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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Key word: Overhydration, Chronic Kidney Disease, Inflammation, E-selectin, Biomarkers

1. Abstract

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

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

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

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

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

2. Introduction

The progressive decline in renal function that characterises advanced stages of chronic kidney disease (CKD) is also associated with marked derangements in salt and water homeostasis[1]. The inability of the kidneys to eliminate salt and water excess leads to their accumulation and predominantly the expansion of the extracellular water (ECW) compartment[1]. This subsequently leads to the development of hypertension, tissue oedema and cardiovascular complications[2–6]. The mechanistic model for this phenomenon has been derived mainly from transcapillary hydrostatic exchange pathways based on Guytonian principles[7,8]. The pathophysiology of salt and water dysregulation and cardiovascular injury may be interlinked but has not been well defined in advanced CKD[1]. The most abundant salt in the body is sodium and its distribution is predominantly extracellular[9]. Hydrostatic balance of salt and water has been recently challenged with proposed putative alternative mechanisms. For example, in pathological states of sodium excess, homeostasis is also maintained through osmotically-inactive salt storage, predominantly within the proteoglycan matrix of tissues such as the skin[10,11].

The study of sodium distribution independent of hydration states is now feasible through the use of non-invasive magnetic resonance (MR) imaging technique, using a ^{23} Sodium (Na) coil[12–14].

Sodium may also be associated with pathological effects on the vasculature and the myocardium that extend beyond those linked to water excess. It is well established that a complex interplay exists between a deregulated or activated endothelium and stimulation of inflammatory pathways. In turn, this causes recruitment of inflammatory cells, an up-regulation of adhesion molecules and potentially release of angiogenic growth factors to stimulate a repair process[15]. States of fluid excess have been linked with elevated inflammatory markers including C-reactive protein (CRP)[16], cytokines (e.g. interleukins; IL)[17] and vascular adhesion molecules (e.g. vascular cell adhesion molecule-1; VCAM-1)[17], while vascular endothelial growth factor C (VEGF-C) is thought to play a key part in the storage and mobilisation of excess sodium in the body[18]. However the link between endothelial dysfunction and inflammation with salt and water balance in CKD, measured by independent techniques remains largely unexplored. Identifying key circulating biomarkers can improve our understanding of the impaired pathways in fluid accumulation in CKD, and potentially influence its overall management.

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

3. Materials and methods

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

Data collection and assessment of dietary sodium and water intake

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

Laboratory analysis

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

Fluid status and its compartmental distribution

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

MR imaging

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

Sodium image acquisition

An adapted version of the protocol developed by Kopp et al.[12] was used to acquire ^{23}Na images of the left lower leg (between the knee and ankle). The imaging protocol included the following sequence: ^{23}Na 3D FLASH acquisition, voxel size= 3 mm x 3 mm x 30 mm, field of view= 232 mm x 232 mm x 240 mm, 8 axial slices, flip angle= 90^0 repetition time= 100 ms, echo time= 1.71 ms. The measured ^{23}Na signal was taken to reflect total Na concentration, that is, the weighted average of the Na concentrations in the intra- and extracellular spaces, with the weights dependent on the volume fraction and MR relaxation properties of each compartment. A short echo time was used to minimise the influence of the short transverse relaxation by the tissue Na.

Sodium imaging analysis

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

24hr ambulatory blood pressure measurement

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

Biomarker analysis

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

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

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

Statistics

Cohort characteristics and measurements were interrogated using descriptive epidemiology. Categorical variables were reported as percentages and ratios. Continuous variables were reported as mean (standard deviation), where distribution was normal, and as median (25; 75 percentile) when distribution was skewed. Normality of distribution was assessed using the Shapiro-Wilk method. Group comparison was performed primarily between the CKD and HC group. Comparison of categorical variables between the study groups was performed using Pearson's Chi^2 test. Group comparison of continuous variables with normal distribution was performed using paired t-test, while for variables with skewed distribution, the Mann-Whitney test was used.

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

4. Results

Subjects, demographics and fluid intake

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

medication burden (median number of medications taken per day: HC= 1 v CKD= 7; p<0.01; Table 1). CKD patients were also differentiated from HC by their biochemical profiles (Table 1). 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).

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

Cardiovascular biomarkers measurements

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

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

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

5. Discussion

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

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

A number of inflammatory and endothelial activation pathways have been linked with both salt[18,36,37] and water[16,17] excess and their pathological endpoints[15,38]. Our previous studies have shown that dialysis-dependant CKD is associated with a disrupted pro-inflammatory biomarker profile and an activated endothelium[39]. In this study we observed a disturbance in the biomarker profile and salt and water distribution in CKD prior to dialysis initiation, suggesting possible links to progressive azotaemia. The biomarker profile of high relevance to salt and water distribution in this study appears to be predominantly the interleukins and adhesion molecules.

High levels of IL-8 correlated with overhydration indices. IL-8 can be secreted by vascular endothelial cells and promotes neutrophil transendothelial migration[40]. TNF-α was also elevated in the CKD participants. Together with IL-8, TNF-α is secreted by macrophages as part of the acute phase response of inflammation[44] and both have been shown to be linked with the microinflammation associated with CKD and haemodialysis[41,45,46]. Previous reports have suggested that high levels of both TNF- α and IL-8 may be associated with longitudinal changes to the endothelial glycocalyx, imaged by sublingual capillaroscopy, indicating endothelial injury[15]. These findings suggest that IL-8 and TNF-α might be implicated in the pathophysiologic pathways of FO and microvascular injury.

The facilitation of transendothelial migration of neutrophils across the vascular barrier is increased in a pro-inflammatory environment, mediated through the expression of adhesion molecules on endothelial cells[47]. ICAM is such an adhesion molecule and showed a significant correlation specifically to SC Na concentrations in our cohort. By binding to inflammatory cells, ICAM promotes an alteration to the endothelial cell cytoskeleton and increasing vascular permeability and thus facilitating inflammatory cell transendothelial migration[49]. Unlike othe adhesion molecules such as VCAM, ICAM is constitutively expressed in healthy controls, and is maintained at a relatively constant level[52]. However, although it does not require cytokine release for its expression, it is likely that the high levels detected in our patient cohort were elevated further due to the inflammatory milieu and the salt/water imbalance. High ICAM levels

have been linked with hypertension[53], early CKD[53] and haemodialysis-dependant CKD[39,54], and has been shown to be a predictor of mortality in both pre- and dialysis-dependant CKD[54]. Although some animal models suggest that high salt intake promotes leukocyte adhesion through ICAM[55], to our knowledge, high levels of ICAM in CKD have not been previously studied with respect to tissue sodium storage.

A biomarker that is linked strongly to FO in the CKD cohort is E-selectin. Similar to ICAM, E-selectin is an adhesion molecule[47] and is expressed by vascular endothelial cells in response to inflammatory cytokines such as TNF-α[52], facilitating transmigration of inflammatory cells across the vessel wall[49]. In general, the levels of E-selectin are increased in CKD[25,56] and are significantly higher in dialysis patients[25]. Paradoxically, we detected a very strong and unique association of FO with low levels of E-selectin. This phenomenon might support the inverse association between E-selectin and all-cause mortality, cardiovascular mortality and morbidity shown by Malatino et al. in their cohort of CKD patients[57]. The reasons behind this remain unclear but low levels of E-selectin may be a useful discriminatory biomarker in bioinformatics-based prediction models for overhydration states and poor outcomes.

The current experimental study has some limitations. It is a relatively small cohort and although all of the associations presented have a strong statistical significance ($p<0.02$), the possibility of type 1 statistical still exist due to the number of measurements performed. Both ^{23}Na MR and BIS are indirect measurements and as such, have their limitations. Although BIS is an accepted method of the clinical assessment of FO in dialysis patients, it does carry a degree of inaccuracy[23], while ^{23}Na MRI remains an experimental tool and an indirect measurement[12].

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

Acknowledgement

We would also like to thank the clinical and technical staff at the participating units, the Manchester Renal Research and Transplant Laboratory and the Welcome Trust Manchester Imaging Facilities for their help and support.

The research reported in this publication was supported by the Cancer Research UK and Engineering and Physical Science Research Council Cancer Image Centre in Cambridge and Manchester and the National Institute for Health Research (NIHR) Devices for Dignity Medtech Co-operative. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Statement of Ethics

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

Disclosure

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

Funding Sources

The study received funded through grants from Kidneys for Life, a local charity organisation, and the University of Manchester Magnetic Resonance Imaging Facilities Grant. None of the above funding bodies had any role in the design of the study, analysis of the data of preparation of this manuscript.

Authors Contribution

Authors' contributions are listed below:

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

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

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

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

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

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

RM: Review and editing of the manuscript.

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

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

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

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

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

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7. Figures and Tables

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

Table 1. Demographic, biochemical and dietary profiles of the study population
Conc= concentration, g= grams, HC= healthy controls, hr= hour, kg= kilogram, L= litre, m= metre, min= minute, ml= millilitre, mmol= millimole, mOsm= millliosmole, Na= sodium. * indicates significance of p-value <0.05.

Table 2 Group comparison of bioimpedance measurements and MR-derived tissue sodium concentrations.
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, SBP= systolic blood pressure, TBW= total body water. * indicates significance of p-value <0.05.

Figure 2. Association between the degree of relative overhydration index (OH/ECW) and MR-derived tissue Na concentration for both the muscle and SC compartments in CKD patients **(Panels A and B)**. **Panels C and D** demonstrate the association between OH/ECW and the cytokine IL-8 and the adhesion 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
Nº of medication		**6 (1.8-9)**	**1 (0-2)**	**7 (6-9)**	**<0.001***
Nº 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/1.73m^2)	**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= millliosmole, 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/m²)	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/m²)	15.2 (SD 8.1)	13.9 (SD 7.0)	15.9 (SD 8.6)	0.507
	BMI (kg/m²)	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.