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Title Page

EFFECT OF A MEDIUM CUT-OFF DIALYSER ON PROTEIN-BOUND URAEMIC TOXINS AND MINERAL METABOLISM MARKERS IN PATIENTS ON HAEMODIALYSIS

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Conflict of interest statement

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Abstract

Introduction: Haemodialysis (HD) with medium cut-off (MCO) dialysers may expand molecular clearance, predominantly larger middle molecules (molecular weight 25-60 kDa). However, the impact of MCO dialysers on long-term clearance of various other components of the uraemic milieu is unknown. The tRial Evaluating Mid cut-Off Value membrane clearance of Albumin and Light chains in HemoDialysis patients (REMOVAL-HD) provided an opportunity to assess the effect of MCO dialysers on protein-bound uraemic toxins and novel markers of mineral metabolism.

Methods: This exploratory sub-study of REMOVAL-HD evaluated changes in protein-bound solutes (total and free indoxyl sulphate [IS] and p-cresyl sulphate [PCS]) and mineral metabolism markers (intact fibroblast growth factor-23 [iFGF23], fetuin-A and endogenous calciprotein particles [CPP-1 and CPP-2]). Mid-week, pre-HD serum samples were collected at baseline and after 12 and 24 weeks of MCO use in stable adult patients. Change from baseline to week 12 and 24 was estimated using linear mixed effects models.

Findings: Eighty-nine participants were studied (mean age 67±15yrs, 38% female, 51% diabetic, median urine output 200mL/24hrs). Serum iFGF23 was reduced at week 12 compared to baseline (-26.8% [95%CI -39.7, -11.1], p=0.001), which was sustained at week 24 (-21.7% [95%CI -35.7, -4.5], p=0.012). There was no significant change in serum IS, PCS, fetuin-A, CPP-1 or CPP-2.

Discussion: Use of a MCO dialyser over 24 weeks was associated with a sustained reduction in FGF23, while other measured components of the uraemic milieu were not significantly altered. Further studies are required to determine whether FGF23 reduction is associated with improved patient outcomes.

Keywords: protein-bound uraemic toxins, calciprotein particles, fetuin-A, fibroblast growth factor 23 (FGF23), medium cut-off dialyser

Background

A hallmark of chronic kidney disease (CKD) is the increased concentration of inorganic and organic molecules in circulating plasma.[1] This is initially related to progressive retention of molecules usually excreted in the urine. Secondary abnormalities in homeostatic controls, such as mineral metabolism, also evolve and become significant in the milieu of CKD.[2, 3]

Globally, haemodialysis (HD) remains the principal therapy for kidney failure.[4] The implicit goal of HD is to correct circulating levels of excess molecules, primarily through dialysis clearance. High flux (HF) dialysers have become standard in HD and allow rapid clearance of small (<500 Da) water soluble molecules, and some so-called *small* middle molecules (0.5-25 kDa). Medium cut-off (MCO) dialysers are a novel class of dialysis membrane with larger pore sizes than HF membranes. The putative advantage of the MCO dialyser is the permissive passage of an expanded range of molecules to include *large* middle molecules (25-60 kDa), whilst retaining essential molecules with higher molecular weights, such as albumin (66 kDa).[5, 6] Dialysis may affect the milieu of circulating molecules directly through clearance, or indirectly by addressing the drivers of secondary metabolic abnormalities.[7] There are limited data on how MCO membranes affect specific subgroups of biologically significant molecules, such as protein-bound uraemic toxins and novel markers of mineral metabolism.

Protein-bound uraemic toxins, such as indoxyl sulphate (IS) and p-cresyl sulphate (PCS), are a group of molecules that are progressively retained in CKD and thought to mediate deleterious effects.[3, 8] Despite most molecules in this class being of small size (<500 kDa), they are poorly cleared by current dialysis technologies, owing to their protein binding. In advanced stages of CKD, elevated levels of total and free IS and PCS have both been linked to a range of pathological effects, including endothelial damage and cardiovascular disease.[8-10]

Fibroblast growth factor-23 (FGF23) is a bone-derived phosphate and vitamin-D-regulating hormone that rises early in CKD, with the highest circulating levels seen in patients on dialysis.[11, 12] FGF23 has been independently and positively associated with a range of adverse events, including all-cause mortality in patients with and without CKD.[12-14] While its role as

a direct cellular toxin is still debated, FGF23 remains of interest as a potential therapeutic target in dialysis patients.[5, 6]

Fetuin-A is a hepatically-derived protein which has a key role in the prevention of ectopic mineralisation.[15] Fetuin-A buffers the constant flux of calcium and phosphate into the circulation by forming colloidal protein mineral complexes.[16] Clusters of calcium and phosphate ions bind to fetuin-A, forming calciprotein monomers, which may self-aggregate into 30-100 nm diameter primary calciprotein particles (CPP-1) before transitioning into larger and denser secondary calciprotein particles (CPP-2) with a diameter between 100-250 nm.[17] This system frequently becomes dysregulated in CKD. Low serum levels of fetuin-A and elevated levels of CPP have been associated with an increased risk of cardiovascular disease and mortality in dialysis patients.[18-21] Fetuin-A has a molecular weight of approximately 58 kDa, and so may ostensibly be lost during HD with an MCO dialyser, although this has not previously been studied. Given the complex and heterogeneous composition of endogenous CPP,[22] it is also unclear whether HF and MCO dialysers may differentially affect levels of CPP in the circulation.

The tRial Evaluating Mid cut-Off Value membrane clearance of Albumin and Light chains in HaemoDialysis patients (REMOVAL-HD) was a multi-centre device study, primarily examining the safety of MCO dialysers over 6 months in comparison to baseline use of HF dialysers.[23] Here we report the results of a pre-planned exploratory analysis of REMOVAL-HD, evaluating the effects of an MCO membrane on the protein-bound uraemic toxins IS and PCS, as well as the novel mineral metabolism markers FGF23, fetuin-A, CPP-1 and CPP-2.

Materials and Methods

The full protocol and the main results for REMOVAL-HD have been previously published.[23, 24] In brief, REMOVAL-HD was a non-randomised, single-arm device study involving nine sites across Australia and New Zealand. Consenting adult patients who had been treated with HD for at least 12 weeks and had a functioning arteriovenous fistula or graft were eligible. All participants were either anuric or oliguric (< 500 mL/24 hours). Key exclusion criteria were anticipated change in dialysis modality, dialysis site or kidney transplant within the study period; presence of chronic infection or inflammatory condition or currently receiving immunosuppressants; life expectancy < 12 months; and serum albumin < 30 g/L within 4 weeks of screening. Ethical approval was obtained from Institutional Ethics Committees for participating sites and REMOVAL-HD was conducted in accordance with the Declaration of Helsinki.

Participants received in-centre HD three times per week throughout the study. A standardised HF dialyser (Revaclear R400; Baxter Healthcare, Sydney, Australia) was used for a four-week wash-in period, followed by 24 weeks with an MCO dialyser (Theranova 400; Baxter Healthcare, Sydney, Australia). Dialysis prescription remained the responsibility of the patient's usual care provider throughout the study, however sites were instructed to maintain the same dialysis prescription for study duration where possible, and to maintain a target blood flow rate > 300 mL/min and a dialysate flow rate of 500 mL/min. Participants received usual care for other CKD and non-CKD related issues.

Outcome measures

Blood samples were collected at baseline after wash-in but before first MCO dialyser use, and then after 12 and 24 weeks of dialysis with the MCO dialyser. All blood samples were collected pre-dialysis prior to the mid-week session. Blood for IS, PCS, FGF23, fetuin-A and CPP was centrifuged and serum aliquots were stored at -80°C until batch analysis. Other biochemistry was performed locally at an accredited laboratory.

Total and free concentrations of the protein-bound uraemic toxins IS and PCS were analysed by ultra-performance liquid chromatography using a fluorescence detection method (Waters Corporation, Milford, MA, USA).[25] We measured intact-FGF23 (iFGF23) and fetuin-A using commercial ELISA kits (Kainos Laboratories, Tokyo, Japan and R&D Systems, Minneapolis,

USA, respectively) according to the manufacturers' instructions. Serum CPP-1 and CPP-2 were measured using a fluorescent probe-based assay running on a BD FACSVerse flow cytometer (BD Biosciences, San Jose, CA, USA) as previously described,[26] with a minor modification where phosphatidyl serine–binding lactadherin-FITC (Haematologic Technologies Inc., Essex Junction, VT, USA) was substituted for PKH67 reagent.[27]

Statistical Analysis

Baseline characteristics and descriptive data are presented as mean (+/− standard deviation [SD]) or, when skewed, median (interquartile range [IQR]) for continuous variables, and as number and percentage for categorical variables. Shapiro-Wilk test and distribution plots were used to assess normality, and right-skewed data were natural log transformed prior to analysis.

The main outcomes of this secondary analysis were change in pre-dialysis serum levels of total- and free- IS and PCS, iFGF23, fetuin-A, CPP-1 and CPP-2 after 12 and 24 weeks of dialysis with an MCO dialyser compared to baseline. For each molecule of interest, a linear mixed effects model was fitted using a restricted maximum likelihood approach. To test for longitudinal change with MCO dialyser use, time was modelled as a fixed effect factor variable and random intercepts were used for each participant to allow for individual variation and repeated measures. Dunnett's test was used when analysing each of the measures to correct for the multiple comparisons that occurred when comparing each follow-up time point to the common control baseline. For these models, iFGF23, CPP-1 and CPP-2 were natural log-transformed, to ensure that the residuals were normally distributed. To aid interpretation, the regression coefficients for iFGF23, CPP-1 and CPP-2 were exponentiated to obtain estimates of percentage change. All available data at each time point were used for the main analysis, but models were also refitted using only participants with complete data to assess for sensitivity to missing data.

In further exploratory analyses, correlation between longitudinal change (from baseline to 24 weeks) in mineral metabolism markers and other variables were examined using Pearson correlation for normally distributed and Spearman rank correlations for skewed data. For these analyses, only participants with data at baseline and 24 weeks were included and no correction for multiple testing was made. All hypothesis tests were assessed at 5% level of significance. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.,

Cary, NC, USA) and R (version 4.0.2). The linear mixed models were fitted in R using the "lmerTest" package.[28]

Results

The full CONSORT participant flow diagram for REMOVAL-HD has been published elsewhere.[23] Eighty-nine participants completed the four-week wash-in with the standardised HF dialyser and commenced treatment with the MCO dialyser. Participant demographics are listed in Table 1. The mean age was 67 ± 15 years, 38% were female and 51% were diabetic. Forty-five percent of participants were anuric, and among oliguric participants, the median urine output was 200 mL/day with an IQR of 130 – 300 mL/day.

All available samples from each of the 89 participants were included in the analysis. Baseline values for protein-bound uraemic toxins, FGF23, fetuin-A and CPP are displayed in Table 2, and summary data at each timepoint are shown in Figure 1. For each analyte, previously reported serum concentrations in apparently healthy adults, and measured using the same assays as in this study, are also provided in Table 2 for reference.

Protein-bound uraemic toxins

There were no significant changes in total or free levels of IS or PCS after 12 or 24 weeks of MCO use compared to baseline (Table 3).

Intact FGF23

There was a significant reduction in pre-dialysis iFGF23 between baseline and 12 weeks (-26.8% [95% CI -39.7, -11.1], adjusted p=0.001) which was sustained at 24 weeks (-21.7% [95% CI -35.7, -4.5] adjusted p=0.012) (Table 3).

Linear mixed models were refitted with complete cases (n=76) to examine the effect of missing data. This sensitivity analysis restricted to complete cases did not change the main finding of reductions in serum iFGF23 levels at 12 and 24 weeks of MCO use (-27.4% [-41.0, -10.8] adjusted p=0.001 and -21.8% [-36.4, -3.9] adjusted p=0.016 respectively).

Fetuin-A and calciprotein particles

There were no significant changes in pre-dialysis serum fetuin-A, CPP-1 or CPP-2 concentrations between baseline and week 12 or baseline and week 24 (Table 3).

Correlations of mineral metabolism markers with selected variables

The correlations between change in novel mineral metabolism markers and other variables were examined for participants that had data available at both baseline and 24 weeks (Table 4). There was no longitudinal correlation between any of the novel mineral metabolism markers and pre-dialysis serum albumin, urea reduction ratio, haemoglobin or high-sensitivity C-reactive protein (hsCRP).

There were moderate positive correlations between iFGF23 and both CPP-1 (r=0.37, p=0.001) and CPP-2 (r=0.33, p=0.004). Fetuin-A negatively correlated with CPP-2 (r=-0.24, p=0.038) but not CPP-1 (r=-0.07, p=0.563). There was also a moderate correlation between CPP-1 and CPP-2 (r=0.38, p=0.001).

Discussion

We report that use of a MCO dialyser over 24 weeks was associated with a sustained reduction in serum concentrations intact FGF23 in patients on HD, while other measured components of the uraemic milieu were not significantly altered.

Despite advances in dialysis technology and techniques, patients requiring dialysis for kidney failure continue to experience significant excess morbidity and mortality compared to those without CKD.[4] Part of this increased risk may be attributable to retained molecules that are incompletely cleared by current therapies, and may directly mediate toxicity or indirectly drive disordered metabolic processes, such as abnormalities in mineral metabolism. MCO dialysers offer the putative advantage over current HF dialysers of expanding the range of molecules cleared during HD. Clinical efficacy studies of MCO dialysers have mainly examined a selection of molecules which are either thought to be clinically significant, or are chosen to give a representative spread of molecule weights.[23, 29-33] These studies are used to inform predictions about how other molecules with similar physiochemical properties may be affected by a MCO dialyser. However, given the complex, and often inter-related, milieu of abnormalities in CKD, it is important to test these predictions, particularly for molecules that may have significant biological and therapeutic implications.

We report that continuous MCO dialyser use was associated with a sustained reduction in pre-dialysis serum iFGF23 concentration. Intact FGF23 is a middle molecule, with an approximate molecular weight of 32 kDa, and would be expected to be cleared more efficiently through dialysis with a MCO than HF membrane. We did not directly measure FGF23 in spent dialysate and so were not able to confirm that clearance was the mechanism behind this observed reduction. There are limited previous data on circulating FGF23 comparing relative effects of HD with a MCO and a standard HF dialyser. Previous studies by Belmoaz *et al* and by Kim *et al* have suggested that a MCO dialyser is associated with a higher reduction ratio of FGF23 compared to a HF dialyser, implying removal through MCO use.[34, 35] Of note though, neither study demonstrated a reduction in pre-dialysis levels. In addition, the trend towards increased reduction ratio in the study by Kim *et al* did not reach statistical significance, however this was a small study (six patients) and used lower blood flow rates (250 mL/min), both of which may have contributed to the equivocal result. This study also used a C-terminal FGF23 assay, in contrast to the intact assay in our study. MCO

membranes are designed to improve clearance of larger middle molecules (>25 kDa). Given that the C-terminal assay measures both intact FGF23 and the smaller C-terminal fragment (12 kDa), use of this assay may be less discriminatory between these membrane types, although in patients receiving dialysis virtually all circulating FGF23 is thought to be intact.[36, 37]

Hypothetically, the observed reduction in FGF23 with MCO dialyser use could be due to mechanisms apart from direct clearance. For instance, phosphate balance is known to correlate with FGF23 production,[38] and we did not measure serum phosphate or phosphate clearance in this cohort. However, it is worth noting that previous studies have suggested there is minimal, if any, difference between MCO and HF dialyser clearance of small solutes, including phosphate.[31] We observed a correlation between FGF23 and CPP, which has previously been demonstrated.[20, 26, 39] It has also recently been shown that CPP induces FGF23 expression in osteoblasts.[40] However, given there was no significant longitudinal change in CPP-1 or CPP-2 in this study, it is less likely that a link between FGF23 and CPP explains the reduction in FGF23.

FGF23 has consistently and positively been linked to adverse outcomes[12-14] and *in vitro* studies have demonstrated biologically plausible mechanisms of cardiovascular toxicity.[41, 42] Taken together, this adds weight to the assertion that elevated levels of FGF23 in CKD may directly mediate adverse outcomes and makes FGF23 a potential therapeutic target in patients on dialysis. However, previous studies have shown mixed benefits of interventions associated with FGF23 reduction,[43, 44] and further studies are required to determine whether the reductions with MCO dialyser use in this study will result in improved patient outcomes. Indeed, the percentage reductions in intact FGF23 observed here are only modestly greater than the biological variation of this hormone in healthy adults.[37]

We found that MCO dialyser use was not associated with a change in pre-dialysis levels of the protein-bound uraemic toxins IS or PCS. Both IS and PCS are predominantly albumin-bound,[45] and previous models of extracorporeal removal have suggested that their clearances are directly related to the free fractions in circulation.[46] It is conceivable that if dialysis membrane types vary in albumin loss, then this could indirectly affect clearance of these compounds. Albumin loss could have the dual effects of removing IS and PCS bound to lost albumin and also reducing the circulating pool of albumin thereby increasing the free fraction

available for diffusion across a dialysis membrane. Given that we did not observe a decrease in albumin in this study,[23] it is not unexpected that IS and PCS levels were also unchanged.

Pore size is a key determinant of the range of molecules that can be cleared by a dialysis membrane. MCO dialysers are specifically manufactured to have a distribution of pore sizes that allow passage of larger middle molecules (25-60 kDa) while restricting loss of albumin (66 kDa).[5, 47] Fetuin-A (58 kDa) has a beneficial role in the prevention of ectopic mineralisation, and given that it has a molecular weight within the upper limit of the cut-off range of a MCO dialyser, it is reassuring that pre-dialysis levels were unchanged across the study period. The large diameter of CPP (50-100 nm for CPP-1 and 100-250 nm for CPP-2), mean it was unlikely that there would be direct clearance by an MCO membrane (effective pore size 3.0-3.5 nm),[47] and so it is unsurprising that levels were unchanged. However, given the complex composition[22] and metabolic pathways[48] of CPP, it is useful to have empiric evidence to confirm this prediction.

As discussed above, one limitation of this study is that we did not directly measure clearance of the study molecules in spent dialysate, and so can only speculate on mechanisms of change (or lack thereof). However, change in serum levels better reflects time-averaged exposure to the molecule of interest, as it accounts for the effects of intercompartmental sequestration and interdialytic generation.[1] This arguably makes pre-dialysis concentrations a more biologically relevant metric than the kinetics of dialysis clearance in isolation. It is also important to note that while this was a pre-planned sub-study, it is in essence an exploratory analysis, and so caution is required when interpreting results. Specifically, in regards to the main finding of a significant reduction in intact FGF23, while we did adjust for multiple hypothesis testing at week 12 and week 24, we did not adjust for multiple hypothesis tests across the various molecules and so we cannot exclude that this result was due to chance. Finally, this was a study of surrogate markers, and while each has a body of evidence supporting biological relevance, only larger long-term outcome studies can properly examine the potential clinical benefits of HD with a MCO dialyser.

Conclusion

This study found that HD with a MCO dialyser was associated with a sustained reduction of intact FGF23 in serum compared to a HF membrane. There were no changes in other markers of mineral metabolism, including no evidence of loss of the beneficial protein fetuin-A. MCO dialyser use did not appear to have a significant impact on serum concentrations of protein-bound uraemic toxins. Whether the reduction in intact FGF23 is associated with improved patient outcomes requires further assessment in future trials.

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Author contributions

RK, ERS, CAH, EMP, CMH, TDH, MJJ, DWJ and NDT designed the study. RK, CAH, CMH, MJJ, MAR, YJC, MGW, AH, CLN, SS, PFM, and NDT were responsible for recruitment, data collection and management. PAPB is the trial coordinator and LAV is the data manager. EGR, EMP and MKT analysed the data. MKT drafted the manuscript; all authors reviewed and edited the manuscript and approved the final version.

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Figure Legends

Figure 1: Box plot of study molecules before first MCO use and after 12 and 24 weeks. Box shows median and interquartile range.

(CPP, calciprotein particles; Fet-A, fetuin-A; iFGF23, intact fibroblast growth factor-23; IS, indoxyl sulphate; PCS, p-cresyl sulphate).

Tables

Table 1: Participant demographics and baseline characteristics

Participant characteristic (n=89)	
Female sex	34 (38)
Age – yrs	67 ± 15
Ethnicity	
Caucasoid	66 (74)
Aboriginal or Torres Strait Islander	1 (1)
New Zealand Māori	8 (9)
Pacific Islander	4 (4.5)
Asian	6 (7)
Other	4 (4.5)
Body mass index – kg/m^2	28.8 ± 5.8
Primary cause of renal disease	
Diabetic nephropathy	33 (37)
Hypertension/vascular	13 (15)
Glomerulonephritis	10 (11)
Reflux nephropathy	3 (3)
Polycystic kidney disease	6 (7)
Other	19 (21)
Unknown	5 (6)
Urine output	
Anuric	40 (45)
Oliguric (<500mL/day)	49 (55)
Residual urine volume (n=49) – mL/24hr	200 [130 – 300]
Diabetes mellitus	45 (51)
Ischemic heart disease	21 (24)
Smoking (current or former)	46 (52)

Mean ± standard deviation, median [interquartile range] or number (%)

Table 2: Baseline serum concentrations of study analyte

Analyte	**Baseline mean or median**	**Previously reported values in healthy adults for assay***
Indoxyl sulphate (n=86)		
Total - µmol/L	138 [96 – 185]	0.7-6.3[25]
Free - µmol/L	11 [7 – 16]	0.0-0.2[25]
p-cresyl sulphate (n=86)		
Total – µmol/L	209 [121 – 314]	0.0-38.4[25]
Free – µmol/L	13 [5 – 22]	0.1-2.4[28]
Intact FGF23 (n=87) – pg/mL	3,985 [1,414 – 18,362]	42.0 ± 9.9[49]
Fetuin-A (n=86) - mg/L	511 ± 148	303-671[50]
CPP-1 (n=87) – $x10^5$ particles/mL	8.2 [3.7 – 13.9]	2.2-17.3[26]
CPP-2 (n=87) – $x10^4$ particles/mL	4.1 [1.9 – 6.6]	1.7-28.2 [26]

Mean ± standard deviation, median [interquartile range], or range minimum-maximum

*Values are serum concentrations previously reported using the same assay for each analyte in apparently healthy adults

Abbreviations: CPP, calciprotein particles; FGF23, fibroblast growth factor-23

Table 3: – Change in protein-bound uraemic toxins and mineral metabolism markers

	Baseline		Week 12				Week 24			
Analyte	***n***	**Mean or median**	***n***	**Mean or median**	**Change from baseline (95% CI)***	**Change at week 12 p-value ****	***n***	**Mean or median**	**Change from baseline (95% CI)***	**Change at week 24 p-value ****
Protein bound uraemic toxins										
Total IS (µmol/L)	*86*	138 [96 - 185]	*82*	138 [101 – 183]	-2.6 (-18.4, 13.3)	0.91	*78*	136 [96 – 185]	2.3 (-13.8, 18.4)	0.93
Free IS (µmol/L)	*86*	11 [7 - 16]	*82*	11 [7 – 18]	0.3 (-1.4, 2.0)	0.92	*78*	10 [6 – 18]	0.2 (-1.6, 1.9)	0.97
Total PCS (µmol/L)	*86*	209 [121 - 314]	*82*	203 [128 – 280]	-11.5 (-39.5, 16.4)	0.56	*78*	191 [114 – 297]	-13.1 (-41.5, 15.3)	0.49
Free PCS (µmol/L)	*86*	13 [5 - 22]	*82*	13 [6 – 19]	-0.4 (-2.9, 2.1)	0.92	*78*	11 [6 – 21]	-0.4 (-2.9, 2.1)	0.92
Mineral metabolism markers										
iFGF23 (pg/mL)	*87*	3,985 [1,414 – 18,362]	*83*	3,594 [688 – 14,888]	-26.8% (-39.7, -11.1)	**0.001**	*79*	4,526 [844 – 14,657]	-21.7% (-35.7, -4.5)	**0.012**
Fetuin-A (mg/L)	*86*	511 ± 148	*80*	500 ± 155	-11.0 (-47.8, 25.8)	0.73	*77*	496 ± 142	-7.2 (-44.5, 30.2)	0.88
CPP-1 ($x10^5$ particles/mL)	*87*	8.2 [3.8 – 13.9]	*83*	8.2 [4.5 – 14.2]	9.1% (-14.0, 38.3)	0.63	*78*	6.4 [3.6 – 10.8]	-16.0% (-34.0, 7.1)	0.20
CPP-2 ($x10^4$ particles/mL)	*87*	4.1 [1.9 – 6.6]	*83*	4.6 [2.6 – 7.9]	11.4% (-17.6 - 50.7)	0.64	*79*	4.2 [1.3 – 7.8]	-6.4% (-31.1, 27.2)	0.85

Mean ± standard deviation or median [interquartile range]

* Change in analytes compared to baseline, estimated from linear mixed model. For iFGF23, CPP-1 and CPP-2, linear models were fitted to natural log transformed data in order to ensure normal distribution of residuals; thereafter the regression coefficients have been exponentiated into percentage change to aid interpretation.

** Adjusted p-value using Dunnett's post estimation test

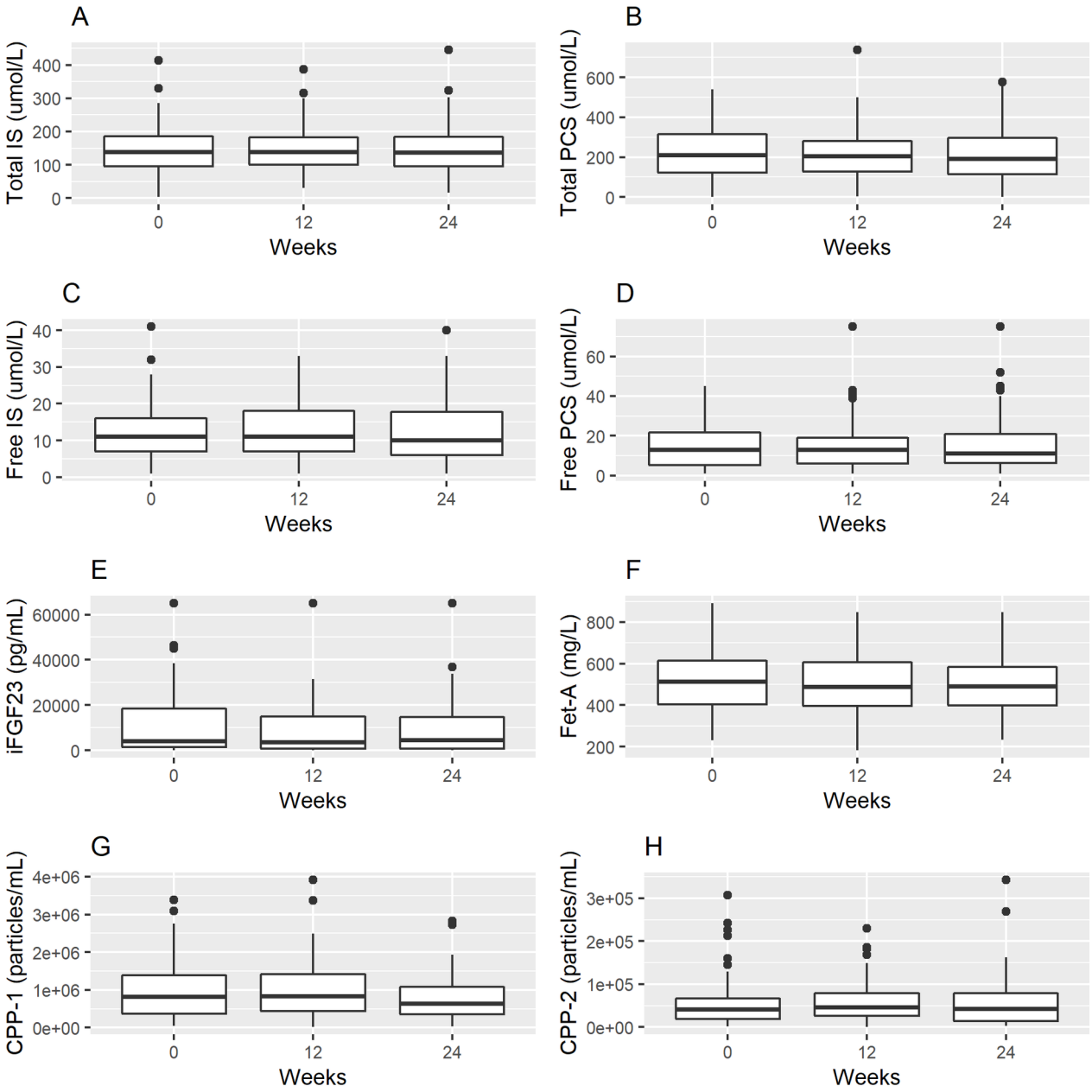
Abbreviations: CI, confidence interval; CPP, calciprotein particles; iFGF23, intact fibroblast growth factor-23; IS, indoxyl sulphate; PCS, p-cresyl sulphate.

Table 4: Correlation between longitudinal change (baseline to 24 weeks) in novel mineral markers and selected variables

	Change in iFGF23		**Change in fetuin-A**		**Change in CPP-1**		**Change in CPP-2**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Change in fetuin-A	-0.08	0.481	-	-	-	-	-	-
Change in CPP-1	**0.37**	**0.001**	-0.07	0.563	-	-	-	-
Change in CPP-2	**0.33**	**0.004**	**-0.24**	**0.038**	**0.38**	**0.001**	-	-
Change in albumin	0.01	0.913	0.10	0.387	0.08	0.491	-0.13	0.261
Change in urea reduction ratio	-0.17	0.182	0.07	0.603	-0.03	0.807	-0.09	0.470
Change in haemoglobin	-0.11	0.356	0.04	0.767	-0.17	0.141	0.10	0.381
Change in hsCRP	0.08	0.471	0.07	0.582	0.01	0.929	0.00	0.973

Urea reduction ratio = (pre-dialysis urea – post dialysis urea)/pre-dialysis urea x100%

Abbreviations: CPP, calciprotein particles; hsCRP, high sensitivity C-reactive protein; iFGF23, intact fibroblast growth factor-23



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