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Salt and water retention associated with microinflammation and endothelial injury in chronic kidney disease

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Short title: Salt and water accumulation linked with inflammation and endothelial injury

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38 1. Abstract

39 **Background:** Progressive chronic kidney disease (CKD) inevitably leads to salt and water
40 retention and disturbances in the macro-and microcirculation.

41 **Objectives:** We hypothesise that salt and water dysregulation in advanced CKD may be linked
42 to inflammation and microvascular injury pathways.

43 **Methods:** We studied 23 CKD stage 5 patients and 11 healthy controls (HC). Tissue sodium
44 concentration was assessed using ²³Sodium magnetic resonance (MR) imaging. Hydration status
45 was evaluated using bioimpedance spectroscopy. A panel of inflammatory and endothelial
46 biomarkers was also measured.

47 **Results:** CKD patients had fluid excess (FO) when compared to HC (Overhydration Index: CKD=
48 $0.5\pm 1.9L$ v HC= $-0.5\pm 1.0L$; $p=0.03$). MR-derived tissue sodium concentrations were
49 predominantly higher in the subcutaneous (SC) compartment [median (interquartile range):
50 CKD= 22.4mmol/l (19.4 ; 31.3) v HC= 18.4mmol/l (16.6 ; 21.3); $p=0.03$], but not the muscle
51 (CKD= $24.9\pm 5.5\text{mmol/L}$ v HC= $22.8\pm 2.5\text{mmol/L}$; $p=0.26$) Tissue sodium in both compartments
52 correlated to FO (muscle: $r= 0.63$, $p<0.01$; SC: $r_s= 0.63$, $p<0.01$). CKD subjects had elevated levels
53 of vascular cell adhesion molecule (VCAM; $p<0.05$), tumour necrosis factor-alpha (TNF- α ;
54 $p<0.01$) and interleukin (IL)-6 ($p=0.01$) and lower levels of vascular endothelial growth factor-C
55 (VEGF-C; $p=0.04$). FO in CKD was linked to higher IL-8 ($r=0.51$, $p<0.05$) and inversely associated
56 to E-selectin ($r= -0.52$, $p=0.01$). Higher SC sodium was linked to higher intracellular adhesion
57 molecule (ICAM) ($r_s= 0.54$, $p=0.02$).

58 **Conclusion:** Salt and water accumulation in CKD appears to be linked with inflammation and
59 endothelial activation pathways. IL-8, E-Selectin (in FO) and ICAM (in salt accumulation) may be
60 specifically involved in the pathophysiology and therefore greater understanding of these
61 pathways could aid the management of FO and merit further investigation.

62

63 2. Introduction

64 The progressive decline in renal function that characterises advanced stages of chronic kidney
65 disease (CKD) is also associated with marked derangements in salt and water homeostasis¹. The
66 inability of the kidneys to eliminate salt and water excess leads to their accumulation and
67 predominantly the expansion of the extracellular water (ECW) compartment¹. This
68 subsequently leads to the development of hypertension, tissue oedema and cardiovascular
69 complications²⁻⁶. The mechanistic model for this phenomenon has been derived mainly from
70 transcapillary hydrostatic exchange pathways based on Guytonian principles^{7,8}. The
71 pathophysiology of salt and water dysregulation and cardiovascular injury may be interlinked
72 but has not been well defined in advanced CKD¹. The most abundant salt in the body is sodium
73 and its distribution is predominantly extracellular⁹. Hydrostatic balance of salt and water has
74 been recently challenged with proposed putative alternative mechanisms. For example, in
75 pathological states of sodium excess, homeostasis is also maintained through osmotically-
76 inactive salt storage, predominantly within the proteoglycan matrix of tissues such as the
77 skin^{10,11}.

78
79 The study of sodium distribution independent of hydration states is now feasible through the
80 use of non-invasive magnetic resonance (MR) imaging technique, using a ²³ Sodium (Na) coil<sup>12-
81 14</sup>.

82
83 Sodium may also be associated with pathological effects on the vasculature and the myocardium
84 that extend beyond those linked to water excess. It is well established that a complex interplay
85 exists between a deregulated or activated endothelium and stimulation of inflammatory
86 pathways. In turn, this causes recruitment of inflammatory cells, an up-regulation of adhesion
87 molecules and potentially release of angiogenic growth factors to stimulate a repair process¹⁵.
88 States of fluid excess have been linked with elevated inflammatory markers including C-reactive
89 protein (CRP)¹⁶, cytokines (e.g. interleukins; IL)¹⁷ and vascular adhesion molecules (e.g. vascular
90 cell adhesion molecule-1; VCAM-1)¹⁷, while vascular endothelial growth factor C (VEGF-C) is
91 thought to play a key part in the storage and mobilisation of excess sodium in the body¹⁸.
92 However the link between endothelial dysfunction and inflammation with salt and water
93 balance in CKD, measured by independent techniques remains largely unexplored. Identifying
94 key circulating biomarkers can improve our understanding of the impaired pathways in fluid
95 accumulation in CKD, and potentially influence its overall management.

96
97 We hypothesised that a disturbance in the salt and water balance may be underpinned by an
98 inflammatory response in CKD. Our aim was to study the interactions between salt, water,
99 inflammation and vascular injury in pre-dialysis CKD.

100 **3. Materials and methods**

101 This is a cross-sectional study examining the association between bioimpedance (BIS)-derived
102 hydration indices, tissue sodium concentration, measured through ²³Sodium MR imaging, and
103 cardiovascular biomarkers in a group of patients with advanced CKD and healthy controls. The
104 study received approval by NHS Research Ethics Committee (15/NW/0471). All adult patients
105 with MDRD estimated glomerular filtration rate (eGFR) <15 ml/min (not on dialysis) and ability
106 to consent, were able to take part in the study. Healthy controls, with no history of CKD, were
107 recruited through posters placed at the participating centres. The only exclusion criteria for
108 both groups were contraindication to MR imaging and limb amputation. The latter was to
109 simplify interpretation of bioimpedance measurements. Written informed consent was obtained
110 prior to enrolment to the study. All measurements were performed during a single study visit.

111 **Data collection and assessment of dietary sodium and water intake**

112 Medical and drug histories were obtained directly from the recruited participants and their
113 electronic medical records. Comorbidity was quantified using the Charlson Comorbidity Index
114 (CCI)¹⁹. Participants were asked to maintain a record of their food and fluid intake for 3
115 consecutive days using a NHS-approved food diary, in order to calculate average salt and water
116 intake. Food diary analysis was performed by a single analyst using the CompEat Pro version
117 5.8.0 software (Nutrition Systems, USA).

118 **Laboratory analysis**

119 Serum electrolytes, urea, creatinine, albumin and osmolality were analysed by the NHS
120 laboratories within the participating units. The laboratory reported eGFR using the MDRD
121 formula. A 24hr urine collection was used to measure urine sodium concentration and total
122 sodium excretion.

123 **Fluid status and its compartmental distribution**

124 Total body water (TBW), ECW, intracellular water (ICW), lean tissue index (LTI), fat tissue index
125 (FTI) and overhydration index (OH) were measured using multifrequency bioimpedance (Body
126 Composition Monitor (BCM®), Fresenius Medical Care, Germany). Overhydration was expressed
127 as absolute OH and relative overhydration (OH/ECW)²⁰⁻²².

128 **MR imaging**

129 Participants' left lower limb was imaged using a 3T MR scanner (Philips 3T Achieva). Imaging
130 time was approximately 40 minutes and during this process, both ²³Na and ¹H images were
131 acquired using a dual-tuned ¹H/²³Na head coil (RAPID Biomedical GmbH, Germany). Analysis
132 was performed using Horos Image Viewer Version 1.7 (www.horosproject.org).

133 ***Sodium image acquisition***

134 An adapted version of the protocol developed by Kopp et al.¹² was used to acquire ²³Na images
135 of the left lower leg (between the knee and ankle). The imaging protocol included the following
136 sequence: ²³Na 3D FLASH acquisition, voxel size= 3 mm x 3 mm x 30 mm, field of view= 232 mm
137 x 232 mm x 240 mm, 8 axial slices, flip angle= 90° repetition time= 100 ms, echo time= 1.71 ms.
138 The measured ²³Na signal was taken to reflect total Na concentration, that is, the weighted
139 average of the Na concentrations in the intra- and extracellular spaces, with the weights
140 dependent on the volume fraction and MR relaxation properties of each compartment. A short
141 echo time was used to minimise the influence of the short transverse relaxation by the tissue
142 Na.

143 ***Sodium imaging analysis***

144 To derive the total Na concentration from the MR signal intensity, five saline calibration
145 phantoms were used, each containing a different Na concentration (15, 45, 80, 115 and 150
146 mmol/L) (Figure 1). Regions of interest (ROI) were drawn over the saline phantoms for each
147 scan at three different slice levels. The averages of signal intensity for each phantom were
148 plotted against the respective Na concentrations. The slope and y-intercept of the line-of-best-fit
149 were used to calculate the Na concentration from tissue signal intensity. Three ROIs were then
150 drawn in the muscle and three in the SC, at different slice levels for each scan, and the average
151 Na concentration for compartments was calculated. All images were analysed by two operators
152 and the reported Na concentration for muscle and SC represents the average of the two
153 measurements. The average of the Na concentration of these two tissue compartments was then
154 reported as total body Na concentration. The average method coefficient of variation for signal
155 intensity calibration was 13.9%. The mean inter-reader variation for muscle was
156 $0.07 \pm 3.5 \text{ mmol/L}$ and for SC $-1.21 \pm 3.9 \text{ mmol/L}$.

157 ***24hr ambulatory blood pressure measurement***

158 Blood pressure was assessed using 24hr ambulatory monitoring (ABMP), performed using an
159 oscillometric technique and the Mobil-O-Graph NG device (I.E.M GmbH, Germany).
160 Measurements of BP were obtained at 30-minute intervals during the day and at 60-minute
161 intervals during the night. Readings were analysed using the hypertension management
162 software of the Mobil-O-Graph NG device. Incomplete measurements (not completing the 24hr
163 period) were included for analysis, provided data were spread uniformly across the day.

164 **Biomarker analysis**

165 Blood samples were collected into both serum gel and lithium heparin tubes, centrifuged at
166 1,500g for 10 minutes at 4° C, aliquoted and frozen at -80°C within 30 minutes from collection,
167 prior to analysis.

168 Luminex array technology (Millipore) was used to determine the levels of the following
169 biomarkers in serum: intracellular adhesion molecule-1 (ICAM-1), placental growth factor
170 (PIGF), VCAM, P-selectin, E-selectin, and VEGF receptor 1 (VEGFR1), using human magnetic
171 bead panels (Angiogenesis/Growth Factor [HAGP1MAG-12K], Cardiovascular Disease 2
172 [CVD2MAG-67K], and Angiogenesis [HANG2MAG-12K], according to manufacturer's
173 instructions (Millipore, UK).

174 Tumour necrosis factor-alpha (TNF α), IL-6, IL-8, VEGF-A and VEGF-C were evaluated in plasma
175 using Magnetic Luminex assay (R&D, UK)

176 **Statistics**

177 Cohort characteristics and measurements were interrogated using descriptive epidemiology.
178 Categorical variables were reported as percentages and ratios. Continuous variables were
179 reported as mean (standard deviation), where distribution was normal, and as median (25; 75
180 percentile) when distribution was skewed. Normality of distribution was assessed using the
181 Shapiro-Wilk method. Group comparison was performed primarily between the CKD and HC
182 group. Comparison of categorical variables between the study groups was performed using
183 Pearson's Chi² test. Group comparison of continuous variables with normal distribution was
184 performed using paired t-test, while for variables with skewed distribution, the Mann-Whitney
185 test was used.

186

187 Associations between BIS measurements, sodium MR measurements and cardiovascular
188 biomarkers were interrogated using bivariate correlation. Pearson's correlation was used when
189 variables were evenly distributed, while Spearman's correlation was used when the distribution
190 of variables was skewed. Statistical analysis was performed using IBM SPSS Statistics, version
191 23 (IBM Corp., USA).

192 **4. Results**

193 **Subjects, demographics and fluid intake**

194 Thirty-four participants enrolled in the study (11 HC and 23 CKD). Thirty (10 HC, 20 CKD) were
195 able to complete the MR imaging. Both cohorts were matched for age, sex and ethnicity. The
196 CKD group had significantly more comorbidity (median CCI: HC= 1 v CKD= 3; p< 0.01) and

197 medication burden (median number of medications taken per day: HC= 1 v CKD= 7; p<0.01;
198 Table 1). CKD patients were also differentiated from HC by their biochemical profiles (Table 1).
199 Serum albumin levels in CKD were also lower (CKD 36.6±2.6 g/L v HC 39.0 ±2.1 g/L; p=0.01).

200
201 Despite significantly lower water intake in CKD than HC (median intake in ml/24hr: CKD= 1892
202 v HC= 2617; p= 0.01; Table 1), they had significant fluid excess (OH: CKD= 0.5±1.9L v HC= -
203 0.5±1.0L; p= 0.03; Table 2). MR-derived tissue Na concentrations in CKD were higher than in
204 HC, predominantly in the SC compartment (median concentrations (interquartile range): CKD=
205 22.4 (19.4; 31.3) v HC= 18.4 (16.6; 21.3); p= 0.03). In the muscle compartment Na
206 concentrations were comparable in the 2 cohorts (CKD= 24.9±5.5 mmol/L v HC= 22.8±2.5
207 mmol/L; p=) (Table 2).

208

209 **Cardiovascular biomarkers measurements**

210 CKD participants had a disrupted vascular endothelial and pro-inflammatory biomarker profile
211 (Supplementary table 1). Serum levels of VCAM [median (interquartile range): CKD= 1256.8
212 (997.4; 1495.5) v HC= 965.5 (811.3; 1104.4); p<0.05] and plasma levels of cytokines TNF- α
213 [median (interquartile range): CKD= 0.85 (0.05; 1.71) v HC= 0; p<0.01] and IL-6 [median
214 (interquartile range): CKD= 1.82 (1.3; 2.8) v HC= 1.04 (0.9; 1.6); p= 0.01] were significantly
215 higher in CKD, while the angiogenic factor VEGF-C was significantly lower [median
216 (interquartile range): CKD= 17.3 (0; 75.7) v HC= 64.5 (29.8; 139.6); p= 0.04].

217

218 **Assessing the interaction between fluid excess (FO), tissue Na concentration and** 219 **cardiovascular biomarkers in CKD**

220 Hydration status, defined by the relative overhydration index OH/ECW, correlated with both
221 muscle ($r= 0.63$, $p<0.01$) and SC ($r_s= 0.63$, $p<0.01$) Na concentrations (Figure 2 A and B). It was
222 also associated with high levels of plasma IL-8 ($r_s= 0.51$, $p= 0.02$) and lower levels of serum E-
223 selectin ($r= -0.52$, $p= 0.01$) (Supplementary Table 2, Figure 2 C and D). Muscle Na concentration
224 did not correlate with any cardiovascular measurements or biomarker levels (Supplementary
225 Table 3). On the other hand, SC Na concentration was linked to higher serum ICAM ($r_s= 0.54$, $p=$
226 0.02) (Supplementary Table 4).

227 **5. Discussion**

228 The study demonstrates high prevalence of salt and water accumulation in advanced CKD,
229 utilising ^{23}Na MRI to image tissue sodium and BIS. We also demonstrate that both salt and water
230 accumulation is associated with endothelial activation and pro-inflammatory pathways.
231 Dahlman et al.¹³ have previously shown that CKD patients on dialysis, especially those over 60

232 years old, tend to have both higher levels of muscle and SC tissue sodium concentration when
233 imaged using a similar protocol to the one used in our study. This state of sodium excess is
234 closely linked to FO. Although both muscle and skin sodium are closely linked with the degree of
235 FO, it is likely that there are differences in the pathophysiological process that influence Na
236 stores in these two compartments. Whether these differences are due to the presence of non-
237 osmotically-active sodium stores within the glycosaminoglycan matrix in the SC
238 compartment^{10,11}, is unknown.

239
240 A number of inflammatory and endothelial activation pathways have been linked with both
241 salt^{18,36,37} and water^{16,17} excess and their pathological endpoints^{15,38}. Our previous studies have
242 shown that dialysis-dependant CKD is associated with a disrupted pro-inflammatory biomarker
243 profile and an activated endothelium³⁹. In this study we observed a disturbance in the
244 biomarker profile and salt and water distribution in CKD prior to dialysis initiation, suggesting
245 possible links to progressive azotaemia. The biomarker profile of high relevance to salt and
246 water distribution in this study appears to be predominantly the interleukins and adhesion
247 molecules.

248
249 High levels of IL-8 correlated with overhydration indices. IL-8 can be secreted by vascular
250 endothelial cells and promotes neutrophil transendothelial migration⁴⁰. TNF- α was also
251 elevated in the CKD participants. Together with IL-8, TNF- α is secreted by macrophages as part
252 of the acute phase response of inflammation⁴⁴ and both have been shown to be linked with the
253 microinflammation associated with CKD and haemodialysis^{41,45,46}. Previous reports have
254 suggested that high levels of both TNF- α and IL-8 may be associated with longitudinal changes
255 to the endothelial glycocalyx, imaged by sublingual capillaroscopy, indicating endothelial
256 injury¹⁵. These findings suggest that IL-8 and TNF- α might be implicated in the pathophysiologic
257 pathways of FO and microvascular injury.

258
259 The facilitation of transendothelial migration of neutrophils across the vascular barrier is
260 increased in a pro-inflammatory environment, mediated through the expression of adhesion
261 molecules on endothelial cells⁴⁷. ICAM is such an adhesion molecule and showed a significant
262 correlation specifically to SC Na concentrations in our cohort. By binding to inflammatory cells,
263 ICAM promotes an alteration to the endothelial cell cytoskeleton and increasing vascular
264 permeability and thus facilitating inflammatory cell transendothelial migration⁴⁹. Unlike othe
265 adhesion molecules such as VCAM, ICAM is constitutively expressed in healthy controls, and is
266 maintained at a relatively constant level⁵². However, although it does not require cytokine
267 release for its expression, it is likely that the high levels detected in our patient cohort were
268 elevated further due to the inflammatory milieu and the salt/water imbalance. High ICAM levels

269 have been linked with hypertension⁵³, early CKD⁵³ and haemodialysis-dependant CKD^{39,54}, and
270 has been shown to be a predictor of mortality in both pre- and dialysis-dependant CKD⁵⁴.
271 Although some animal models suggest that high salt intake promotes leukocyte adhesion
272 through ICAM⁵⁵, to our knowledge, high levels of ICAM in CKD have not been previously studied
273 with respect to tissue sodium storage.

274
275 A biomarker that is linked strongly to FO in the CKD cohort is E-selectin. Similar to ICAM, E-
276 selectin is an adhesion molecule⁴⁷ and is expressed by vascular endothelial cells in response to
277 inflammatory cytokines such as TNF- α ⁵², facilitating transmigration of inflammatory cells
278 across the vessel wall⁴⁹. In general, the levels of E-selectin are increased in CKD^{25,56} and are
279 significantly higher in dialysis patients²⁵. Paradoxically, we detected a very strong and unique
280 association of FO with low levels of E-selectin. This phenomenon might support the inverse
281 association between E-selectin and all-cause mortality, cardiovascular mortality and morbidity
282 shown by Malatino et al. in their cohort of CKD patients⁵⁷. The reasons behind this remain
283 unclear but low levels of E-selectin may be a useful discriminatory biomarker in bioinformatics-
284 based prediction models for overhydration states and poor outcomes.

285
286 The current experimental study has some limitations. It is a relatively small cohort and although
287 all of the associations presented have a strong statistical significance ($p < 0.02$), the possibility of
288 type 1 statistical still exist due to the number of measurements performed. Both ²³Na MR and
289 BIS are indirect measurements and as such, have their limitations. Although BIS is an accepted
290 method of the clinical assessment of FO in dialysis patients, it does carry a degree of
291 inaccuracy²³, while ²³Na MRI remains an experimental tool and an indirect measurement¹².

292
293 In conclusion, this study demonstrates that advanced CKD is associated with a high prevalence
294 of salt and water excess and features of endothelial activation and microinflammation. FO in
295 pre-dialysis CKD is associated with progressively higher tissue sodium accumulation in muscle
296 and skin. The fluid excess in body compartments appears to be related to levels of inflammatory
297 biomarkers. The specific biomarker profiles of IL-8 and E-selectin in water balance and ICAM in
298 salt distribution may play a significant and discriminatory role. Further studies to elucidate
299 these pathways are necessary for a better understanding of fluid imbalance and poor
300 cardiovascular outcomes in advanced CKD.

301

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306
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311 NHS, the NIHR or the Department of Health.

312 **Statement of Ethics**

313 The study received approval by NHS Research Ethics Committee (15/NW/0471). All
314 participation was voluntary and subject to the ability to consent. Written informed consent was
315 obtained prior to enrolment to the study. Subjects were enrolled to the study following
316 informed written consent.

317 **Disclosure**

318 GJMP is a shareholder and director in Bioxydyn, a company with an interest in quantitative MRI.

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323 of this manuscript.

324 **Authors Contribution**

325 Authors' contributions are listed below:

326 **NM:** Grant holder, study design, data collection and study measurements, image analysis,
327 statistical analysis, data interpretation and main author to the manuscript.

328 **FMSA:** Laboratory biomarker analysis, review and editing of the manuscript.

329 **DM:** Imaging acquisition and analysis protocol development, manuscript review and editing.

330 **FW:** Laboratory biomarker analysis, data interpretation, review and editing of the manuscript.

331 **LS:** Laboratory biomarker analysis, data interpretation, review and editing of the manuscript.

332 **JA:** Analysis of food diaries, review and editing of the manuscript.

333 **RM:** Review and editing of the manuscript.

334 **AS:** Imaging analysis, review and editing of the manuscript.

335 **PB:** Supervision, facility provision, review and editing of the manuscript.

336 **GJMP:** Supervision, imaging acquisition and analysis protocol development, data interpretation,
337 manuscript review and editing.

338 **YA:** Supervision, facility provision, laboratory biomarker analysis, data interpretation, review
339 and editing of the manuscript.

340 **SM:** Study Chief Investigator, Supervisor, facility provision, data interpretation, review and
341 editing of the manuscript

342

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447 **7. Figures and Tables**

448 **Figure 1. ²³Sodium Magnetic Resonance Image (MRI) of the left lower limb of a patient with**
449 **advanced chronic kidney disease.** Underneath the calf muscle the five saline phantoms are shown.

450

451 **Table 1. Demographic, biochemical and dietary profiles of the study population**

452 Conc= concentration, g= grams, HC= healthy controls, hr= hour, kg= kilogram, L= litre, m= metre, min=
453 minute, ml= millilitre, mmol= millimole, mOsm= milliosmole, Na= sodium. * indicates significance of p-
454 value <0.05.

455

456 **Table 2 Group comparison of bioimpedance measurements and MR-derived tissue sodium**
457 **concentrations.**

458 ABPM= ambulatory blood pressure monitoring, BMI= body mass index,, DBP= diastolic blood pressure,
459 ECW= extracellular water, FTI= fat tissue index, ICW= intracellular water, LTI= lean tissue index, LTM=
460 lean tissue mass, MAP= mean arterial pressure, mmHg= millimetres of Mercury, MRI= magnetic
461 resonance imaging, OH=overhydration index, SBP= systolic blood pressure, TBW= total body water. *
462 indicates significance of p-value <0.05.

463

464 **Figure 2.** Association between the degree of relative overhydration index (OH/ECW) and MR-derived
465 tissue Na concentration for both the muscle and SC compartments in CKD patients (**Panels A and B**).

466 **Panels C and D** demonstrate the association between OH/ECW and the cytokine IL-8 and the adhesion
467 molecule E-selectin in CKD patients. * indicates significance of p-value < 0.05.

Table 1. Demographic, biochemical and dietary profiles of the study population

		Entire Cohort	HC	CKD	p-value
N		34	11	23	
Age		52.8 (SD 10.3)	51.6 (SD 12.7)	53.4 (SD 9.2)	0.641
Sex (Male)		18 (52.9%)	5 (45.5%)	13 (56.5%)	0.545
Ethnicity	White	31 (91.2%)	10 (90.9%)	21 (91.3%)	0.970
	Black	3 (8.8%)	1 (9.1%)	2 (8.7%)	
Charlson Comorbidity Index		2.5 (2-3.3)	1 (0-2)	3 (2-4)	<0.001*
Cardiovascular disease		1 (2.9%)	0	1 (4.3%)	0.483
Diabetes mellitus		4 (11.8%)	1 (9.1%)	3 (13%)	0.738
Hypertension		27 (79.4%)	4 (36.4%)	23 (100%)	<0.001*
Smoking		5 (14.7%)	2 (18.2%)	3 (13%)	0.692
N° of medication		6 (1.8-9)	1 (0-2)	7 (6-9)	<0.001*
N° of blood pressure medication		2 (0-3)	0 (0-1)	3 (2-3)	0.001*
Diuretic treatment		8 (23.5%)	1 (9.1%)	7 (30.4%)	0.170
Serum biochemistry	Na (mmol/L)	140.7 (SD 2.3)	140.6 (SD 2.5)	140.8 (SD 2.2)	0.784
	Potassium (mmol/L)	4.8 (SD 0.6)	4.6 (SD 0.4)	4.8 (SD 0.6)	0.266
	Bicarbonate (mmol/L)	21.9 (SD3.4)	25.2 (SD 2.6)	20.5 (SD 2.6)	<0.001*
	Urea (mmol/L)	17.3 (SD 9.8)	5.1 (SD 1.2)	23.1 (SD 5.8)	<0.001*
	Creatinine (umol/L)	382.7 (SD 204.1)	70.1 (SD 9.8)	452.4 (SD 114.1)	<0.001*
	eGFR (ml/min/1.73m²)	35.3 (SD 35.7)	85.7 (SD 7.5)	11.2 (SD 2.7)	<0.001*
	Albumin (g/L)	37.3 (SD 2,7)	39.0 (SD 2.1)	36.6 (SD 2.6)	0.013*
	Osmolality (mOsm/kg)	303 (292-308)	289 (280-292)	307 (302-308)	<0.001*
Average salt and water dietary intake	Na (mmol/day)	96.8 (SD 34.0)	99.2 (SD 44.4)	95.6 (SD 28,9)	0.774
	Na in grams of salt/day	5.7 (SD 2.0)	5.8 (SD 2.6)	5.6 (SD 1.7)	0.771
	Water (ml/24hr)	1984 (1795-2620)	2617 (2295-3660)	1892 (1734-2160)	0.011*
Average salt and water excretion	N	34	10	23	
	Na Conc (mmol/L)	64.5 (SD 35.1)	79.4 (SD 59.3)	58.0 (SD 14.6)	0.108
	Total Na (mmol/24hr)	97 (81-153)	93.5 (77-262)	114 (80-150)	0.984
	Volume (ml)	2075 (SD 772)	2173 (SD 1052)	2033 (SD 639)	0.639

Conc= concentration, g= grams, HC= healthy controls, hr= hour, kg= kilogram, L= litre, m= metre, min= minute, ml= millilitre, mmol= millimole, mOsm= milliosmole, Na= sodium. * indicates significance of p-value <0.05.

Table 2 Group comparison of bioimpedance measurements, MR-derived tissue sodium concentrations and cardiovascular measurements.

Hydration, blood pressure and Na MRI measurements		Entire Cohort (n=34)	HC (n=11)	CKD (n=23)	p-value
Body Composition	OH (L)	0.2 (SD 1.3)	-0.5 (SD 1.0)	0.5 (SD 1.9)	0.029*
	TBW (L)	35.8 (SD 6.3)	34.5 (SD 6.5)	36.5 (SD 6.3)	0.390
	ECW (L)	16.7 (SD 3.1)	15.6 (SD 3.2)	17.3 (SD 3.0)	0.153
	OH/ECW (%)	0.6 (SD 7.8)	-3.5 (SD 6.3)	2.5 (SD 7.8)	0.032*
	ECW/TBW	0.47 (SD 0.04)	0.45 (SD 0.03)	0.47 (SD 0.04)	0.108
	ECW/ICW	0.89 (SD 0.13)	0.83 (SD 0.09)	0.91 (SD 0.14)	0.089
	LTM (kg)	37.0 (SD 10.2)	36.9 (SD 9.4)	37.0 (SD 10.7)	0.990
	LTI (kg/m ²)	12.87(10.8; 14.5)	11.3 (10.7; 14.5)	12.7 (10.8; 15.6)	0.731
	Fat Mass (kg)	32.5 (SD 15.1)	29.5 (SD 14.8)	34.0 (SD 15.3)	0.420
	FTI (kg/m ²)	15.2 (SD 8.1)	13.9 (SD 7.0)	15.9 (SD 8.6)	0.507
BMI (kg/m ²)	28.4 (25.0; 31.5)	25.1 (22.2; 32.4)	29.8 (26.1; 31.4)	0.204	
Na MRI		30	10	20	
Average Muscle Na Conc (mmol/L)		24.2 (SD 4.8)	22.8 (SD 2.5)	24.9(SD 5.5)	0.257
Average SC Na Conc (mmol/L)		21.2 (17.7; 29.7)	18.4 (16.6; 21.3)	22.4 (19.4; 31.3)	0.031*
24hr ABMP	N	32	10	22	
	SBP (mmHg)	130 (124; 139)	127 (122; 131)	133 (127; 142)	0.064
	DBP (mmHg)	88 (SD 11.8)	78 (SD 13.2)	85 (SD 10.4)	0.104
	MAP (mmHg)	101 (99; 110)	100 (94; 101)	105 (100; 111)	0.051
	% Nocturnal Dip	6.0 (-0.4; 10.3)	8.2 (1.1; 11.2)	4.2 (-3.6; 9.4)	0.170
PWV m/s	N	33	11	22	
		7.6 (SD 1.8)	7.1 (SD 2.2)	7.9 (SD 1.6)	0.247
Capillaroscopy	N	34	11	23	
	PBR 5-25	2.04 (SD 0.26)	1.94 (SD 0.28)	2.08 (SD 0.24)	0.142
	PBR 5-9	0.99 (SD 0.08)	1.00 (SD 0.07)	0.99 (SD 0.08)	0.755
	PBR 10-19	2.19 (SD 0.29)	2.11 (SD 0.33)	2.25 (SD 0.26)	0.225
	PBR 20-25	2.64 (SD 0.46)	2.44 (SD 0.47)	2.74 (SD 0.43)	0.075

ABPM= ambulatory blood pressure monitoring, BMI= body mass index,, DBP= diastolic blood pressure, ECW= extracellular water, FTI= fat tissue index, ICW= intracellular water, LTI= lean tissue index, LTM= lean tissue mass, MAP= mean arterial pressure, mmHg= millimetres of Mercury, MRI= magnetic resonance imaging, OH=overhydration index, PBR= perfused boundary region of the endothelial glycocalyx, PWV= pulse wave velocity, s= second, SBP= systolic blood pressure, TBW= total body water. * indicates significance of p-value <0.05.

