

Hemodialysis Patients with Cardiovascular Disease Reveal Increased Tissue Na⁺ Deposition

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Keywords

Hemodialysis · Cardiovascular disease · Tissue Na⁺ ·
²³Na-magnetic resonance imaging

Abstract

Background: The relationship between Na⁺ balance and cardiovascular disease (CVD) in hemodialysis (HD) patients is not yet fully understood. We hypothesized that HD patients co-diagnosed with CVD show increased tissue Na⁺ accumulation compared to HD patients without CVD. **Methods:** In our observational study, 52 HD patients were divided into a group with (23 subjects) or without (29 subjects) a positive history of cardiovascular events. We used ²³Na-magnetic resonance imaging (²³Na-MRI) at 3.0 Tesla to quantify Na⁺ content in skin and muscle of both groups directly before and after HD. Additionally, total body fluid distribution was determined by bioimpedance spectroscopy (BIS) and laboratory parameters were assessed. **Results:** Compared to HD patients without CVD, ²³Na-MRI detected an increased Na⁺ content in skin (21.7 ± 7.3 vs. 30.2 ± 9.8 arbitrary units (a.u.), *p* < 0.01) and muscle tissue (21.5 ± 3.6 vs. 24.7 ± 6.0 a.u., *p* < 0.05) in patients with previous CVD events. Simultaneously

measured fluid amount by BIS, including excess extracellular water (1.8 ± 1.7 vs. 2.2 ± 1.7 L, *p* = 0.44), was not significantly different between both groups. Tissue Na⁺ accumulation in HD-CVD patients was paralleled by a higher plasma concentration of the inflammation marker interleukin-6 (5.1, IQR 5.8 vs. 8.5, IQR 7.9 pg/mL, *p* < 0.05). **Conclusion:** In our cohort, HD patients with CVD showed higher tissue Na⁺ content than HD patients without CVD, while no difference in body water distribution could be detected between both groups. Our findings provide evidence that the history of a cardiovascular event is associated with disturbances in tissue Na⁺ content in HD patients.

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Introduction

The relationship between salt intake, Na⁺ balance, and cardiovascular disease (CVD) is imperfectly understood [1–3]. Both high and low urinary Na⁺ levels have been

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associated with increased cardiovascular mortality [4]. In patients with chronic kidney disease (CKD), disturbed Na^+ homeostasis has been associated with hypertension as well as CVD [5]. Specifically, a dysfunctional regulation of tissue bound Na^+ appears to play a significant role in developing salt-sensitive hypertension [6–8]. Tissue Na^+ deposition has been discovered as a relevant mechanism of Na^+ -homeostasis alongside extracellular fluid expansion [6]. Patients with end-stage CKD depend on hemodialysis (HD) treatment for Na^+ excretion, thereby regulating blood pressure and reducing CVD risk [9, 10]. However, recent studies suggest that total body Na^+ content behaves independently of Na^+ intake and does not correlate with water retention, weight gain, or renal excretion [11, 12]. A quantifiable marker for Na^+ deposition in tissue therefore would be desirable for a more precise estimation of total body Na^+ content. Implementation of ^{23}Na -magnetic resonance imaging (^{23}Na -MRI) allows quantifying tissue Na^+ content in humans noninvasively [13–18]. In several studies, it could be shown that HD patients are tissue Na^+ overloaded, particularly those co-diagnosed with type 2 diabetes mellitus [15, 19–22]. Furthermore, skin Na^+ content in patients with CKD correlates with left ventricular mass [23], a surrogate factor of CVD. We hypothesized that an association between tissue Na^+ deposition and CVD exists in HD patients and that tissue Na^+ may represent a novel risk marker.

Methods

As tissue Na^+ is age-dependent [14] and cardiovascular events most commonly occur in elderly patients, we conducted an observational study in a cohort of 52 HD patients over the age of 50 years. A further inclusion criterion was HD vintage time of at least 6 months. Exclusion criteria were active malignancy, severe heart failure (NYHA IV), severe liver disease (CHILD C), acute infectious situation, and recent major surgical procedures (<3 months). Additionally, patients with pacemakers, implants, or other MRI contraindications were excluded. Scans were conducted at the Institute of Radiology, University Hospital of Erlangen.

Study Protocol

Fifty-two HD patients ≥ 50 years of age were divided into 2 groups in regard to preexisting cardiovascular events as defined by the American Heart Association [24]. For this purpose, we surveyed medical history, including available echocardiography, duplex sonography of arteries or electrocardiogram, for events of coronary artery disease (myocardial infarction or previous bypass surgery/stent implantation), heart failure (NYHA III), hypertensive heart disease, persisting arrhythmia, history of stroke or TIA, carotid artery stenosis >70%, peripheral arterial disease (stage III/IV), deep venous thrombosis, or pulmonary embolism. All HD

patients with such an event in their medical history were placed in the “cardiovascular group” (HD-CVD, $n = 23$, detailed information is shown in online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000520821). The remaining subjects served as controls (HD-Controls, $n = 29$). Each patient’s dialysis regimen including interdialytic weight gain, residual diuresis, prescribed dialysate composition, and applied ultrafiltration volume were documented. Preceding pre-dialytic ^{23}Na -MRI scans, medical exams determining a subject’s height, weight, blood pressure, and body fluids were performed, followed by a venous blood sample. Blood pressure was measured after 5 minutes of rest in a seated position using an automated oscillometric device (Dinamap; Critikon, Carlsbad, CA, USA) prior to HD. In patients without fistula, blood pressure was analyzed on both upper arms and the arm with higher pressure was chosen. Three consecutive measurements were averaged. Venous blood samples were taken before HD treatment, centrifuged, and stored at -80°C . Plasma determination of interleukin-6 (IL-6) and N-terminal-prohormone of brain natriuretic peptide (NT-proBNP) concentration were performed by electro-chemiluminescence immunoassay, high-sensitive troponin I (hs-TnI) by chemiluminescence assay and high-sensitive C-reactive protein by nephelometric assay. Serum vascular endothelial growth factor-C concentration was determined by enzyme-linked immune-sorbent assays. ^{23}Na -MRI scans were conducted pre-dialysis and assessed for tissue Na^+ content. To analyze tissue Na^+ mobilization by HD treatment, we additionally performed ^{23}Na -MRI scans after HD treatment in 27 HD-control and 23 HD-CVD patients. Na^+ mobilization (ΔNa^+) was calculated by subtracting tissue Na^+ amount after HD from the pre-dialysis tissue Na^+ content.

^{23}Na -MRI Assessment

Na^+ tissue content of the skin and the M. triceps surae were measured in a conventional 3 Tesla ^1H -MRI-scanner (Magnetom Verio or Magnetom Skyra; Siemens Healthineers, Erlangen, Germany), using a volume coil (Stark-Contrast, Erlangen, Germany) positioned around the left calf, centered at the area of the largest circumference. Blood vessels were excluded from the regions of interest (ROI). ^{23}Na -MR images were acquired using a 2D gradient-echo sequence (total acquisition time TA = 13.7 min, echo time TE = 2.07 ms, repetition time TR = 100 ms, flip angle FA = 90, 128 averages, resolution: $3 \times 3 \times 30 \text{ mm}^3$). Images were analyzed using validated software (ImageJ, University of Wisconsin, Madison, WI, USA). Due to the higher resolution, ROI are first outlined in the ^1H -images and subsequently projected onto the ^{23}Na image. Four tubes containing NaCl-solutions (10, 20, 30, and 40 mmol/L Na^+) in the coil base were used to calibrate signal intensities to Na^+ concentrations.

The low in-plane resolution of ^{23}Na -MR imaging results in partial volume effects and thus in an underestimation of the Na^+ skin content, since the skin thickness is approximately 1 mm. In addition, the Na^+ concentration of subcutaneous fat tissue influences the measured skin Na^+ , and the fast decay of the ^{23}Na -MRI signal can result in an underestimation of tissue Na^+ amount. To account for these limitations, we labeled the tissue Na^+ concentration obtained by the calibration as arbitrary units (a.u.).

Bioimpedance Spectroscopy

We used multifrequency bioimpedance spectroscopy (BIS) to noninvasively assess the patient’s fluid status (Body composition

Table 1. Clinical characteristics of HD-CVD and HD-control patients

	HD-control	HD-CVD	<i>p</i> value
Demographics			
Individuals, <i>n</i>	29	23	
Women's quota, %	31	26	
Age, years	62.8±8.8	65.3±7.2	0.28
BMI, kg/m ²	28.1±4.9	28.8±4.0	0.60
Diabetes mellitus, <i>n</i>	5	6	
Hypertension, <i>n</i>	29	23	
BP medication	3.0±1.5	3.0±1.4	0.92
SBP, mm Hg	140±23	132±17	0.17
DBP, mm Hg	75±11	69±12	0.09
HD-related parameter			
HD vintage, years	1.6 (IQR 3.4)	2.5 (IQR 4.8)	0.22
HD technique	24 BHD/5 HDF	20 BHD/3 HDF	
Treatment time, h	4.5 (IQR 0.75)	4.75 (IQR 0.75)	0.39
Residual diuresis, mL/d	500 (IQR 950)	300 (IQR 500)	0.30
IDWG, kg	1.8±1.1	1.8±1.5	0.94
Ultrafiltration, L	2.2±1.1	2.3±1.2	0.71
Dialysate Na ⁺ , mmol/L	138 (IQR 3)	138 (IQR 0)	0.39
Dialysate bicarbonate, mmol/L	32 (IQR 3)	32 (IQR 3)	0.95
Laboratory data			
Plasma Na ⁺ , mmol/L	138±2	139±3	0.19
Plasma K ⁺ , mmol/L	5.6±0.9	5.6±0.8	0.95
hs-CRP, mg/L	3.1 (IQR 3.4)	3.8 (IQR 6.6)	0.51
VEGF-C, pg/ml	3,720±900	3,170±1,380	0.13
BIS data			
Total body water, L	38.2±5.9	40.6±7.7	0.25
ECW, L	18.7±3.2	20.0±3.4	0.18
ICW, L	19.5±3.2	20.6±4.6	0.37
Ratio ECW/ICW	0.96±0.12	0.99±0.11	0.43

Variables are presented as mean ± SD or as median and interquartile range (IQR). BMI, body mass index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; IDWG, Interdialytic Weight Gain; BHD, bicarbonate hemodialysis; HDF, hemodiafiltration; hs-CRP, high-sensitive C-reactive protein; VEGF-C, vascular endothelial growth factor-C; ECW, extracellular water; ICW, intracellular water.

monitor (BCM); Fresenius Medical Care, Bad Homburg, Germany). This technique uses bioelectrical impedance to differentiate between extracellular water and intracellular water. Additionally, it calculates the overhydration (OH), i.e., the excess extracellular fluid compared to healthy individuals of the same age. As we implemented this technique later on in the trial, we only have this information for 25 HD-controls and 22 HD-CVD subjects.

Statistics

All data were analyzed by IBM SPSS statistics (version 24). The Kolmogorov-Smirnov test was used to assess the distribution of our data. All normal distributed data were subsequently analyzed using the independent Student's *t* test, data with a skewed distribution were analyzed using the Mann-Whitney *U* test. Results were expressed as mean ± SD for normally distributed data and as median and IQR for the data lacking a normal distribution. A *p* value <0.05 was considered significant and two-sided tests of hypotheses were used throughout. Pearson's correlation was used to compute the correlation coefficient in normally distributed samples and

Spearman's correlation in non-normally distributed samples. For all correlations, the Bonferroni correction was applied and an adjusted **p* value (*p* value <0.05/*n*; *n* = 5) <0.01 was considered significant while two-sided tests of hypotheses were used throughout.

Results

All demographic data of our study are outlined in Table 1. There were no differences in age, body mass index, blood pressure values, or relevant dialysis-specific parameters. Detailed information on primary renal disease, prescribed antihypertensive, antidiabetic, and HD-related medication is listed in online supplementary Table 2. Figure 1a shows representative ²³Na-MR images obtained before HD treatment of a HD-

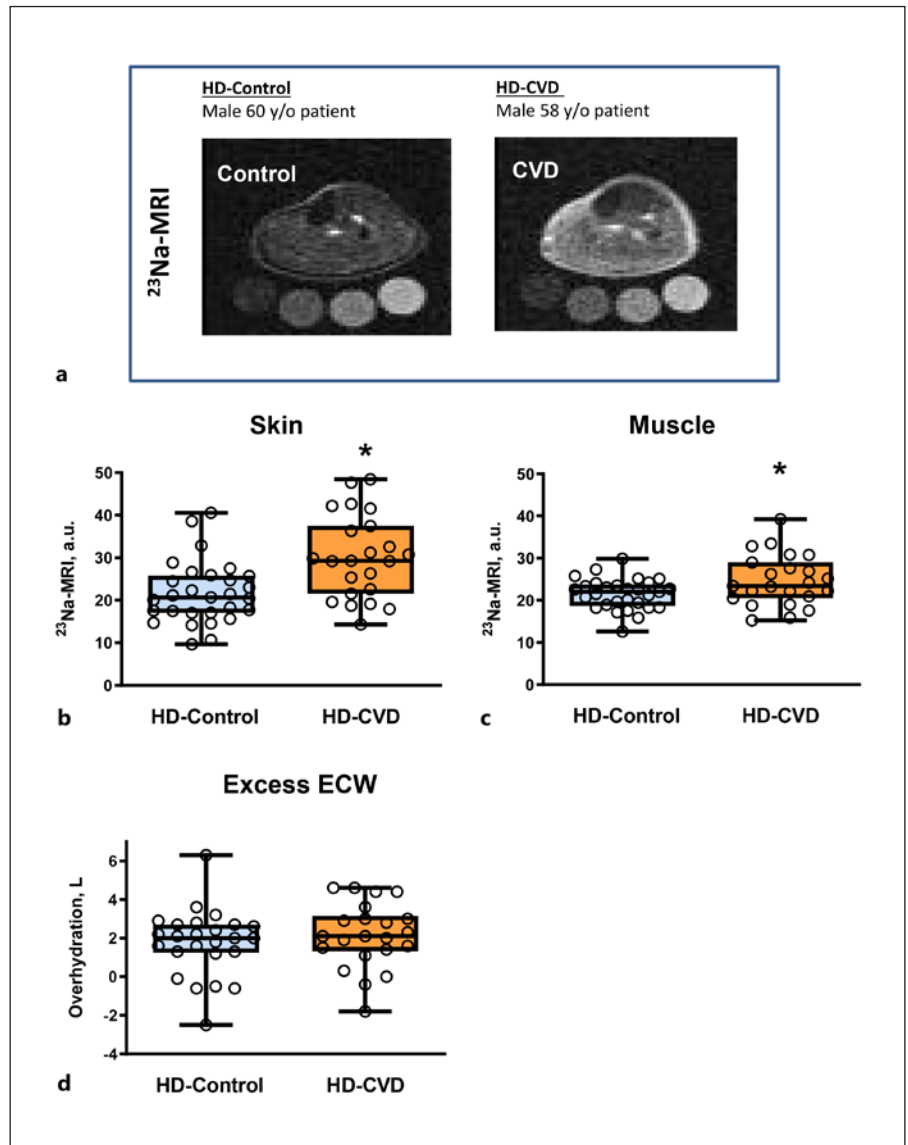


Fig. 1. Elevated tissue Na⁺ content in HD-CVD patients. **a** Representative ²³Na-MRI of the left calf. Left panel: male HD-control patient, 60 years old. Right panel: male HD-CVD patient, 58 years old. Calibration tubes with 10, 20, 30, and 40 mmol/L NaCl are placed below the leg; the brightness of the resonance signal reflects the Na⁺ amount. HD-CVD patients (orange box, *n* = 23) showed significantly higher skin (**b**) and muscle (**c**) Na⁺ content compared with HD-control patients (blue box, *n* = 29) as determined by ²³Na-MRI. **d** No significant difference of excess extracellular volume between HD-CVD patients (*n* = 22) and HD-controls (*n* = 25). **p* < 0.05; OH, overhydration.

CVD patient (right panel) and an age- and gender-matched HD-control patient (left panel), indicating a higher Na⁺ signal intensity in the HD-CVD patient. Means of all ²³Na-MRI scans conducted prior to HD treatment, as shown in Figure 1b and c, revealed that HD-CVD patients presented with significantly higher Na⁺ contents in skin (21.7 ± 7.3 vs. 30.2 ± 9.8 a.u., *p* < 0.01), as well as in muscle tissue (21.5 ± 3.6 vs. 24.7 ± 6.0 a.u., *p* < 0.05). The obtained fluid status detected by BIS did not render a significant difference in excess extracellular volume (OH) 1.8 ± 1.7 versus 2.2 ± 1.7 L, *p* = 0.44, as shown in Figure 1d. In Figure 2 laboratory results of both groups reveal no difference in plasma concentrations of NT-proBNP (HD-control: 3,410

[IQR 16,360] vs. HD-CVD: 6,860 [IQR 25,690] pg/mL, *p* = 0.24, Fig. 2a); whereas hs-TnI levels were higher in the HD-CVD group (8.8 [IQR 5.6] vs. 12.4 [IQR 11.9] pg/mL, *p* < 0.01, Fig. 2b). The observed Na⁺ accumulation in HD-CVD patients was accompanied by elevated plasma concentrations of the inflammatory cytokine IL-6 in the HD-CVD group (5.1 [IQR 5.8] vs. 8.5 [IQR 7.9] pg/mL, *p* < 0.05, Fig. 2c), while high-sensitive CRP did not show a significant difference (Table 1).

Table 2 presents correlations of tissue Na⁺ content with HD-related parameters as well as laboratory values of our HD cohort (combined in A and group specific in B/C). Besides the known age-dependency, we found correlations between tissue Na⁺ content and OH in all sub-

Fig. 2. Cardiovascular and inflammatory parameters. **a** No difference in NT-proBNP plasma levels between both groups (logarithmic scale, HD-CVD patients $n = 23$, HD-controls $n = 24$). **b** Elevated hs-TnI plasma levels in HD-CVD patients ($n = 23$) compared to HD-controls ($n = 24$). **c** Higher IL-6 plasma concentrations in HD-CVD patients ($n = 23$) compared to HD-controls ($n = 24$). $*p < 0.05$.

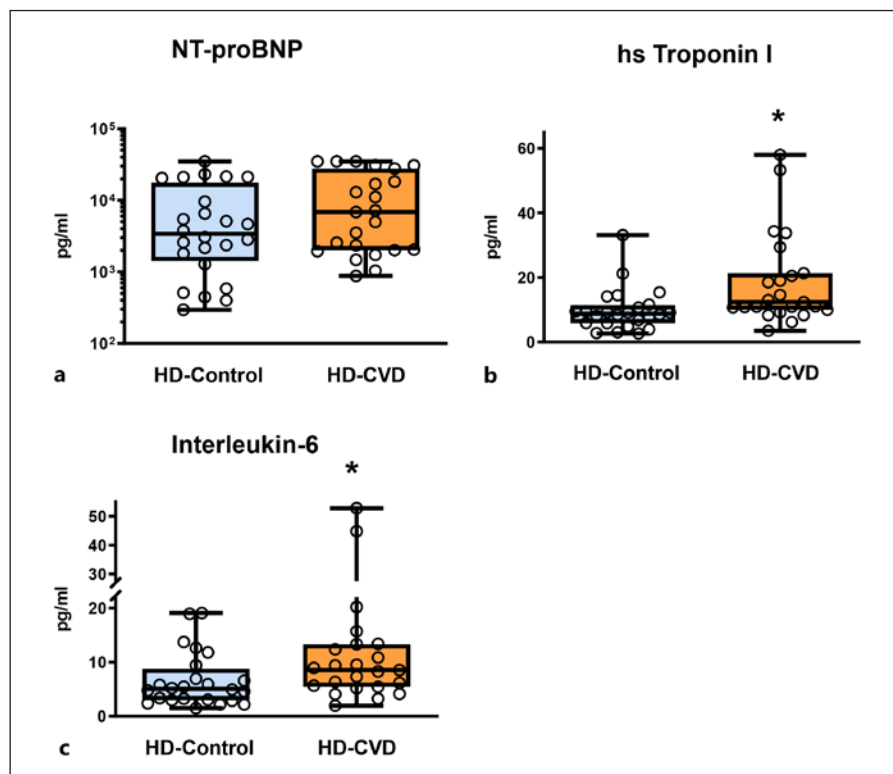
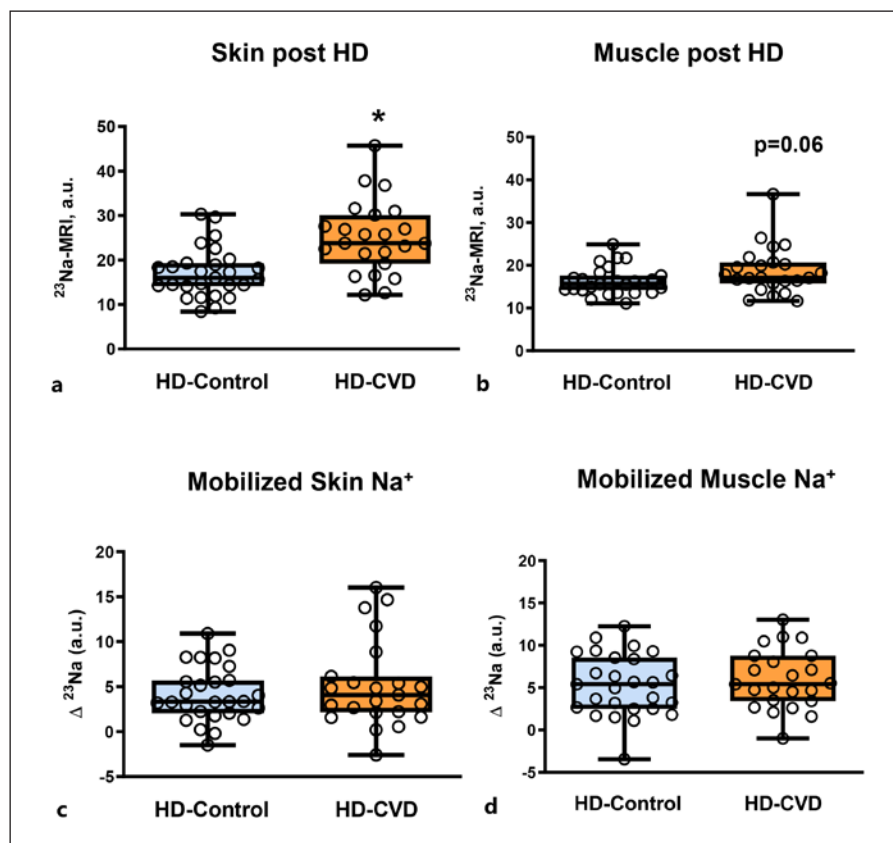


Table 2. Correlation analysis between skin and muscle sodium content with age, HD-related and laboratory parameter, combined HD cohort (A), HD-controls (B) and HD-CVD patients (C)

Parameters	Muscle Na ⁺ <i>r</i> (95% CI)	<i>p</i> value	Skin Na ⁺ <i>r</i> (95% CI)	<i>p</i> value
A. Combined HD cohort (CVD + controls)				
Age	0.312 (0.043; 0.539)	0.024	0.504 (0.268; 0.683)	0.000
OH	0.585 (0.358; 0.747)	0.000	0.403 (0.131; 0.619)	0.005
HD vintage	0.047 ^a (-0.229; 0.316)	0.740	0.139 ^a (-0.139; 0.397)	0.327
NT-proBNP	0.257 ^a (-0.033; 0.507)	0.081	0.285 ^a (-0.002; 0.529)	0.052
IL-6	0.222 ^a (-0.070; 0.479)	0.133	0.448 ^a (0.185; 0.651)	0.002
B. HD-control group				
Age	0.473 (0.129; 0.716)	0.010	0.556 (0.238; 0.766)	0.002
OH	0.501 (0.132; 0.748)	0.011	0.379 (-0.019; 0.673)	0.062
HD vintage	-0.126 ^a (-0.471; 0.252)	0.516	-0.098 ^a (-0.448; 0.279)	0.613
NT-proBNP	0.487 ^a (0.104; 0.744)	0.016	0.316 ^a (-0.100; 0.638)	0.133
IL-6	0.156 ^a (-0.264; 0.526)	0.468	0.419 ^a (0.019; 0.704)	0.041
C. HD-CVD group				
Age	0.137 (-0.292; 0.520)	0.533	0.451 (0.048; 0.728)	0.031
OH	0.661 (0.332; 0.847)	0.001	0.420 (-0.002; 0.715)	0.052
HD vintage	0.208 ^a (-0.223; 0.571)	0.341	0.323 ^a (-0.103; 0.649)	0.132
NT-proBNP	0.002 ^a (-0.411; 0.414)	0.993	0.244 ^a (-0.187; 0.596)	0.261
IL-6	0.220 ^a (-0.211; 0.580)	0.312	0.449 ^a (0.045; 0.727)	0.032

Pearson's correlation was used to compute the correlation coefficient (*r*) in normally distributed samples. ^aSpearman's correlation was used to compute the correlation coefficient (*q*) in non-normally distributed samples. Bold *p* values: considered significance adjusted for Bonferroni correction ($* p < 0.01$); OH, overhydration.

Fig. 3. HD induced tissue Na⁺ mobilization. Persistent elevated tissue Na⁺ content in skin (a) and tendency in muscle tissue (b) in HD-CVD patients (orange box, *n* = 23) compared with HD-control patients (blue box, *n* = 27) post-HD treatment. Tissue Na⁺ amount removed during HD treatment (Δ Na⁺) in skin (c) and muscle tissue (d) do not differ between HD-CVD patients (orange box, *n* = 23) and HD-controls (blue box, *n* = 27). **p* < 0.05.



analyses, while IL-6 correlated only with skin Na⁺ content. In contrast, HD-vintage time showed no correlation with tissue Na⁺.

To determine the effect of HD treatment on tissue Na⁺ mobilization, we conducted ²³Na-MRI scans following a regular dialysis procedure. Post-HD scans revealed that – although dialysis was able to remove some of the Na⁺ load – the surplus remained amongst HD-CVD patients, as shown in Figure 3. The difference in Na⁺ content continued to be significant in skin tissue (17.1 ± 5.6 vs. 25.0 ± 8.2 a.u., *p* < 0.001, Fig. 3a), while in muscle tissue data suggested a trend towards Na⁺ accumulation (16.3 ± 3.2 vs. 18.7 ± 5.7 a.u., *p* = 0.06, Fig. 3b). Mobilized tissue Na⁺ amount (Δ Na⁺) was not different between both groups (Fig. 3c, d).

Discussion

Based on recent findings concerning tissue Na⁺ accumulation, fluid overload, and blood pressure regulation, we intended to explore the potential link between tissue Na⁺ deposition and CVD in dialysis patients [25, 26]. This

seems to be particularly relevant as in patients with moderately impaired renal function tissue Na⁺ content is closely correlated to left ventricular hypertrophy, a surrogate parameter of CVD [23]. We focused in our study on elderly HD patients as they are tissue Na⁺ overloaded [15] – and seriously affected by a high risk for CVD [27]. We were able to demonstrate that HD patients with a history of cardiovascular events reveal a pronounced tissue Na⁺ accumulation compared to age-matched HD patients without CVD. Clinical data and HD-related parameter did not differ between groups (Table 1). In particular, HD-vintage time was not different between HD-control and HD-CVD patients, nor was there any correlation between HD-vintage time and tissue Na⁺ content (Table 2). This is especially relevant as longtime HD treatment may lead to an increased CVD risk [28].

Long-term fluid overload is known to be associated with CVD development in dialysis patients [29]. Excess extracellular water (OH) as an indicator for edema could play a causal role in the explanation of increased tissue Na⁺ signals [30]. To explore this hypothesis, we complementary assessed the fluid status of our patients via BIS technique. In studies on dialysis patients, BIS has been

widely utilized to determine fluid status. Increased cardiovascular mortality was thereby associated with even moderate fluid overload [31, 32]. In our study, either HD group presented with a distinct fluid overload (Table 1; Fig. 2d), which correlated to tissue Na^+ content (Table 2). This correlation is in line with previous observations of tissue Na^+ and BIS in dialysis patients [33, 34]. However, in our study, none of the BIS-determined parameters were different between both groups. As the extent of fluid overload did not reflect the CVD risk in our cohort of HD patients, ^{23}Na -MRI detected tissue Na^+ accumulation could turn out to be a more sensitive marker for CVD in HD patients than extracellular fluid assessment using BIS.

To determine the effect of impaired cardiac function on tissue Na^+ deposition we measured the NT-proBNP in both groups. NT-proBNP is released by cardiomyocytes upon ventricular stretch and plays an important role as diagnostic marker for congestive heart failure [35, 36]. Natriuretic BNP is almost always increased in HD patients and has been shown to be of diagnostic value for systolic dysfunction in dialysis patients [37]. However, residual renal function and dialysis modality are important confounders for its interpretation making it difficult to define cutoff values [38]. In our study, no significant difference in NT-proBNP could be detected between HD-CVD and HD-control patients, indicating that Na^+ retention due to heart failure does not explain the observed tissue Na^+ overload in HD-CVD patients.

Recent investigations shed light on a salt-related regulation of the immune system. Increased Na^+ thereby resulted in an exacerbation of autoimmune disease whereas in infectious disease clearance of pathogens was facilitated by higher Na^+ concentrations [39–42]. In HD patients, a close link between subclinical inflammation and predialysis fluid overload is present [43]. Besides water overload, Na^+ might be also relevant for micro-inflammation in patients with CKD [33]. To assess if inflammation is present in our HD-CVD cohort and whether it is associated with Na^+ accumulation, plasma concentrations of the pro-inflammatory cytokine IL-6 were determined. We found higher levels of IL-6 in HD patients with a CVD history that correlated with skin Na^+ amount. Whether tissue Na^+ plays a direct role in the development of chronic inflammation or even CVD in dialysis patients remains unclear and has to be assessed by basic research and prospective interventional trials. We additionally plan a longitudinal study to follow-up our cohort in future.

Our data suggest that HD is able to mobilize tissue Na^+ independently of CVD history. The amount of tissue Na^+

removed by a single HD procedure (ΔNa^+) did not differ between both groups, resulting in a sustained condition of tissue Na^+ overload in HD-CVD patients post-HD (Fig. 3). Therefore, nephrologists should consider additional aspects of Na^+ balance in these patients like dietary salt restriction or adaption of dialysate Na^+ concentration. In future, ^{23}Na -MRI could serve as a noninvasive diagnostic tool in the assessment of individualized dialysis regimens in HD-CVD patients.

We are aware of general limitations of our study. Besides the observational approach, our cohort of HD patients presented with several comorbidities, which might explain outliers in both groups. As the prevalence of asymptomatic coronary artery disease in HD patients is relatively high, we cannot exclude occult forms of CVD in our HD-control group [44]. However, the level of plasma hs-TnI as a marker for cardiac damage was significantly higher in the HD-CVD group, indicating a higher prevalence of coronary artery disease in our CVD cohort [45]. Additionally, solely Caucasians were investigated in our study. Results should therefore not be generalized to all ethnicities, which is important as for instance African Americans are known to accumulate higher amounts of tissue Na^+ [22].

In summary, we present evidence that HD-CVD patients present with elevated tissue Na^+ content, which cannot be mobilized completely by a single HD procedure. In our study, this overload is independent of HD-vintage time, not paralleled by changes in OH or markers for heart failure, but associated with subclinical inflammation detected by IL-6 elevation. Based on these data, we cannot distinguish between “chicken or egg”: Is tissue Na^+ overload involved in the development of CVD or does CVD itself lead to tissue Na^+ retention? Prospective long-term cohort studies have to reveal whether CVD is causal for Na^+ deposition or vice versa and further continuative basic research is required to identify underlying mechanisms.

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Statement of Ethics

The local Ethics Committee of the University of Erlangen-Nürnberg, Germany approved the study (No. 3948 and 271_17B), which was conducted according to the declaration of Helsinki principles. All participants provided written informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.D. and C.K. conceived and designed the study. P.L. implemented, conducted, and analyzed the MRI measurements. A.-C.F., S.H., and D.R. enrolled the participants, conducted the BCM measurements, conceived and carried out laboratory tests, and collected the data. A.D. and C.K. analyzed and interpreted the data. A.-C.F. drafted the manuscript, A.D. and C.K. contributed to the writing of the manuscript. M.U., A.M.N., and M.S. contributed to the study design and critically revised the manuscript. All authors approved the final version of the manuscript.

Data Availability Statement

Full data set is provided on request by Anke Dahlmann (anke.dahlmann@uk-erlangen.de).

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