibition in the kidney are not obtained and are not associated with vascular calcification. In the present study, we did find a weak but significant positive correlation between Δ CPP and Δ FGF23 in blood (Fig. 2e, 2f). FGF23 in blood is probably produced by osteoblasts and osteocytes upon primary CPP stimulation, while secondary CPP acts on vascular calcification because it cannot pass through bone sinuses due to its large size.^{12,18} As compared to AD, CD has the potential to improve prognosis in patients on maintenance haemodialysis.¹⁹ This may be due to the inhibition of secondary CPP production by citrate.¹² CPP is thought to induce FGF23 secretion,^{12,18} and FGF23 is associated with mortality and cardiovascular disease in patients on maintenance haemodialysis.²⁰ An in vivo rat study has reported that recombinant FGF23 directly causes cardiac hypertrophy and that there was a significant positive correlation between blood FGF23 and cardiac hypertrophy assessed by echocardiography in patients with CKD.²¹ Similar to our findings, the use of CD has previously been reported to inhibit CPP ripening, as indicated by T50 (T50 is 1/2 of the time it takes for the turbidity of primary CPP in the blood to rapidly increase to secondary CPP).^{22,23} Based on previous reports, primary CPP excess, in other words, excess of both Pi and Ca in the blood is thought to be associated with worse prognosis via FGF23 secretion, and secondary CPP is thought to be associated with worse prognosis via vascular calcification.

Another reason for the greater efficacy of CD than AD in suppressing arterial calcification may be related to the following: An ex vivo model showed that citric acid, at concentrations of 733 μ mol/L or higher, inhibited rat aortic ring calcification.²⁴ The post-dialysis blood citrate concentration in haemodialysis patients using CD has been reported to be 771.6 ± 184.3 μ mol/L, which was approximately five times higher than that of AD.²⁴ Moreover, bicarbonate promoted rat aortic ring calcification in a concentration-dependent manner.²⁴ The use of AD in haemodialysis patients was reported to result in a higher post-dialysis blood bicarbonate concentrations and lower citric acid concentrations than those obtained with CD.²⁴ Moreover, it was reported that no excess bicarbonate correction was observed after dialysis with CD.²⁵ Switching from AD to CD was reported to significantly reduce malondialdehyde-modified LDL (MDA-LDL) (an indicator of lipid oxidation) and pentosidine (an indicator of glycoxidation) level in the blood.²⁶ Therefore, it is likely that CD inhibits lipid oxidation, which is associated with coronary artery calcification,²⁷ as well as glycation, which causes atherosclerosis²⁸ in patients on maintenance haemodialysis, thereby exerting anti-atherosclerotic effects. In subgroup analysis, CPP dissolution from bone was enhanced in patients with secondary hyperparathyroidism,²⁹ suggesting a significant benefit of CD use in patients with controlled PTH (iPTH \leq 132 pg/mL) ($P = 0.036$) (Fig. 3). If Pi is controlled ($Pi \leq 6$ mg/dL), PTH will also be suppressed, and the anti-calcification effect of CD use will be more effective ($P = 0.008$). Typically, patients on maintenance haemodialysis have chronic inflammation due to the uremic environment, low nutrition, the dialysis equipment used, and the use of AD.³⁰ In vitro analysis showed that CD significantly inhibited the induction of oxidative stress in the blood of these patients, as compared to AD.³¹ Thus, CD is less inflammatory than AD and may inhibit the transition to a vicious cycle of atherosclerosis (i.e. malnutrition-inflammation-atherosclerosis syndrome). Therefore, if there is persistent inflammation in hemodialysis patients, AD may further induce inflammation, and CD may reduce inflammation. Subgroup analysis showed that CD was significantly more

favorable than AD in inhibiting the progression of vascular calcification when CRP levels were ≥ 0.1 mg/dL ($P=0.041$). Under the conditions of low Ca levels and no CaCO₃ use, the chelating effect of citric acid on Ca ions was more effective, and CD was thought to have a more anti-calcification effect than AD ($P=0.044$ under $cCa < 9$ mg/dL, $P=0.036$ without CaCO₃ use). CD had a significant anti-calcification effect compared to AD when the AoACS was < 19 (%). The reason for this is that it has been reported that when calcification is already highly advanced, treatment is unlikely to have an inhibitory effect on progression.³²

Warfarin induces vascular calcification by inhibiting matrix Gla protein, an inhibitor of calcification in arteries, in both models of CKD³³ and in clinical trials of patients on haemodialysis.³⁴ Therefore, patients on warfarin were excluded from our study. Patients using bisphosphonates were also excluded as bisphosphonate use reportedly reduced the coronary artery calcification scores in patients on maintenance haemodialysis³⁵ and inhibited rat aortic calcification by reducing bone-derived CPP.²⁹

There are several limitations in the present study. First, we did not measure blood Mg levels. Administration of magnesium oxide (MgO) significantly inhibited the progression of coronary artery calcification in patients with CKD,³⁶ and basic experiments in vascular smooth muscle cells showed that Mg dose-dependently inhibited calcification,³⁷ Mg citrate treatment reduced the carotid intima-media thickening in haemodialysis patients,³⁸ and elevation of Mg concentration in the dialysate prolonged the T50.³⁹ Thus, blood Mg levels and oral medications containing Mg are associated with vascular calcification and atherosclerosis in dialysis patients. Nevertheless, none of the patients in this study were receiving oral medications containing Mg. Second, blood bicarbonate and citrate levels may be associated with vascular calcification, but these were not measured in this study. Third, observational study is not of high quality as a research method, and the number of patients included in this study was small.

In conclusion, for patients on maintenance haemodialysis, CD is more effective than AD in inhibiting the progression of vascular calcification as assessed by Δ AoACS. Larger randomised controlled trials are warranted to validate the anti-atherosclerotic results obtained with the use of CD, and to elucidate the mechanism of this therapeutic effect.

Methods

Study design and population

This was a retrospective observational study. We used a 12-month observation period, from October 2017 to October 2018. Eligibility criteria were as follows: 1) having undergone maintenance haemodialysis at Joban Hospital (Fukushima, Japan); 2) patients who, upon introduction of dialysis treatment, have given written informed consent to save the remaining plasma collected for routine testing and subsequently use it for research; 3) those on continuous outpatient maintenance dialysis for 12 months; 4) those who had available data; 5) ≥ 18 years of age; 6) no active cancer; and 7) those stable for at least 3 months after dialysis induction at baseline. Patients using warfarin or bisphosphonates and those with a baseline AoACS of 100% were excluded.

We have obtained written informed consent for renal biopsy from all patients, and clinical data were obtained from all recently recruited patients, who had the opportunity to opt-out from this study.

Treatment interventions

The dialysis centre had two floors; originally, all patients on maintenance haemodialysis were on AD. Patients on the 1st floor remained on AD, while patients on the 2nd floor were assigned to start CD from October 2017. AoACS was compared between two groups: one in which AD (Kindary 4E®; Fuso Pharmaceutical Co., LTD, Osaka, Japan) remained unchanged and the other in which AD was changed to CD (Carbostar®; Ajinomoto Pharmaceutical Co., LTD, Tokyo, Japan). All relevant investigators were blinded to the treatment groups.

Composition of the dialysis solution

According to their respective product descriptions, the composition of AD is 140 mEq/L of sodium, 2 mEq/L potassium, 2.75 mEq/L calcium (Ca), 1 mEq/L magnesium (Mg), 112.25 mEq/L chloride, 8 mEq/L acetic acid, 0 mEq/L citrate, 27.5 mEq/L bicarbonate, and 125 mg/dL glucose. The composition of the CD was the same as above, except for the following: 3 mEq/L Ca, 111 mEq/L chloride, 0 mEq/L acetic acid, 2 mEq/L citrate, 35 mEq/L bicarbonate, and 150 mg/dL glucose (Supplementary Table).

Comparison method and outcomes

AoACS progression was defined as ΔAoACS (12-month AoACS – baseline [0-month] AoACS) > 0% over the 12-month observation period and was compared between the AD and CD groups. Thus, $\Delta\text{AoACS} = 0$ was interpreted as no AoACS progression during the 12-month observation period, and $\Delta\text{AoACS} < 0$ was interpreted as improvement in AoACS.

Evaluation scale and evaluator

Chest X-ray imaging was performed after dialysis, and the X-ray image was divided into 16 sections using the previously described method.² The presence of one calcification was calculated as 1 point divided by 16 sections \times 100 (%) = 6.25 (%); thus, 1 point = 6.25%. A clinical engineering technician at Joban Hospital performed the AoACS measurements and was available for consultations at the Department of Radiology at Tokyo Women's Medical University as needed.

Sample collection

In the non-fasting state, blood samples were taken at the beginning of the week, 2 days after the last dialysis, and were obtained before beginning the dialysis procedure. Isolated plasma samples were promptly stored at -80°C until they were used in experiments. The baseline and follow-up clinical data, such as age, sex, diabetes mellitus (DM) as the primary disease of end-stage renal disease, dialysis vintage (number of years on dialysis), mean arterial blood pressure (MAP), body mass index (BMI), dry weight, and use of Ca-containing phosphate-binders, vitamin D, calcimimetics, renin–angiotensin system (RAS) inhibitors, dialysis modality (haemodialysis, online haemodiafiltration, intermittent infusion haemodiafiltration), Kt/V, haemoglobin (Hb), and erythropoietin-stimulating agents were determined. Biochemical tests, using a plasma sample assayed the following: inorganic phosphorus (Pi), Ca, $\text{Ca} \times \text{Pi}$ product, intact parathyroid hormone (iPTH), alkaline phosphatase (ALP), C-reactive protein (CRP), albumin (Alb), normalised protein catabolic rate (nPCR), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), triglycerides (TG), blood urea nitrogen (BUN), and creatinine (Cre) levels were obtained from the patients' medical records. Corrected Ca (cCa) was calculated when the blood Alb was < 4, based on Payne's formula: $\text{cCa} = \text{blood Ca} + 4 - \text{Alb}$. For the calculation of Kt/V, the single-pool $\text{Kt/V}_{\text{urea}}$ was calculated using Daugirdas' formula: $\text{Kt/V} = -\text{Ln}(\text{Ct}/\text{Co} - 0.008t) + (4 - 3.5\text{Ct}/\text{Co}) \times \Delta\text{BW}/\text{DW}$, (Ct: post-dialysis BUN, Co: pre-dialysis BUN, t: dialysis time [h], ΔBW : weight loss during dialysis, DW: post-dialysis weight). MAP was calculated as $(\text{systolic blood pressure} - \text{diastolic blood pressure}) / 3 + \text{diastolic blood pressure}$. The erythropoietin resistance index (ERI) was calculated as erythropoiesis stimulating agent dose / (post-dialysis weight \times Hb) values. The

conversion ratio was calculated as recombinant human erythropoietin (rHuEPO): darbepoetin α (DA): epoetin β pegol (C. E. R. A.) = 200: 1: 1.

Ethics

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Medical Ethics Committee at the Joban Hospital, Iwaki-city, Fukushima, Japan (# 5104). Since this was an observational study and no invasive procedures were performed, patient consent was obtained in an opt-out format for the use of residual plasma in regular practice.

Statistical analyses

Data were expressed as means \pm standard deviations if normally distributed; otherwise, they were reported as median and interquartile range (25–75 percentile) for non-normally distributed data. The baseline characteristics of the two groups were compared using analysis of variance as a parametric or a nonparametric test. Comparisons between proportions were made using chi-square tests. In case of two-group comparisons of normally distributed data, equal variance tests were performed for quantitative variables, by Student's *t*-test for equal variances, and Welch's *t*-test for unequal variances. For two-group comparisons of non-normally distributed data, Wilcoxon's signed-rank test was used for quantitative variables. The baseline characteristics of the two groups were subjected to univariate and multivariate logistic regression analyses to assess the impact of factors on AoACS progression. Odds ratios (ORs) were expressed with 95% confidence intervals (CIs). *P*-value < 0.05 was considered statistically significant.

A subgroup analysis was used to examine the characteristics involved in the suppressive effect of CD on AoACS progression. As this was an observational study and prone to various confounders, we performed 1:1 propensity score matching to adjust for covariates and estimate causal association. We created a model that adjusted for all baseline characteristics (Table 4) as covariates, for the outcome of AoACS progression due to AD and CD treatment interventions. The model was used to perform propensity score matching. The analyses were performed with JMP version 15 (SAS Institute Inc., Cary, NC).

Declarations

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Authors' Contributions:

N.H., K.A., Y.M., K.K., and T.M. designed the study, K.A. collected, analysed, interpreted the data, and wrote the draft of the manuscript. Y.M. and M.K. contributed to the measurement of CPP and FGF23 in the blood and the interpretation of the data. T.M., N.H., K.A., Y.M., K.K., M.S., Y.I., H.S., H.K., K.U., K.T., and K.N. contributed to the interpretation of the data and critically reviewed the manuscript. All authors have read and provided approval of the version of the manuscript to be submitted.

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All the authors declared no competing interests.

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Figures

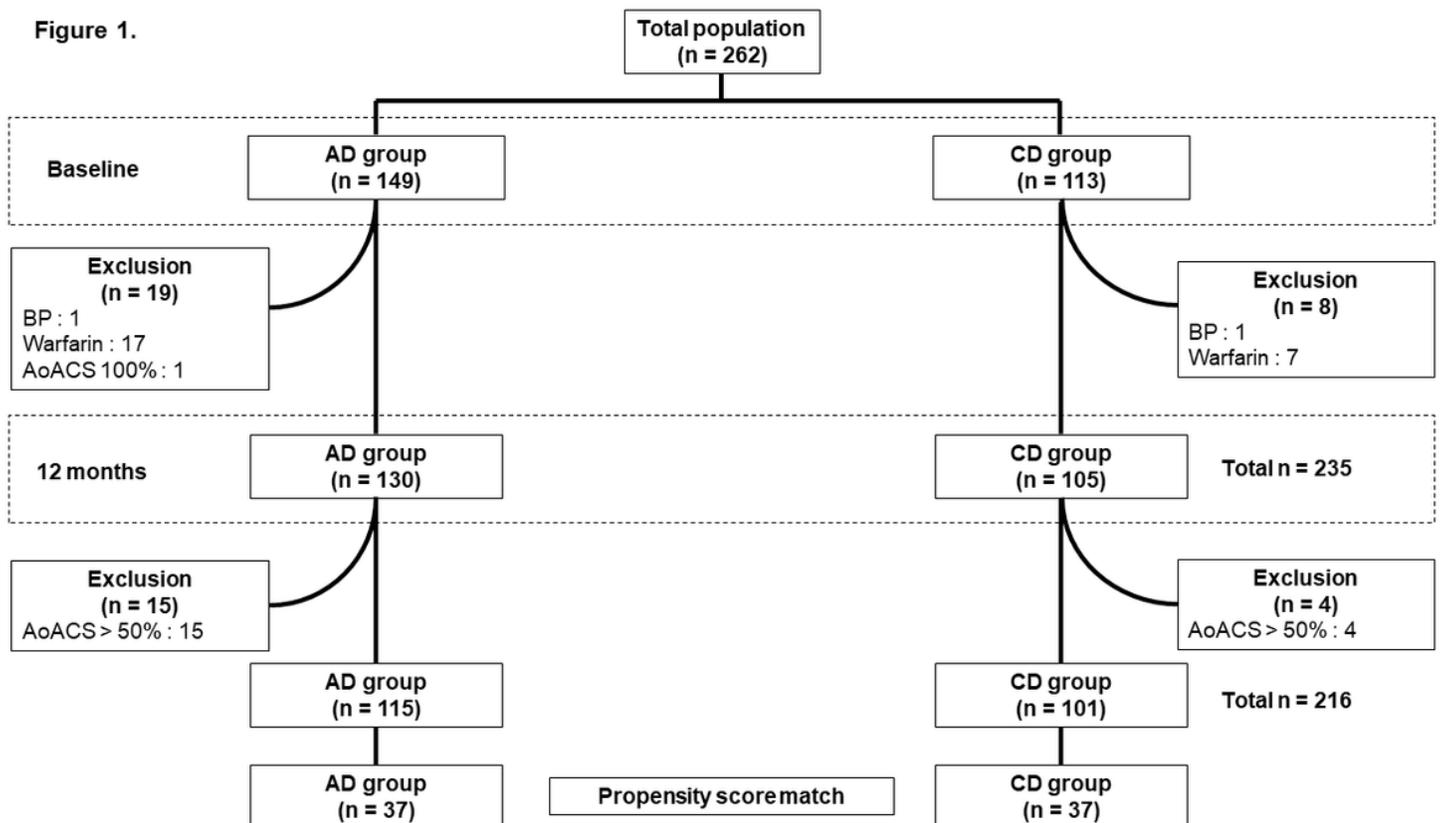


Figure 1

Flow diagram of study participants. AD, acetate acid-based bicarbonate dialysate; CD, citric acid-based bicarbonate dialysate; HD, haemodialysis; O-HDF, online hemodiafiltration; I-HDF, intermittent infusion hemodiafiltration; PSM, propensity score matching.

Figure 2.

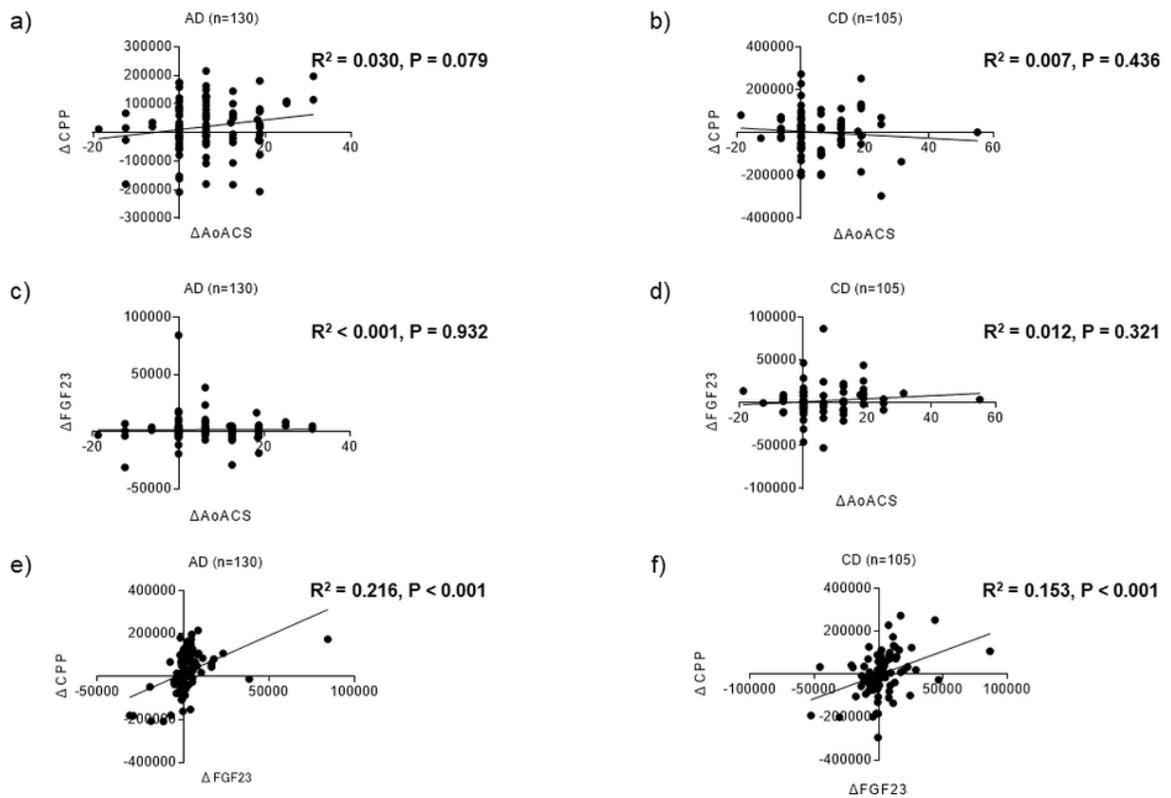


Figure 2

Relationship between blood CPP and AoACS and blood FGF23 (n = 235). a) Correlation of ΔCPP and ΔAoACS in the AD group. b) Correlation of ΔCPP and ΔAoACS in the CD group. c) Correlation of ΔFGF23 and ΔAoACS in the AD group. d) Correlation of ΔFGF23 and ΔAoACS in the CD group. e) Correlation of ΔFGF23 and ΔCPP in the AD group. f) Correlation of ΔFGF23 and ΔCPP in the CD group. CPP, calciprotein particle; AoACS, aortic arch calcification score; AD, acid-based bicarbonate dialysate; CD, citric acid-based bicarbonate dialysate; FGF23, fibroblast factor-23. Note: Statistically significant P-values are in bold.

Figure 3.

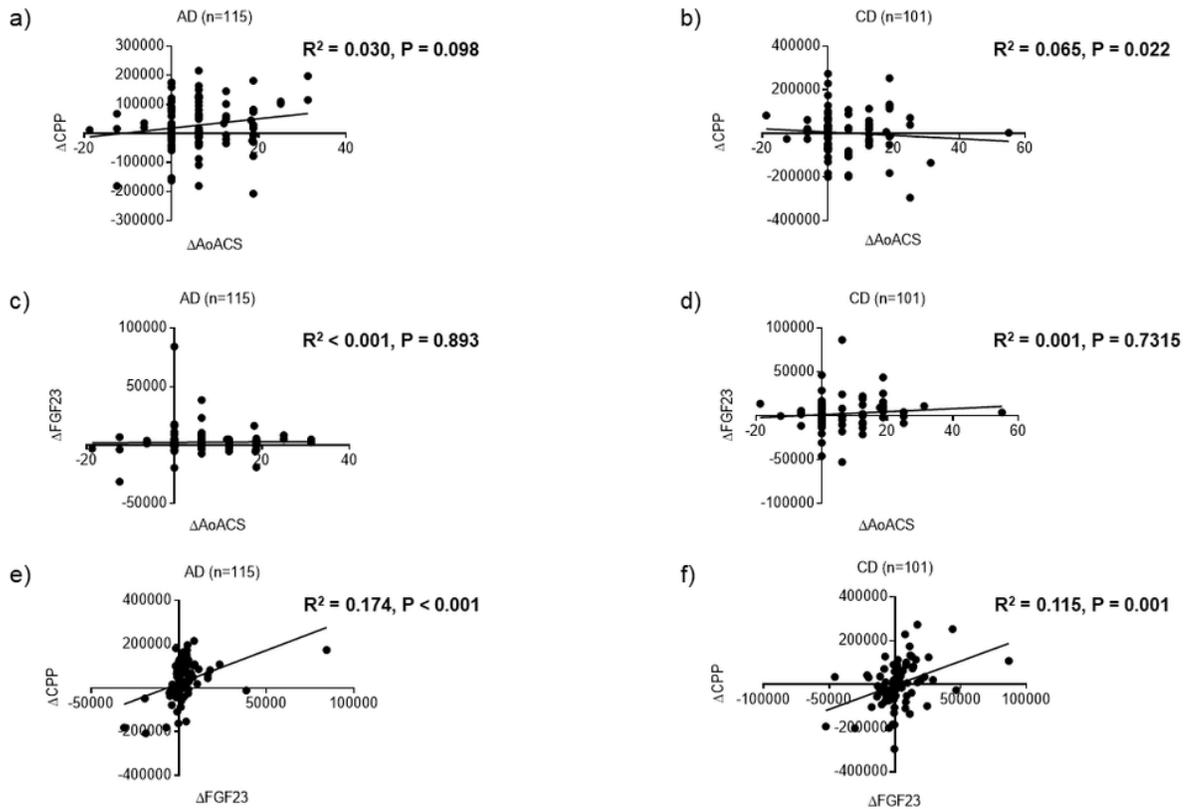


Figure 3

Relationship between blood CPP and AoACS and blood FGF23 (n = 216). a) Correlation of ΔCPP and ΔAoACS in the AD group. b) Correlation of ΔCPP and ΔAoACS in the CD group. c) Correlation of ΔFGF23 and ΔAoACS in the AD group. d) Correlation of ΔFGF23 and ΔAoACS in the CD group. e) Correlation of ΔFGF23 and ΔCPP in the AD group. f) Correlation of ΔFGF23 and ΔCPP in the CD group. CPP, calciprotein particle; AoACS, aortic arch calcification score; AD, acid-based bicarbonate dialysate; CD, citric acid-based bicarbonate dialysate; FGF23, fibroblast factor-23. Note: Statistically significant P-values are in bold.

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