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Original Article

ORIGINAL ARTICLE

The effect of sevelamer on serum calcification propensity in patients with chronic kidney disease: the results of a multicentre, double-blind, placebo-controlled, randomized clinical trial

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ABSTRACT

Background. The serum calcification propensity test (or T50 test) might become a standard tool for the assessment of vascular calcification risk and T50 might be a valuable biomarker in clinical trials of treatments intended to slow the progression of vascular calcification. Literature data suggest that non-calcium-containing phosphate binders can influence T50 in chronic dialysed patients. However, it is not clear whether similar interventions are effective in patients at earlier stages of chronic kidney disease (CKD).

Methods. The FGF23 Reduction: Efficacy of a New phosphate binder in CHronic kidney disease (FRENCH) trial was a multicentre, double-blind, placebo-controlled, randomized trial of sevelamer carbonate in participants with stage 3b/4 CKD. In this subanalysis of the FRENCH data, T50 and other laboratory variables (including fetuin-A and ionized and total magnesium) were measured centrally at baseline and after 12 weeks of treatment.

Results. A total of 96 patients were screened and 78 (55 men and 23 women) met the inclusion criteria and were randomized to receive placebo ($n = 39$) or sevelamer carbonate ($n = 39$). The median patient age was 66 years [interquartile range (IQR) 56–72], the median eGFR was 25 ml/min/1.73 m^2 (IQR 21–30) and the mean T50 was 335 minutes (standard deviation 82). In a linear regression model, T50 was independently associated with serum ionized magnesium, fetuin-A and bicarbonate levels and inversely associated with phosphate concentration. The within-group changes in the mean T50 between week 0 and week 12 were not significant in the sevelamer group or the placebo group {4.6 minutes [95% confidence interval (CI) −13.6–22.8; $P = .61$] and 7.8 minutes [95% CI −16.4–32.1; $P = .51$], respectively}. Furthermore, we did not observe significant changes in fetuin-A and magnesium levels.

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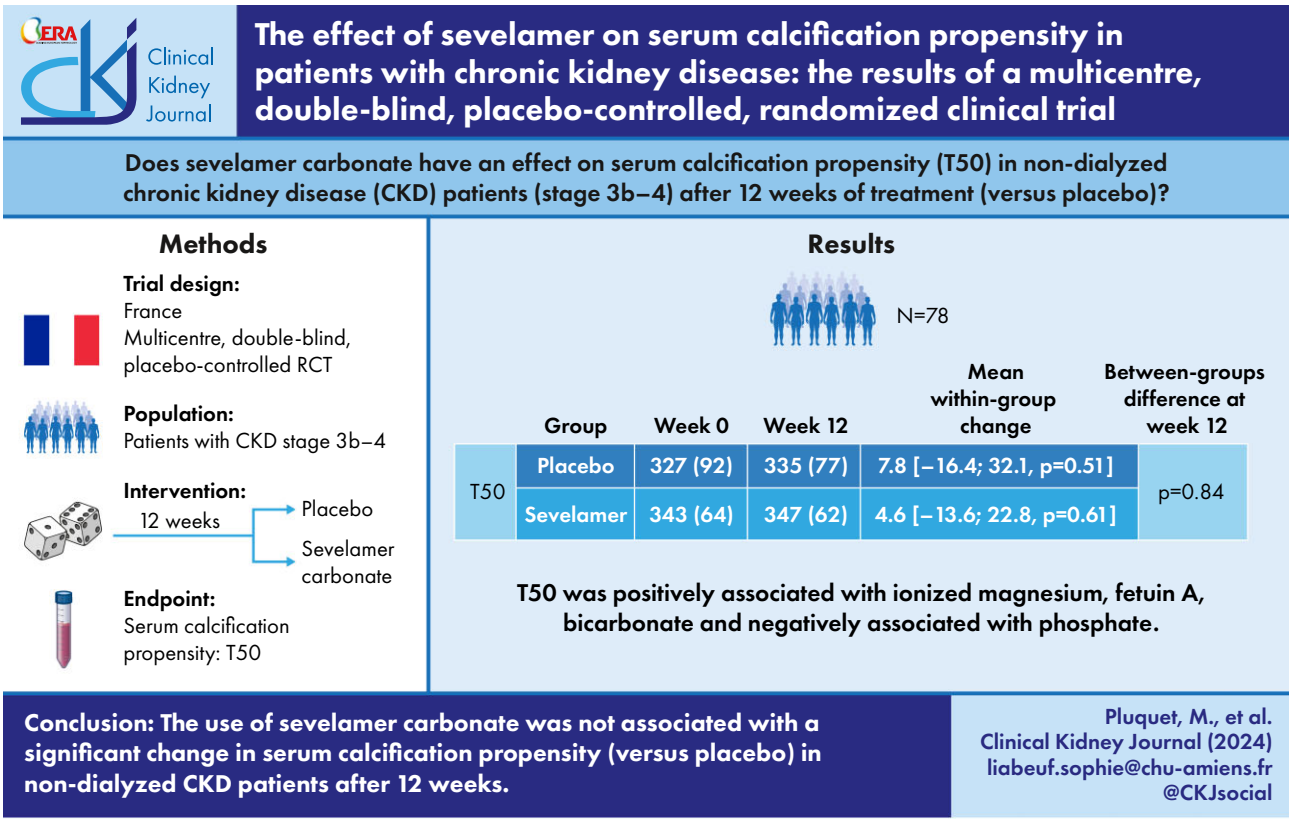
Conclusion. A 12-week course of the non-calcium-containing phosphate binder sevelamer carbonate was not associated with a significant change in T50 in patients with stage 3b/4 CKD. Phosphate binders might not be an effective strategy for modifying serum calcification propensity in non-dialysis-dependent patients with CKD.

GRAPHICAL ABSTRACT



Keywords: calcification propensity, magnesium, phosphate binders, sevelamer, T50

KEY LEARNING POINTS

What was known:

- The serum calcification propensity test (or T50 test) reflects the capacity of an individual's serum to resist the formation of hydroxyapatite crystals when supraphysiological amounts of calcium and phosphate are added.
- T50 is inversely associated with cardiovascular events and all-cause mortality in non-dialysis CKD patients.
- In patients with CKD, the administration of phosphate binders has given conflicting results.

This study adds:

- T50 was associated with serum concentrations of ionized magnesium, fetuin-A and bicarbonate and inversely associated with phosphataemia.
- Sevelamer carbonate had no significant effect on serum calcification propensity in non-dialysis CKD patients compared with placebo.

Potential impact:

- Phosphate binders might not be effective for modifying serum calcification propensity in non-dialysis CKD patients.

INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem worldwide [1]. The signs and symptoms of CKD are closely linked to the progressive decline in the kidney's ability to maintain homeostasis, which leads to alterations in ion balances and mineral metabolism. Although the mortality rate from

cardiovascular disease is significantly higher in patients with CKD than in the general population [2], the underlying pathophysiological mechanisms are not fully understood. Indeed, conventional cardiovascular risk factors do not appear to fully explain the elevated cardiovascular risk in patients with CKD.

On the one hand, vascular calcification contributes to cardiovascular morbidity and mortality in patients with CKD [3], while on the other, CKD increases the severity and progression of vascular calcification [4]. Vascular calcification is a cell-mediated process [5] and features both the passive and active deposition of calcium phosphate in the arterial wall. Under physiological conditions, inhibitors of active mineralization protect blood vessels from the formation of stable hydroxyapatite crystals [6].

Various biomarkers have been identified as potential therapeutic targets or predictors of cardiovascular events and/or vascular calcification [7]. However, these biomarkers are mainly used in research rather than in routine clinical practice. Each marker reflects a single, specific calcification pathway and cannot be used alone to monitor the overall calcification propensity.

Under physiological circumstances, the precipitation of calcium and phosphate in the serum is prevented by the formation of primary calciprotein particles (CPP I), which can then be transformed into more harmful secondary CPP (CPP II). CPP II are internalized by endothelial and vascular cells and induce a pro-inflammatory response, cellular dysfunction, cell death and calcification of vascular smooth muscle cells. In turn, these events contribute to the development of atherosclerosis and vascular calcification [8]. The CPP I to CPP II conversion time reflects the serum's endogenous capacity to prevent the precipitation of calcium phosphate. The serum calcification propensity test (also known as the T50 test) was recently developed as a functional assay of the ability of an individual's serum to resist the formation of hydroxyapatite crystals when supraphysiological amounts of calcium and phosphate ions are added [9, 10].

In the literature, various laboratory variables were positively associated with T50, such as albumin, magnesium, bicarbonate, triglycerides, high-density lipoprotein (HDL)-cholesterol, creatinine, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], 25-hydroxyvitamin D [25(OH)D], fetuin-A and pyrophosphate, or inversely associated with T50, such as phosphate, ionized calcium, parathyroid hormone (PTH), urea and C-reactive protein (CRP) [11–15].

In patients with CKD, the administration of phosphate binders has produced conflicting results, and the majority of studies were performed in dialysis patients. In a small randomized controlled trial (RCT) in haemodialysis patients, the use of calcium carbonate, sevelamer hydrochloride and sevelamer carbonate was associated with a longer T50 [16]. In contrast, the results of a 2-year prospective study of peritoneal dialysis patients showed that the use of sevelamer was not significantly associated with changes in serum calcification propensity [17].

The primary objective of the present post hoc analysis was to evaluate the effect of sevelamer carbonate versus placebo on serum calcification propensity (the change in T50) in non-dialysed patients with CKD (stage 3b/4) after 12 weeks of treatment. The secondary objective was to investigate factors associated with serum calcification propensity (phosphocalcic biomarkers, in particular).

MATERIALS AND METHODS

Study design

The FGF23 Reduction: Efficacy of a New phosphate binder in CHronic kidney disease (FRENCH) study was an interventional, multicentre, double-blind, placebo-controlled RCT. FRENCH included 78 adult patients with CKD stage 3b/4 [estimated glomerular filtration rate (eGFR) 15–45 ml/min/1.73 m^2) and normal phosphataemia [3.1 mg/dl (1.0 mmol/l)–5.6 mg/dl (1.8 mmol/l)]. The primary objective of FRENCH was to investigate the impact of 12 weeks of treatment with sevelamer carbonate on the FGF23 level in non-dialysis-dependent patients with CKD [18]. The present report describes one of the study's secondary objectives, the evaluation of sevelamer carbonate's effect (versus placebo) on levels of phosphocalcic biomarkers.

The main exclusion criteria were the use of phosphate binders, active vitamin D and calcimimetics; kidney transplantation or parathyroidectomy; serum phosphate >1.8 mmol/l (5.6 mg/dl); serum 25(OH)D <20 ng/ml; serum PTH <30 or >600 pg/ml and serum calcium <2.1 mmol/l (8.4 mg/dl).

Patients avoided taking any of the following drugs for the duration of the study: antacids and phosphate binders containing aluminium, magnesium, calcium or lanthanum and treatment of hyperparathyroidism (active vitamin D and calcimimetics). In addition, at randomization, all patients received 100 000 IU of native vitamin D in the form of cholecalciferol.

The study's objectives and procedures were approved by an independent ethics committee (CPP Nord Ouest II, Amiens, France; reference 2010/19, 2 June 2010) and the French Drug Agency (reference A100615-32, 30 July 2010). Each patient gave his/her written informed consent to participate in the study. The study was registered at ClinicalTrials.gov (NCT01220843). Details of the study population and study design have been published elsewhere [18].

Study procedures

After a screening visit, participants completed a 1- to 2-week run-in period during which baseline blood and urine samples were collected. This was followed by a 12-week treatment period. Centralized randomization was performed by the clinical research unit at Amiens-Picardie University Hospital (Amiens, France). Upon randomization, the study personnel and participants were blinded to the use of phosphate binder. The study participants took a dose of 4.8 g of sevelamer or placebo three times a day for 12 weeks. Adherence to study medication was monitored via pill counts [18].

All patients had an interview with a physician (to establish their personal medical history) and underwent a clinical examination. Sociodemographic, clinical and biochemical variables and medications taken by patients were documented. Fasting blood samples were collected after inclusion in the study and after 12 weeks of treatment.

Main outcome evaluation

For the present substudy, blood samples for the T50 test were collected at baseline and after 12 weeks in dry tubes, centrifuged to collect serum, aliquoted and stored frozen at −80°C at the Biobanque de Picardie (Amiens-Picardie University Hospital, Amiens, France). Serum samples were sent to Calciscon (Biel, Switzerland) for measurement of T50. The biological and chemical principles behind this test have been described previously

[9]. Nephelometry is used to detect changes over time in turbidity (light scattering) and thus the transformation of CPP I into CPP II. T50 corresponds to the time at which 50% of the change in relative nephelometric units is observed; the longer the T50, the slower the transition from CPP I to CPP II and the lower the calcification propensity.

Evaluation of other laboratory variables

Ionized magnesium assays were performed in the MP3CV laboratory (Jules Verne University of Picardie, Amiens, France) using direct potentiometry with an ion-selective electrode (Stat Profile PRIME ES Comp; Nova Biomedical, Waltham, MA, USA). In a cohort of healthy patients, the serum ionized magnesium range was 0.43–0.54 mmol/l [19]. In a meta-analysis, the estimated reference range for ionized magnesium was 0.40–0.68 mmol/l for healthy populations and 0.40–0.76 mmol/l in patients with kidney disease [20]. Photometric assays of total magnesium (using a modified xylidyl blue reaction) were performed on the same serum samples at Amiens-Picardie University Hospital (Atellica CH; Siemens Healthcare Diagnostics, Tarrytown, NY, USA; reference range for total magnesium 0.66–1.07 mmol/l). Fetuin-A was assayed with a sandwich enzyme-linked immunosorbent assay (ELISA) [Fetuin-A (AHSG) Human ELISA; BioVendor R&D, Brno, Czech Republic]. During the FRENCH study, the samples were also assayed for C-terminal FGF23 (Human FGF23 C-Terminal ELISA Kit; Immutopics International, San Clemente, CA, USA). Serum intact FGF23 (Human FGF23 Intact ELISA Kit; Immutopics International) and human soluble α-klotho (Human Soluble α-Klotho Assay Kit; Immuno Biologic Laboratories, Fujioka, Japan) were also assayed.

Statistical analyses

The main outcome of interest was the effect of sevelamer carbonate on T50 at 12 weeks. All randomized patients were included in the intention-to-treat (ITT) analysis. The per-protocol analysis included all randomized patients who complied with the protocol for 12 weeks. The treatment groups (sevelamer carbonate versus placebo) were compared. Data for continuous variables were expressed as the mean and standard deviation (SD) or the median and interquartile range (IQR), depending on the distribution. Data for categorical variables were expressed as the frequency and percentage. Intergroup comparisons were performed using Student's *t*-test or the Mann–Whitney test for continuous variables and a chi-squared test or Fisher's exact test for categorical variables.

Correlations between T50 and laboratory variables at baseline (notably serum concentrations of phosphocalcic biomarkers) were measured. The determinants of T50 at baseline were assessed using multivariate linear regression models and expressed as non-standardized coefficients with 95% confidence intervals (CIs) and standardized coefficients. The selection of variables included in the multivariate models was based on literature data and a *P*-value <.2 in the univariate analysis.

The sevelamer and placebo groups were compared with regard to T50 values, serum concentrations of ionized and total magnesium and fetuin-A at week 12 in separate analyses of covariance (ANCOVA), with adjustment for each variable's baseline concentration. A Student's *t*-test was also used to compare the group's T50 values at week 12. Within-group variations in these parameters between baseline and week 12 were assessed using a paired Student's *t*-test or a paired Wilcoxon test.

All tests were two-tailed and the threshold for statistical significance was set to $P < .05$. All statistical analyses were performed with R version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) [21].

RESULTS

Characteristics of the patients at baseline

A total of 78 patients were randomized to receive either sevelamer carbonate ($n = 39$) or placebo ($n = 39$). The ITT population comprised 78 patients. The per-protocol analysis included 31 patients from the sevelamer group and 33 patients from the placebo group (Supplementary Fig. S1). In the ITT population, the mean adherence rate was 88% in the placebo group and 86% in the sevelamer group. There was no change in the prescribed dose of sevelamer during the study period.

At baseline, the two treatment groups did not differ significantly with regard to sociodemographic, clinical and biochemical variables, with the exception of higher 25(OH)D and CRP levels in the sevelamer group than in the placebo group (Table 1). The median patient age was 66 years (IQR 56–72). The median eGFR was 25 ml/min/1.73 m^2 (IQR 21–30), the mean T50 was 335 minutes (SD 82) and the mean phosphate level was 1.24 mmol/l (SD 0.17).

Factors associated with T50

At baseline, serum levels of fetuin-A ($r = 0.60$, $P < .001$), ionized magnesium ($r = 0.27$, $P = .02$), α-klotho ($r = 0.33$, $P = .005$) and bicarbonate ($r = 0.33$, $P = .005$) significantly and positively correlated with T50, whereas serum concentrations of phosphate ($r = -0.25$, $P = .03$) and chloride ($r = -0.23$, $P = .047$) were significantly but inversely correlated with T50 (Supplementary Table S1). In a linear regression model, T50 was independently associated with the serum ionized magnesium, fetuin-A and bicarbonate levels and inversely associated with phosphate concentration (Table 2, Supplementary Table S2).

Effect of sevelamer on T50 and other variables

The within-group change in the mean T50 between week 0 and week 12 was not significant in either treatment group. In the placebo group, the mean change in T50 was 7.8 minutes (95% CI −16.4–32.1) ($P = .51$) (Supplementary Table S3) and in the sevelamer group, the mean change in T50 was 4.6 minutes (95% CI −13.6–22.8) ($P = .61$). There was no difference between the groups in T50 at week 12 ($P = .84$) using an ANCOVA adjusted for the T50 at week 0 (Fig. 1A) or a Student's *t*-test ($P = .61$).

The within-group difference in the mean serum ionized magnesium concentration after 12 weeks of treatment was not significant in the sevelamer group or the placebo group [0.02 mmol/l (95% CI −0.01–0.04), $P = .23$ and 0.01 mmol/l (95% CI −0.01–0.04), $P = .18$, respectively; Supplementary Table S3]. After adjustment for the ionized magnesium concentration at week 0, there was no difference in ionized magnesium between the groups after 12 weeks ($P = .99$) (Fig. 1B).

The within-group difference in the mean serum total magnesium concentration after 12 weeks of treatment was not significant in the sevelamer group or the placebo group [0.01 mmol/l (95% CI −0.02–0.05), $P = .50$ and 0.01 mmol/l (95% CI −0.03–0.06), $P = .60$, respectively; Supplementary Table S3]. Again, after adjustment for the total magnesium concentration at week 0, there

Table 1: Baseline characteristics of the study population.

Characteristics	Total ($N = 78$)	Sevelamer ($n = 39$)	Placebo ($n = 39$)	P-value
T50 (minutes), mean (SD)	335 (82)	331 (74)	339 (91)	.67
Demographics				
Age (years), median (IQR)	66 (56–72)	67 (56–70)	65 (57–74)	.92
Men, %	71	69	72	.80
Body mass index (kg/m^2), mean (SD)	28.0 (5.1)	28.0 (5.3)	28.0 (5.0)	.97
Clinical characteristics				
Diabetes, %	26	33	18	.12
Causes of nephropathy, %				.37
Vascular	27	28	26	
Polycystic kidney disease	14	21	8	
Glomerulonephritis	10	8	13	
Interstitial nephritis	6	5	8	
Other	23	15	31	
Systolic blood pressure (mmHg), median (IQR)	141 (123–155)	144 (124–154)	140 (124–156)	.76
Diastolic blood pressure (mmHg), mean (SD)	79 (10)	78 (10)	80 (10)	.53
Electrolytes, mean (SD)				
Ionized magnesium (mmol/l)	0.58 (0.10)	0.58 (0.07)	0.58 (0.12)	.97
Total magnesium (mmol/l)	0.77 (0.13)	0.77 (0.11)	0.77 (0.16)	.95
Phosphate (mmol/l)	1.24 (0.17)	1.23 (0.18)	1.25 (0.16)	.70
Calcium (mmol/l)	2.34 (0.11)	2.35 (0.11)	2.33 (0.11)	.49
Sodium (mmol/l)	140 (2.5)	140 (2.8)	140 (2.3)	.96
Potassium (mmol/l)	4.8 (0.6)	4.7 (0.6)	4.8 (0.6)	.65
Bicarbonate (mmol/l)	24.4 (4.2)	24.5 (3.5)	24.2 (4.9)	.78
Chloride (mmol/l)	106 (4.0)	106 (3.9)	106 (4.2)	.39
Other biological parameters				
eGFR (ml/min/1.73 m^2), median (IQR)	24.9 (20.9–29.9)	24.8 (20.8–27.4)	27.2 (21.0–32.0)	.23
Fetuin-A (g/l), mean (SD)	0.22 (0.05)	0.22 (0.05)	0.22 (0.05)	.91
Klotho (pg/ml), median (IQR)	810 (677–977)	830 (728–899)	784 (653–1050)	.57
Intact FGF-23 (pg/ml), median (IQR)	42.2 (18.8–81.4)	42.2 (22.6–76.9)	40.6 (12.9–86.0)	.75
C-terminal FGF-23 (RU/ml), median (IQR)	157 (120–238)	166 (121–248)	142 (121–202)	.39
Creatinine (µmol/l), median (IQR)	222 (179–267)	224 (187–264)	220 (171–268)	.38
Urea (mmol/l), median (IQR)	16.7 (13.1–21.2)	17.2 (12.5–21.8)	15.8 (13.4–19.4)	.55
Albumin (g/l), mean (SD)	41.7 (3.4)	41.9 (3.1)	41.5 (3.7)	.65
Glucose (mmol/l), median (IQR)	5.4 (4.9–6.7)	5.4 (4.8–6.8)	5.4 (5.0–6.5)	.92
Intact PTH (pg/ml), median (IQR)	97 (61–127)	91 (59–122)	104 (73–137)	.17
25(OH)D (ng/ml), mean (SD)	31.4 (12.3)	34.3 (13.1)	28.5 (10.8)	.03
1,25(OH)$_2$D (ng/ml), median (IQR)	28.0 (22.0–38.8)	26.0 (20.5–39.9)	31.0 (23.7–38.5)	.45
Triglycerides (mmol/l), median (IQR)	1.6 (1.1–2.2)	1.5 (1.0–1.8)	1.7 (1.1–2.8)	.30
Total cholesterol (mmol/l), median (IQR)	4.5 (4.0–5.5)	4.4 (3.9–5.5)	4.5 (4.2–5.3)	.53
HDL-cholesterol (mmol/l), median (IQR)	1.3 (1.1–1.6)	1.3 (1.2–1.6)	1.3 (1.0–1.5)	.55
LDL-cholesterol (mmol/l), median (IQR)	2.3 (1.8–3.0)	2.4 (1.9–3.1)	2.2 (1.7–2.8)	.43
Haemoglobin (g/dl), mean (SD)	12.6 (1.43)	12.7 (1.51)	12.5 (1.34)	.41
CRP (mg/l), median (IQR)	3.1 (2.5–7.8)	4.8 (3.0–10)	3.0 (2.2–4.0)	.02
Osteocalcin (ng/ml), median (IQR)	28.8 (20.0–45.8)	28.2 (17.0–40.9)	31.4 (22.4–54.5)	.46
Indoxyl sulphate (µg/ml), median (IQR)	3.6 (1.3–6.2)	4.3 (1.8–7.1)	3.4 (1.1–5.2)	.12
Para-cresyl sulphate (µg/ml), median (IQR)	10.6 (6.3–17.5)	11.3 (8.0–16.0)	10.3 (5.7–20.9)	.69
Indole acetic acid (µg/ml), mean (SD)	1.04 (0.54)	1.05 (0.49)	1.04 (0.60)	.95
Alkaline phosphatase (IU/l), median (IQR)	71.5 (58.0–87.8)	67.0 (57.5–84.0)	72.0 (59.5–90.5)	.34
GGT (IU/l), median (IQR)	26.0 (19.0–55.5)	27.5 (23.3–58.3)	22.0 (17.0–46.0)	.17
Bone alkaline phosphatase (IU/l), median (IQR)	11.5 (8.6–15)	11.7 (9.0–14)	11.4 (8.6–17)	.90

CRP: C-reactive protein; FGF-23: fibroblast growth factor 23; GGT: gamma-glutamyltransferase; LDL: low-density lipoprotein.

was no difference in total magnesium between the groups after 12 weeks ($P = .96$) (Fig. 1C).

Lastly, there was a small but significant difference in the mean serum fetuin-A concentration after 12 weeks of treatment in the sevelamer group [−0.01 g/l (95% CI −0.02–0.0002), $P = .046$]. There was no significant difference in the mean serum fetuin-A concentration in the placebo group [−0.01 g/l (95% CI −0.02–0.001), $P = .08$; Supplementary Table S3]. After adjustment for the fetuin-A concentration at week 0, there was no difference in fetuin-A between groups after 12 weeks ($P = .97$) (Fig. 1D).

The per-protocol analysis yielded results similar to those of the ITT analysis for T50, ionized magnesium, total magnesium and fetuin-A (Supplementary Table S4, Supplementary Fig. S2).

DISCUSSION

In the FRENCH multicentre, double-blind, placebo-controlled RCT of patients with stage 3b/4 CKD, we did not observe a significant difference in the change in serum calcification propensity when comparing 12 weeks of treatment with seve-

Table 2: Factors associated with T50 at baseline.

	Multivariable model		
Factors	Non-standardized coefficients	P-value	Standardized coefficients[a]
Ionized magnesium (mmol/l)	513.8 (246.2–781.4)	<.001	0.63
Fetuin-A (g/l)	632.3 (282.2–982.4)	<.001	0.37
α-Klotho (pg/ml)	0.04 (−0.01–0.09)	.15	0.14
Indoxyl sulphate (μg/ml)	1.70 (−1.81–5.20)	.33	0.10
Phosphate (mmol/l)	−162.6 (−287.5 to −37.8)	.01	0.33
Albumin (g/l)	2.89 (−2.30–8.07)	.27	0.12
Bicarbonate (mmol/l)	7.74 (0.55–14.9)	.04	0.40
Chloride (mmol/l)	3.78 (−3.09–10.6)	.27	0.18
GGT (IU/l)	−0.12 (−0.06–0.30)	.20	0.12
Osteocalcin (ng/ml)	0.11 (−0.40–0.62)	.67	0.05

GGT: gamma-glutamyltransferase.

[a]The standardized coefficient is measured in standard deviation units and is shown as an absolute value here. A change of 1 SD in a given variable is associated with a change in the 'standardized coefficients' standard deviation of T50.

lamer carbonate versus placebo. Our findings suggest that 3-month sevelamer carbonate therapy might not be an effective strategy for modifying serum calcification propensity in non-dialysis-dependent patients with CKD.

Serum calcification propensity has recently emerged as a promising predictive biomarker of vascular calcification and cardiovascular outcomes. The present study contributed in-depth information on factors associated with serum calcification propensity [10]. Indeed, we found that T50 was correlated positively with serum α-klotho, bicarbonate, ionized magnesium and fetuin-A and inversely with serum phosphate and chloride. In a linear regression model, T50 was independently associated with serum levels of ionized magnesium, fetuin-A and bicarbonate and inversely with phosphate. According to the literature, T50 is associated positively with fetuin-A [11], total magnesium [13, 15] and bicarbonate [12] and inversely with phosphate [11–15]. However, the link observed here between T50 on the one hand and α-klotho and chloride on the other has not previously been reported. Furthermore, the present study is the first to document the association between calcification propensity and ionized magnesium (i.e. the active form of magnesium). Indeed, we found an association between serum ionized magnesium concentration and T50. Magnesium is known to be a calcification inhibitor [22]. Magnesium reduces phosphate uptake in the intestine, inhibits hydroxyapatite crystal formation in the vessel by interfering with amorphous calcium phosphate and CPP I maturation into CPP II and it prevents the transdifferentiation of vascular smooth muscle cells (VSMCs) toward an osteogenic phenotype [23]. However, the literature data are contradictory with regard to a putative influence of magnesium supplementation on the progression of coronary artery calcification in CKD [24, 25]. Although oral magnesium supplementation was associated with a lower serum calcification propensity in one study of CKD patients [26], increasing the magnesium concentration in the dialysate did not lengthen T50 in haemodialysis patients in a recent RCT [27]. Fetuin-A is also one of the main calcification inhibitors and is a key player in the formation of CPP I. Fetuin-A scavenges complexes of calcium phosphate, and its interaction with matrix gla protein (MGP) integrates calcium phosphate into CPP I, preventing calcium phosphate from precipitating [8]. In the original article presenting the T50 test, alkaline pH inhibited the transitional ripening step from CPP I into CPP II, which may explain the link between serum bicarbonate concentration and T50 [9]. Finally, phosphate is known to be one of the main promoters of calcification; at high concentrations, it directly stimulates VSMCs to undergo phenotypic changes that predispose to calcification [28]. In the FRENCH study, the mean T50 was 335 minutes (SD 82); this value is similar that those reported by Eelderink *et al.* [29] for the general population [329 minutes (SD 58)] and found in CKD patients stages II–IV [313 minutes (SD 79)] [30]. In haemodialysis patients, T50 levels are much lower [12]. The T50 values in the CKD stages 3–4 patients in our study might already have been too high to be influenced by sevelamer.

The literature data on the impact of phosphate binder use on serum calcification propensity are contradictory. In a small RCT in haemodialysis patients, calcium carbonate, sevelamer hydrochloride and sevelamer carbonate increased T50 to a similar extent over 24 weeks [16]. In another small RCT in haemodialysis patients with hyperphosphataemia, the use of sucroferric oxyhydroxide significantly increased T50 after 6 weeks [31]. However, in a prospective, randomized pilot study in 60 patients on peritoneal dialysis, 2 years of sevelamer treatment did not yield a significant difference in T50 [17]. In a recent clinical trial in a CKD cohort, lanthanum carbonate was not associated with a reduction in CPP at 96 weeks versus placebo [32]. In our study of non-dialysed CKD patients, a 12-week course of sevelamer carbonate had no influence on T50 versus placebo. A potential influence of phosphate binders on serum calcification propensity might be exerted mainly through an effect on phosphataemia. In our study, the patients were normophosphataemic and sevelamer use had no effect on phosphate levels; only phosphaturia decreased, which perhaps explains (at least in part) the limited effect of sevelamer on T50 [18]. Furthermore, we did not observe significant changes in magnesium levels, and there was no difference in fetuin-A concentrations between both groups after 12 weeks of treatment, suggesting that the decrease in fetuin-A was not due to the sevelamer. In our study, the level of α-klotho was correlated with T50 but did not change after 12 weeks of sevelamer treatment [18]. In the absence of other measurable effects of sevelamer carbonate on mineral metabolism in this trial, the lack of a treatment effect on T50 is consistent with these other observations.

The main strength of the present study is its design: FRENCH is a nationwide, multicentre, double-blind, placebo-controlled RCT. The multicentric design provides a more representative overview of CKD care in France and thus reduces bias due to patient care pratices in a single centre.

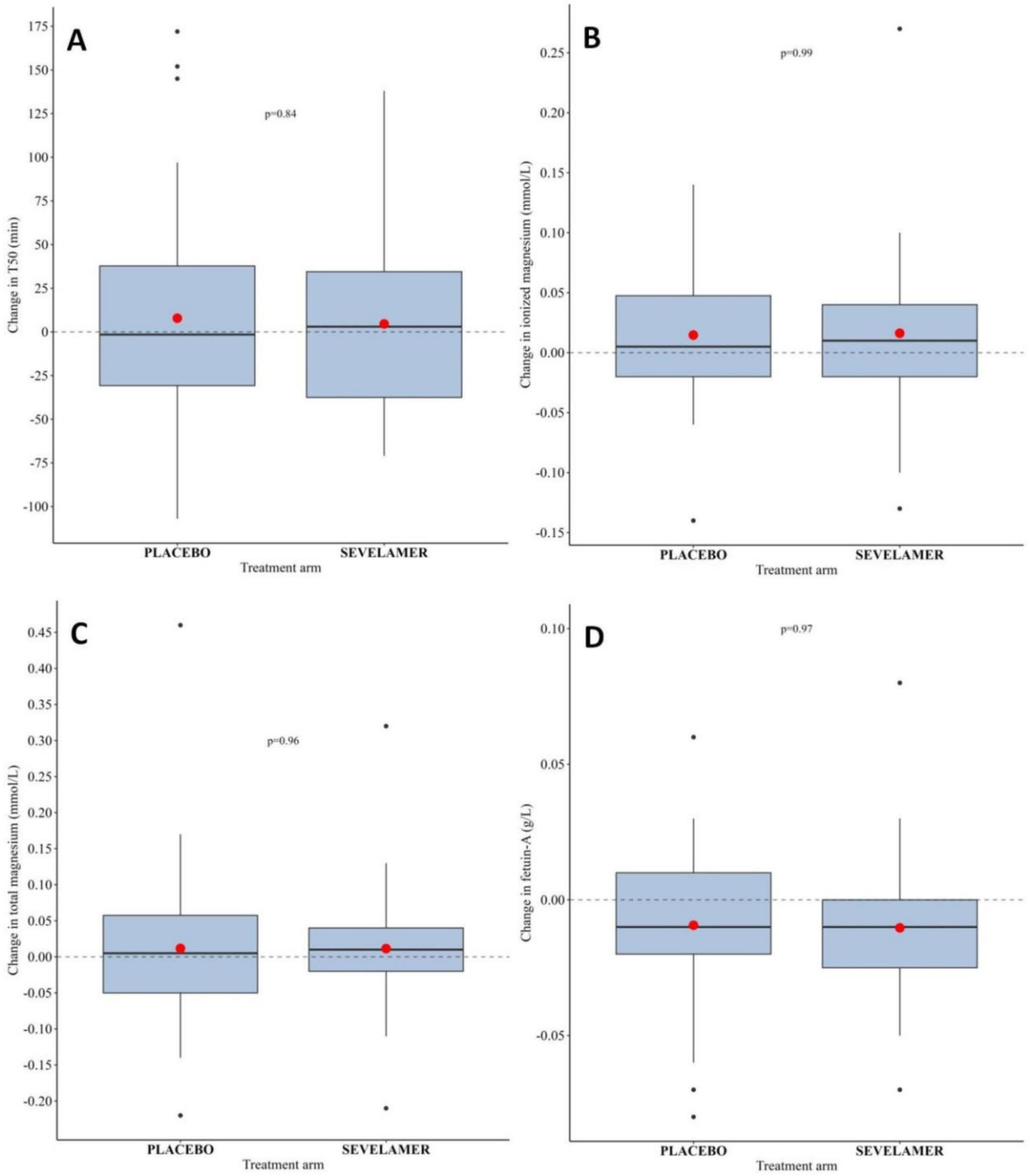


Figure 1: Mean changes in laboratory variables between week 0 and week 12, by treatment group, in an ITT analysis: **(A)** T50, **(B)** ionized magnesium, **(C)** total magnesium and **(D)** fetuin-A. The line within each box indicates the median, the upper and lower boundaries of a box indicate the first and third quartiles and the red dot indicates the mean.

Furthermore, treatment adherence was monitored. This aspect was particularly valuable because study participants had to take up to six pills per day, which might have increased the risk of non-adherence. T50 measurements were performed centrally (by the company that created the calcification propensity test), along with assays of ionized magnesium, total magnesium and fetuin-A; this probably limited variability in the results.

Our study also had some limitations. First, the 12-week course of sevelamer treatment might not have been long enough to produce an effect in the participants. Second, data on food intake and the time of sample collection were not collected routinely in our study, which prevented us from testing for potential associations with these factors. Third, we cannot rule out underdosing, poor compliance (even though 12 weeks of sevelamer treatment led to a decrease in urinary phosphate and cholesterol

levels [18]) and low statistical power (due to the small sample size).

In conclusion, the use of the non-calcium-containing phosphate binder sevelamer carbonate in the FRENCH multicentre, randomized, double-blind, placebo-controlled RCT was not associated with a significant change in serum calcification propensity. Hence, orally administered sevelamer carbonate might not be effective in modifying serum calcification propensity in non-dialysis-dependent patients with CKD.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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AUTHORS' CONTRIBUTIONS

Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work—even in which the author was not directly involved—are appropriately investigated and resolved, including with documentation in the literature if appropriate.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available due to legal restrictions.

CONFLICT OF INTEREST STATEMENT

G.C. was the lead investigator of the FRENCH study and declares that for this trial Sanofi Genzyme provided financial assistance, drugs and placebo. The other authors have no conflicts of interest.

REFERENCES

1. Bikbov B, Purcell CA, Levey AS *et al*. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2020;**395**:709–33. https://doi.org/10.1016/S0140-6736(20)30045-3
2. Go AS, Chertow GM, Fan D *et al*. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004;**351**:1296–305. https://doi.org/10.1056/NEJMoa041031
3. Tian WB, Zhang WS, Jiang CQ *et al*. Aortic arch calcification and risk of all-cause mortality and cardiovascular disease: the Guangzhou Biobank Cohort Study. *Lancet Reg Health West Pac* 2022;**23**:100460. https://doi.org/10.1016/j.lanwpc.2022.100460
4. Moe SM, Chen NX. Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol* 2008;**19**:213–6. https://doi.org/10.1681/ASN.2007080854
5. Shanahan CM. Mechanisms of vascular calcification in CKD—evidence for premature ageing? *Nat Rev Nephrol* 2013;**9**:661–70. https://doi.org/10.1038/nrneph.2013.176
6. Bäck M, Aranyi T, Cancela ML *et al*. Endogenous calcification inhibitors in the prevention of vascular calcification: a consensus statement from the COST action EuroSoftCalcNet. *Front Cardiovasc Med* 2019;**5**:196. https://doi.org/10.3389/fcvm.2018.00196
7. Liabeuf S, Delanaye P, Cavalier É *et al*. Cardiovascular calcification inhibitors. *Ann Biol Clin (Paris)* 2015;**73**:315–22. https://doi.org/10.1684/abc.2015.1047
8. Kutikhin AG, Feenstra L, Kostyunin AE *et al*. Calciprotein particles. *Arterioscler Thromb Vasc Biol* 2021;**41**:1607–24. https://doi.org/10.1161/ATVBAHA.120.315697
9. Pasch A, Farese S, Gräber S *et al*. Nanoparticle-based test measures overall propensity for calcification in serum. *J Am Soc Nephrol* 2012;**23**:1744–52. https://doi.org/10.1681/ASN.2012030240
10. Pluquet M, Kamel S, Choukroun G *et al*. Serum calcification propensity represents a good biomarker of vascular calcification: a systematic review. *Toxins* 2022;**14**:637. https://doi.org/10.3390/toxins14090637
11. Smith ER, Ford ML, Tomlinson LA *et al*. Serum calcification propensity predicts all-cause mortality in predialysis CKD. *J Am Soc Nephrol* 2014;**25**:339–48. https://doi.org/10.1681/ASN.2013060635
12. Pasch A, Block GA, Bachtler M *et al*. Blood calcification propensity, cardiovascular events, and survival in patients receiving hemodialysis in the EVOLVE trial. *Clin J Am Soc Nephrol* 2017;**12**:315–22. https://doi.org/10.2215/CJN.04720416
13. van Dijk PR, Hop H, Waanders F *et al*. Serum calcification propensity in type 1 diabetes associates with mineral stress. *Diabetes Res Clin Pract* 2019;**158**:107917. https://doi.org/10.1016/j.diabres.2019.107917
14. Kim H, Kim AJ, Ro H *et al*. Serum calcification propensity and its association with biochemical parameters and bone mineral density in hemodialysis patients. *Kidney Res Clin Pract* 2022;**42**:262–71. https://doi.org/10.23876/j.krcp.22.059
15. van der Vaart A, Eelderink C, van Goor H *et al*. Serum T50 predicts cardiovascular mortality in individuals with type 2 diabetes: a prospective cohort study. *J Intern Med* 2024;**295**:748–58. https://doi.org/10.1111/joim.13781
16. Smith ER, Pan FFM, Hewitson TD *et al*. Effect of sevelamer on calciprotein particles in hemodialysis patients: the Sevelamer versus Calcium to Reduce Fetuin-A-containing Calciprotein Particles in Dialysis (SCaRF) randomized controlled trial. *Kidney Int Rep* 2020;**5**:1432–47. https://doi.org/10.1016/j.ekir.2020.06.014
17. Wang AYM, Pasch A, Wong CK *et al*. Long-term effects of sevelamer on vascular calcification, arterial stiffness, and calcification propensity in patients receiving peritoneal dialysis: the randomized pilot SERENE (Sevelamer on Vascular Calcification, Arterial Stiffness) trial.

Kidney Med 2022;**4**:100384. https://doi.org/10.1016/j.xkme.2021.10.002
18. Liabeuf S, Ryckelynck JP, Esper NE *et al*. Randomized clinical trial of sevelamer carbonate on serum klotho and fibroblast growth factor 23 in CKD. *Clin J Am Soc Nephrol* 2017;**12**:1930–40. https://doi.org/10.2215/CJN.03030317
19. Rwayane K, Carpentier M, Piver E *et al*. P1594. Ionized magnesium: reference range values in adults patients. *Clin Chem Lab Med* 2023;**61**(Suppl 1):S1588–747. https://doi.org/10.1515/cclm-2023-7056
20. Ansu Baidoo VY, Cara KC, Dickinson SL *et al*. Systematic review and meta-analysis to estimate a reference range for circulating ionized magnesium concentrations in adult populations. *J Nutr* 2023;**153**:3458–71. https://doi.org/10.1016/j.tjnut.2023.10.006
21. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing, 2022. https://www.R-project.org/
22. Hénaut L, Massy ZA. Magnesium as a calcification inhibitor. *Adv Chronic Kidney Dis* 2018;**25**:281–90. https://doi.org/10.1053/j.ackd.2017.12.001
23. ter Braake AD, Shanahan CM, de Baaij JHF. Magnesium counteracts vascular calcification. *Arterioscler Thromb Vasc Biol* 2017;**37**:1431–45. https://doi.org/10.1161/ATVBAHA.117.309182
24. Sakaguchi Y, Hamano T, Obi Y *et al*. A randomized trial of magnesium oxide and oral carbon adsorbent for coronary artery calcification in predialysis CKD. *J Am Soc Nephrol* 2019;**30**:1073–85. https://doi.org/10.1681/ASN.2018111150
25. Bressendorff I, Hansen D, Schou M *et al*. The effect of magnesium supplementation on vascular calcification in CKD: a randomized clinical trial (MAGiCAL-CKD). *J Am Soc Nephrol* 2023;**34**:886–94. https://doi.org/10.1681/ASN.0000000000000092
26. Bressendorff I, Hansen D, Schou M *et al*. Oral magnesium supplementation in chronic kidney disease stages 3 and 4: efficacy, safety, and effect on serum calcification propensity—a prospective randomized double-blinded placebo-controlled clinical trial. *Kidney Int Rep* 2017;**2**:380–9. https://doi.org/10.1016/j.ekir.2016.12.008
27. Cejka D, Thiem U, Blinzler E *et al*. Citrate-buffered, magnesium-enriched dialysate on calcification propensity in hemodialysis patients – the CitMag study. *Kidney Int Rep* 2024;**9**:1765–73. https://doi.org/10.1016/j.ekir.2024.03.023
28. Jono S, McKee MD, Murry CE *et al*. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 2000;**87**:e10–7. https://doi.org/10.1161/01.RES.87.7.e10
29. Eelderink C, te Velde-Keyzer CA, Frenay ARS *et al*. Serum calcification propensity and the risk of cardiovascular and all-cause mortality in the general population. *Arterioscler Thromb Vasc Biol* 2020;**40**:1942–51. https://doi.org/10.1161/ATVBAHA.120.314187
30. Bundy JD, Cai X, Mehta RC *et al*. Serum calcification propensity and clinical events in CKD. *Clin J Am Soc Nephrol* 2019;**14**:1562–71. https://doi.org/10.2215/CJN.04710419
31. Thiem U, Soellradl I, Robl B *et al*. The effect of phosphate binder therapy with sucroferric oxyhydroxide on calcification propensity in chronic haemodialysis patients: a randomized, controlled, crossover trial. *Clin Kidney J* 2021;**14**:631–8. https://doi.org/10.1093/ckj/sfaa154
32. Tiong MK, Smith ER, Pascoe EM *et al*. Effect of lanthanum carbonate on serum calciprotein particles in patients with stage 3–4 CKD—results from a placebo-controlled randomized trial. *Nephrol Dial Transplant* 2023;**38**:344–51. https://doi.org/10.1093/ndt/gfac043

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