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RESEARCH ARTICLE

Sodium storage in human tissues is mediated by glycosaminoglycan expression

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Fischereder M, Michalke B, Schmöckel E, Habicht A, Kunisch R, Pavelic I, Szabados B, Schönermarck U, Nelson PJ, Stangl M. Sodium storage in human tissues is mediated by glycosaminoglycan expression. *Am J Physiol Renal Physiol* 313: F319–F325, 2017. First published April 26, 2017; doi:10.1152/ajprenal.00703.2016.—The current paradigm regarding sodium handling in animals and humans postulates that total body sodium is regulated predominately via regulation of extracellular volume. Active sodium storage independent of volume retention is thought to be negligible. However, studies in animals, hypertensive patients, and healthy humans suggest water-free storage of sodium in skin. We hypothesized that tissue sodium concentrations ($[Na]_T$) found in humans vary and reflect regulation due to variable glycosaminoglycan content due to variable expression of XYLT-1. Twenty seven patients on dialysis and 21 living kidney transplant donors free of clinically detectable edema were studied. During surgery, abdominal skin, muscle, and arteries were biopsied. $[Na]_T$ was determined by inductively coupled plasma-optical emission spectrometry, semiquantitative glycosaminoglycan content with Alcian stain, and XYLT-1 expression by real-time PCR. $[Na]_T$ of arteries were ranging between 0.86 and 9.83 g/kg wet wt and were significantly higher in arteries (4.52 ± 1.82 g/kg) than in muscle (2.03 ± 1.41 g/kg; $P < 0.001$) or skin (3.24 ± 2.26 g/kg wet wt; $P = 0.038$). For individual patients $[Na]_T$ correlated for skin and arterial tissue ($r = 0.440$, $P = 0.012$). $[Na]_T$ also correlated significantly with blinded semiquantitative analysis of glycosaminoglycans staining ($r = 0.588$, $P = 0.004$). In arteries XYLT-1 expression was also correlated with $[Na]_T$ ($r = 0.392$, $P = 0.003$). Our data confirm highly variable $[Na]_T$ in human skin and muscle and extend this observation to $[Na]_T$ in human arteries. These data support the hypothesis of water-independent sodium storage via regulated glycosaminoglycan synthesis in human tissues, including arteries.

artery; skin; sodium handling; XYLT1

IMPLICATIONS OF SODIUM HANDLING for health and disease are vividly discussed, and there is an ample body of literature suggesting a contribution of increased sodium intake to the development of hypertension and cardiovascular disease, including aortic stiffness, smooth muscle hypertrophy, and fibrinoid media necrosis of arteries via a variety of mechanisms (1, 7, 10). In the current pathophysiological understanding, sodium is thought to be closely linked to body water, primarily the extracellular compartment. Although this model is well suited to explain many responses to sodium loading, it only partially explains the extremes of the spectrum, i.e., compensation of sodium losses in sodium-deplete populations or sodium handling in individuals subjected to sodium loading despite impaired limited renal excretion capacity such as infants or anuric patients on dialysis. Interestingly, experimental studies in rats exposed to deoxycorticosterone suggest the possibility of water-free storage of sodium via incorporation in glycosaminoglycans (18). Recently, water-free sodium storage has been proposed for hypertensive and even healthy humans (9, 14). These studies focused on skin and muscles and demonstrated increased tissue sodium concentrations ($[Na]_T$), scattered over a wide range from ~40 to 110 mmol/kg wet wt (9).

We hypothesized that patients with advanced renal disease requiring dialysis exhibit a decreased capacity to excrete sodium. When exposed to a Western diet they might experience constant sodium loading, resulting in expanded sodium stores due to increased glycosaminoglycan synthesis. Because one prominent clinical manifestation of sodium loading is via arterial pathology, we also wondered if variable and tissue-specific sodium concentrations can be found in humans.

To test this hypothesis we measured $[Na]_T$ of skin, muscle, and arteries in dialysis patients and healthy kidney donors. To further investigate possible mechanisms of osmotically inactive sodium storage, we studied glycosaminoglycan content and expression of XYLT-1, the enzyme initiating glycosaminoglycan synthesis.

MATERIALS AND METHODS

Patients. Dialysis patients ($n = 27$), mostly recipients of living related kidney transplants, and healthy controls ($n = 21$), i.e., living kidney donors at the time of organ donation, were included in this study. Demographic data were extracted from the patients' medical records. Laboratory data were obtained on the day before surgery except for intact parathyroid hormone and serum phosphate for which the most recent value from the preceding 6 mo was used. Serum osmolality was calculated from serum concentrations of sodium, glucose, and urea. Blood pressure was measured in clinic before

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Table 1. *Demographic data of dialysis patients and healthy kidney donors*

Parameter	Donors (n = 21)	Dialysis Patients (n = 27)	P Value
Sex, %male	45	74	0.042
Age, yr	55.1 ± 10.9	51.9 ± 14.7	0.416
Weight, kg	74.0 ± 16.4	72.7 ± 10.0	0.744
BMI, kg/m^2	24.9 ± 4.2	24.3 ± 3.1	0.569
Dialysis vintage, yr		1.5 ± 1.7	
Diuresis, ml/day	full	1,625 ± 1,372	
Blood pressure systolic/diastolic, mmHg			
Office	124 ± 10.3/76 ± 5.3	136 ± 16.4/79 ± 9.4	0.010 0.241
24 h	123 ± 8.7/74 ± 7.5	138 ± 11.7/78 ± 7.6	0.001 0.210
Office pulse pressure, mmHg	49 ± 8.4	58 ± 16	0.030
No. of antihypertensives	0.5 ± 1.0	2.8 ± 1.6	<0.001
Diuretic, %use	16	47	0.037
Creatinine, mg/dl	1.0 ± 0.2	7.5 ± 2.4	<0.001
Urea, mg/dl	33.7 ± 8.53	126 ± 38.8	<0.001
Sodium, mmol/l	140 ± 1.97	139 ± 2.30	0.183
Potassium, mmol/l	4.5 ± 0.3	5.1 ± 0.8	0.002
Phosphate, mg/dl	3.5 ± 0.5	4.9 ± 1.7	0.005
iPTH, pg/ml	39.9 ± 18.4	261 ± 259	0.006
Glucose, mg/dl	96.0 ± 12.8	118 ± 63.9	0.132
Bicarbonate, mmol/l	24.9 ± 1.82	24.5 ± 2.64	0.696
Osmolality calculated, mosm/l	291 ± 3.69	305 ± 7.72	<0.001
Hemoglobin, g/dl	14.0 ± 1.18	11.6 ± 1.41	<0.001
CRP, mg/dl (median; range)	0.2 (0; 3.2)	0.3 (0; 13.6)	0.029
Tissue concentration, mmol/g dry wt			
Skin	0.287 ± 0.176	0.308 ± 0.146	0.526
Artery	0.378 ± 0.213	0.412 ± 0.269	0.723
Muscle	0.195 ± 0.127	0.204 ± 0.92	0.790

Data are reported as means ± SD; n, no. of subjects. BMI, body mass index; iPTH, intact parathyroid hormone; CRP, C-reactive protein.

hospital admission. Kidney donors were also routinely examined by 24-h ambulatory blood pressure monitoring.

Study approval. The study protocol was approved by the institutional ethics committee, and all human participants gave written informed consent.

Tissue biopsies. Only patients free from clinically detectable edema were included. During surgery tissue samples were obtained from the abdominal incision from skin, muscle, and epigastric artery. Sodium-containing fluids for preparation or irrigation were strictly avoided. Immediately after excision of the biopsy, a small aliquot (~2 × 3 × 3 mm) was stored in RNAlater (Ambion, Darmstadt, Germany) until further analysis. The larger portion of the biopsy specimen was divided. One part was fixed and stored in 4% neutral buffered formalin for histopathological analysis, and one part was frozen at −20°C without further additives for measurement of $[Na]_T$.

Measurement of $[Na]_T$. $[Na]_T$ was determined from frozen specimens by inductively coupled plasma-optical emission spectroscopy. The samples (100 mg) were properly weighed in quartz vessels. Subsequently, 1 ml HNO_3, suprapure, subboiling distilled (Merck, Darmstadt, Germany) was added. The vessels were closed and introduced into a pressure digestion system (Seif, Unterschleissheim, Germany) for 10 h at 170°C. The resulting clear solution was filled up

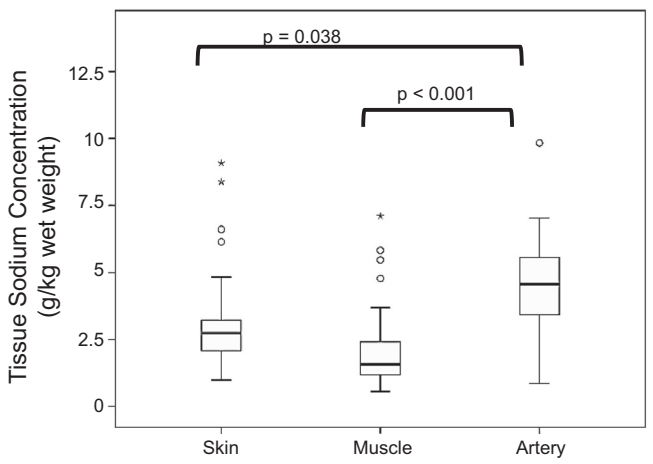


Fig. 1. Tissue sodium concentrations measured in muscle biopsies were significantly lower than in skin and arteries ($P < 0.001$). The highest tissue sodium concentrations were determined in arterial samples ($P = 0.038$ compared with skin biopsies; skin $n = 48$, muscle $n = 47$, artery $n = 32$).

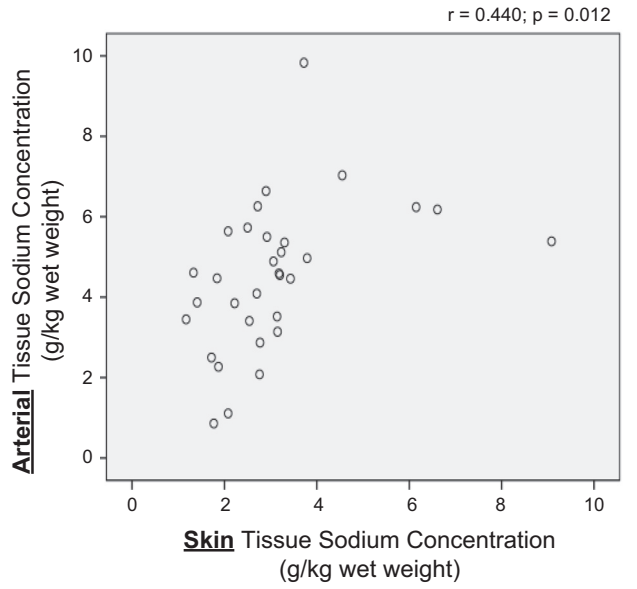
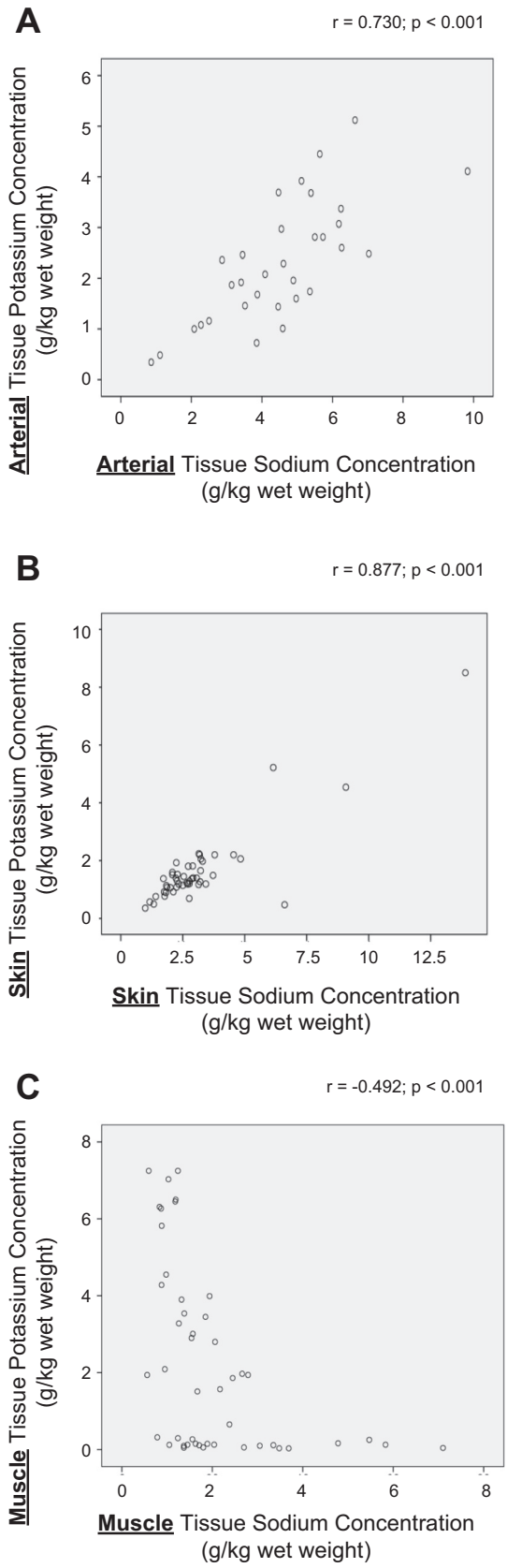


Fig. 2. Tissue sodium concentrations in skin and arterial biopsies. Tissue concentrations determined in skin and arterial biopsies are plotted for each individual ($r = 0.440$; $P = 0.012$, $n = 32$).



exactly to 5 ml with Milli-Q H_2O and was then ready for element determination.

An inductively coupled plasma atomic emission spectrometer "Optima 7300" system (Perkin Elmer, Rodgau-Jügesheim, Germany) was used for sodium and potassium determination in samples. Sample introduction was carried out using a peristaltic pump (flow rate 0.8 ml/min) connected to a Seaspray nebulizer with a cyclon spray chamber. The measured spectral element line was 589.592 nm for sodium.

The radiofrequency power was set to 1,350 watts, the plasma gas was 15 l Ar/min, and the auxiliary gas was 0.2 l Ar/min, whereas the nebulizer gas was 600 ml Ar/min. For every 10 measurements, 3 blank determinations and a control determination of a certified Na standard were performed. Calculation of results was carried out on a computerized lab-data management system, relating the sample measurements to calibration curves, blank determinations, control standards, and the weight of the digested sample.

Estimation of glycosaminoglycan expression in biopsy samples. Glycosaminoglycan content was assessed in skin, muscle, and arterial samples by standardized Alcian Blue-Periodic acid-Schiff (PAS) staining. In brief, the protocol followed consisted of deparaffinization in xylol and hydration through alcohols, rinsing in distilled water, staining with Alcian Blue (pH 2.5) for 10 min, rinsing in distilled water, staining with 0.5% Periodic acid for 5 min, rinsing in distilled water, optimized Schiff's solution for 5 min, rinsing in tap water for 5 min, staining with hemalaun for 5 min, rinsing in running water for 5 min, rinsing in distilled water, and dehydrating through alcohol and xylol.

The intensity of Alcian Blue staining was scored semiquantitatively by a pathologist blinded to patient identification and $[Na]_T$.

XYLT1 expression. To examine regulation of glycosaminoglycan synthesis, XYLT1 expression was analyzed in aliquots of the respective biopsies by RT-PCR. RNA isolation was performed as described previously (2). Of the total RNA, 1 μg was used for cDNA synthesis by Superscript I/II reverse transcriptase (Invitrogen, Karlsruhe, Germany) with hexanucleotides as primers (Roche, Mannheim, Germany). RT-PCR products from 25 arteries and 31 muscle biopsies were obtained. qPCR was performed by an ABIPrism7000 Sequence detection system (Applied Biosystems, Darmstadt, Germany) (20).

In vitro induction of XYLT1 expression. Stimulation of XYLT1 expression by various external stimuli such as hyperosmolality, increased extracellular phosphate concentrations, and inflammatory stimuli was studied in an in vitro model using K4IM cells, a human dermal fibroblast cell line, immortalized by SV40 (5). In brief, cells were grown to confluence under standard conditions, i.e., Dulbecco's Modified Eagle's Medium (GIBCO, Darmstadt, Germany) with 10% fetal calf serum (Invitrogen, Darmstadt, Germany) and antibiotic additive (pencicillin/streptomycin; Invitrogen). Next, cells were exposed to standard medium (149 mmol sodium, 0.87 mmol phosphate), hyperosmolar medium (200 mmol sodium, 0.87 mmol phosphate), increased phosphate concentrations (149 mmol sodium, 8 mmol phosphate), or standard medium with the addition of TGF-β (10 mg/ml; Sigma, St. Louis, MO) for 48 h. Next, RNA was extracted with lysis buffer (Novex; Invitrogen, Carlsbad, CA). cDNA synthesis and qPCR were performed as described above. XYLT1 expression was normalized to 18S RNA.

Statistical analysis. Descriptive statistics were used to summarize the baseline characteristics of donors and recipients and were compared using univariate ANOVA. Data are reported as means ± SD.

Fig. 3. Tissue potassium concentrations and tissue sodium concentrations in the respective tissue are positively correlated when measured in arteries (n = 25; r = 0.877, $P < 0.001$; *A*) and inversely correlated when measured in muscle (n = 31; r = −0.492, $P < 0.001$; *C*). The measured tissue concentrations are reported in g/kg wet wt.

$[Na]_T$ between various tissues were compared with a two-sided *t*-test. Pearson's correlation was used to determine the relationship between $[Na]_T$ in various tissues, glycosaminoglycan staining and XYLT expression, and to determine the relationship between clinical parameters and arterial XYLT-1 expression. For in vitro experiments, unpaired *t*-test was used to analyze data between two groups. All analyses were performed with IBM SPSS Statistical Software Version 22. $P < 0.05$ was considered significant.

RESULTS

Demographic data. Descriptive demographic data are given for dialysis patients and healthy controls in Table 1. Patients and living kidney donors were well matched with respect to age, weight, and body mass index. As expected, dialysis patients were more likely to be male and exhibited significantly higher systolic blood pressure on both office and 24-h measurements as well as higher pulse pressure. Dialysis patients also received significantly more antihypertensive medications.

Serum-creatinine, urea, potassium, phosphate, calculated serum osmolality, C-reactive protein (CRP), and intact parathyroid hormone were significantly higher in dialysis patients, whereas hemoglobin was significantly lower. Serum concentrations for sodium, glucose and bicarbonate were not different between the groups (Table 1).

Tissue specific sodium concentrations. Adequate samples for analysis were available from skin in 48 patients, muscle in 47 patients, and artery in 32 patients.

$[Na]_T$ exhibited substantial interindividual variability, ranging between 0.9 and 9.8 g/kg wet wt for arteries, 0.6 and 7.1 g/kg wet wt for muscle, and 1.0 and 14 g/kg wet wt for skin. There was an 11- to 14-fold increase in $[Na]_T$ between the lowest and highest measurements. Also, mean measured $[Na]_T$ were significantly lower in muscle with 2.0 ± 1.4 g/kg than in skin biopsies with 3.2 ± 2.3 g/kg ($P < 0.001$). Highest mean $[Na]_T$ of 4.5 ± 1.8 g/kg wet wt were measured in arterial tissue ($P < 0.001$ vs. muscle; $P = 0.038$ vs. skin; Fig. 1).

$[Na]_T$ were not different between dialysis patients or healthy controls ($P = 0.723$ for arteries; $P = 0.804$ for skin). However, $[Na]_T$ were significantly correlated intraindividually between skin and arteries ($r = 0.440$, $P = 0.012$; Fig. 2).

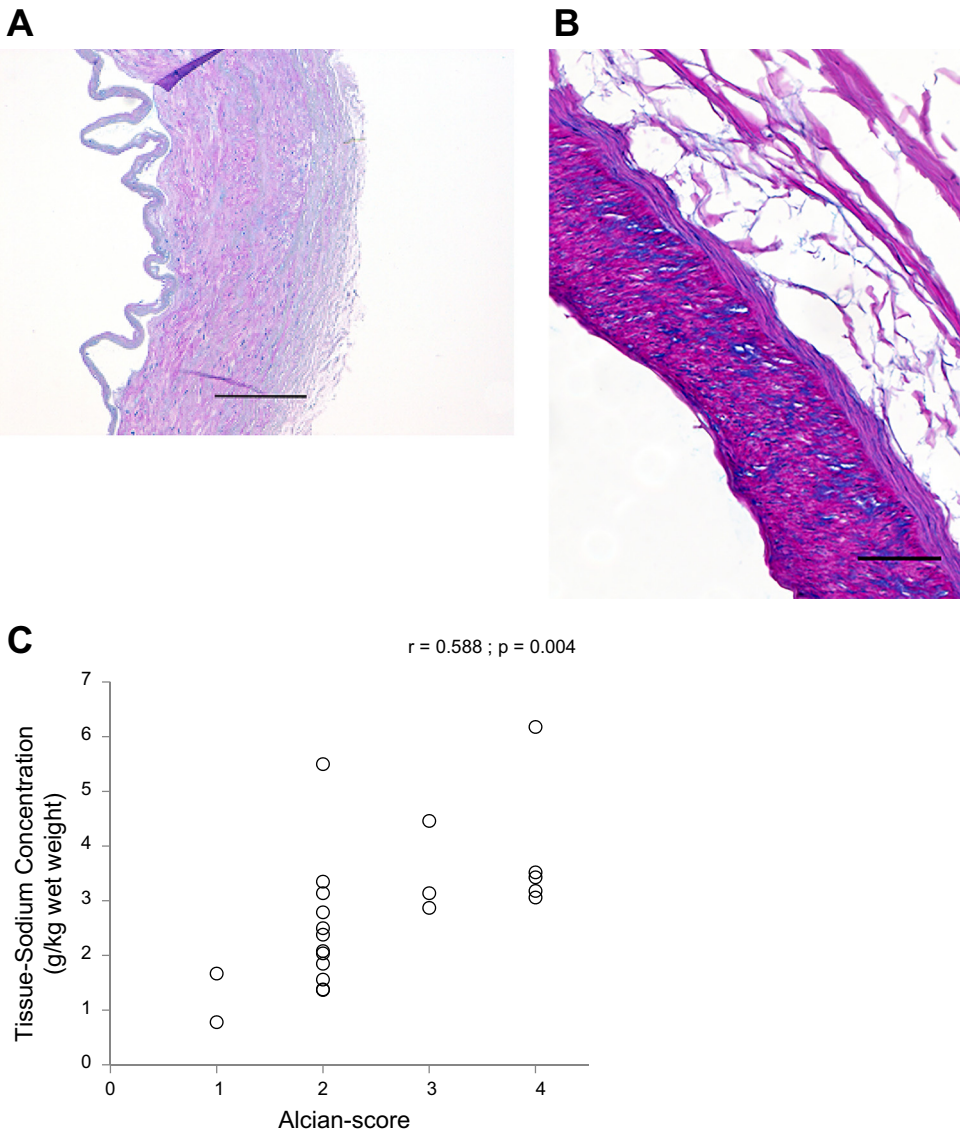


Fig. 4. Glycosaminoglycan staining of one representative arterial biopsy specimen for patients with low (*A*) or high (*B*) tissue sodium concentrations. The measured tissue sodium concentrations are reported in g/kg wet wt. *C*: results of measured tissue sodium concentrations according to the intensity of Alcian-Periodic acid-Schiff staining in skin ($n = 6$), muscle ($n = 10$), and arteries ($n = 6$) per blinded scoring of biopsy specimens ($n = 22$; $r = 0.588$; $P = 0.004$).

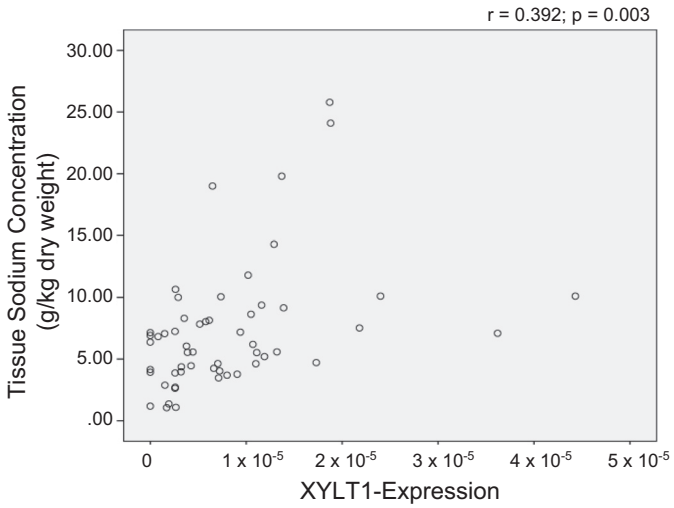


Fig. 5. Tissue sodium concentrations measured in muscles ($n = 31$) and arteries ($n = 25$) are correlated to XYLT1 expression of the respective biopsy sample ($r = 0.392$; $P = 0.03$).

The respective $[Na]_T$ concentrations were for skin 0.295 ± 0.159 (donors: 0.287 ± 0.176 mmol/g dry wt; dialysis patients 0.308 ± 0.146 mmol/g dry wt, $P = 0.526$), for arteries 0.402 ± 0.250 (donors: 0.378 ± 0.213 mmol/g dry wt; dialysis patients 0.412 ± 0.269 mmol/g dry wt, $P = 0.723$), for muscle 0.200 ± 0.108 (donors: 0.195 ± 0.127 mmol/g dry wt; dialysis patients 0.204 ± 0.92 mmol/g dry wt, $P = 0.790$) mmol/g dry wt.

Tissue specific potassium concentrations. Tissue specific potassium concentrations ($[K]_T$) were for skin 0.045 ± 0.028 (donors: 0.039 ± 0.031 mmol/g dry wt; dialysis patients 0.049 ± 0.025 mmol/g dry wt, $P = 0.821$), for arteries 0.119 ± 0.081 (donors: 0.107 ± 0.063 mmol/g dry wt; dialysis patients 0.125 ± 0.089 mmol/g dry wt, $P = 0.997$), for muscle 0.194 ± 0.251 (donors: 0.136 ± 0.167 mmol/g dry wt; dialysis patients 0.241 ± 0.297 mmol/g dry wt, $P = 0.415$) mmol/g dry wt.

Intraindividually, $[K]_T$ exhibited a strong positive correlation with $[Na]_T$ for arteries ($r = 0.730$, $P < 0.001$; Fig. 3*A*) and skin ($r = 0.877$, $P < 0.001$; Fig. 3*B*). In contrast, for muscle $[K]_T$ and $[Na]_T$ were inversely correlated ($r = -0.492$, $P < 0.001$; Fig. 3*C*).

Alcian Blue-PAS staining. When intensity of Alcian Blue-PAS staining was scored semiquantitatively by a pathologist blinded to the results of $[Na]_T$, again substantial variations were observed. Representative micrographs are shown for an artery with a low $[Na]_T$ of 2.1 g/kg wet wt (Fig. 4*A*) and an artery with a high $[Na]_T$ of 6.2 g/kg wet wt (Fig. 4*A*).

As shown in Fig. 4*C*, measured $[Na]_T$ were higher in specimens with higher intensity of Alcian-PAS staining ($r = 0.588$; $P = 0.004$).

XYLT1 expression. Also XYLT1 expression relative to 18S RNA varied greatly between the samples studied, ranging from 2.9×10^{-6} to 4.4×10^{-5} in arteries and $<1 \times 10^{-9}$ to 1.2×10^{-5} in muscles. As with intensity of glycosaminoglycan staining on histopathological analysis, higher $[Na]_T$ were observed in samples with increasing XYLT1 expression ($r = 0.392$; $P = 0.003$; Fig. 5).

In vivo, arterial XYLT1 expression was correlated to calculated osmolality ($r = 0.558$, $P = 0.004$), serum bicarbonate ($r = -0.523$, $P = 0.031$), and serum phosphate ($r = 0.664$, $P = 0.001$). In vitro, XYLT1 expression was induced 12-fold compared with baseline by incubation with TGF-β ($6.1 \times 10^{-6} \pm 4.8 \times 10^{-6}$ vs. $7.2 \times 10^{-5} \pm 4.0 \times 10^{-5}$; $P = 0.030$; Fig. 6). Medium with hypertonic sodium or elevated extracellular phosphate concentration was without significant effect on XYLT1 expression.

DISCUSSION

In this human study we demonstrate significant amounts of sodium in various tissues that substantially exceed the concentrations to be expected from sodium concentrations in extracellular fluid and intracellular concentrations. Furthermore, measured $[Na]_T$ vary between tissues such as muscle, skin, and arteries, with the highest amounts of sodium to be found in arterial tissue. We also report a similar degree of variability in $[Na]_T$ compared with studies in rats and humans from other groups (9, 18). In contrast to this wide range of interindividual variation, $[Na]_T$ were tightly correlated within one individual. As well as reported by Kopp et al., this wide range of variability observed in our study is far beyond the physiological variability in extracellular volume (9).

Thus, water-free sodium storage appears as another possible explanation for this observation. Indeed, the presence of such a slow exchangeable sodium pool has been suspected from equilibration studies using radioactive sodium half a century ago (16). As a potential site of sodium storage, previously, incorporation into glycosaminoglycans has been suspected. This is based on glycosaminoglycan measurements in the skin of Wistar rats with and without chronic sodium loading, structural analysis of hyaluronan, and binding studies in glycosaminoglycan-rich tissues (6, 11, 21). Our study also reports a significant correlation of the intensity of glycosaminoglycan staining of tissue biopsies and $[Na]_T$. Although similar variability of glycosaminoglycan expression in arteries and myocardium have been reported by others, our observations link this observation to $[Na]_T$.

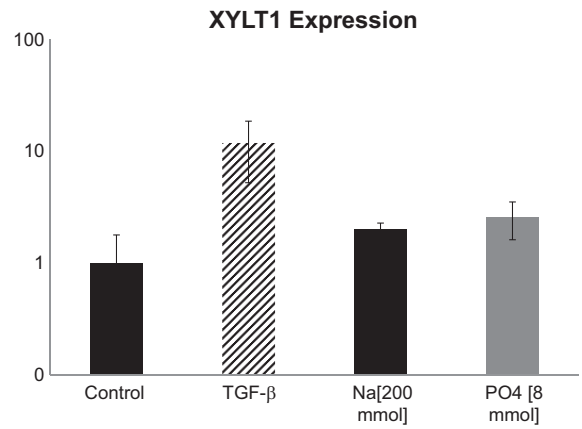


Fig. 6. Relative induction in XYLT1 expression compared with control. XYLT1 expression was determined by RT-PCR from human skin fibroblasts, incubated with medium or medium with either 200 mmol sodium, addition of 8 mmol phosphate, or 10 ng/ml TGF-β. The data for control ($n = 16$) and incubation with TGF-β ($n = 14$) represent results from four sets of independent experiments. Exposure to hyperosmolar medium ($n = 7$) and increased phosphate concentration ($n = 7$) represent results from two independent experiments. Data are shown as means ± SD.

Aside from water-free sodium storage, osmotically neutral sodium potassium exchange also has to be considered as an explanation for our observations. However, in our patients, $[K]_T$ and $[Na]_T$ showed a highly significant positive correlation in skin and artery. Because glycosaminoglycans may incorporate both cations, sodium and potassium, in their tertiary structure, this observation adds further to the assumption of intraindividually different glycosaminoglycan expression.

This raises the question if glycosaminoglycan expression and $[Na]_T$ as a consequence thereof reflect an individual constant or a process of active regulation. The latter appears likely, since one previous study in rats demonstrated significant increases in glycosaminoglycan expression on Western blot of the skin along with increased $[Na]_T$ in rats exposed to sodium loading (6, 19). Our results extend this observation in various aspects. We show such an effect in human tissue, namely arteries, and furthermore demonstrate variable amounts of glycosaminoglycans in the respective tissues that were again closely correlated to $[Na]_T$. Furthermore, we studied the expression of XYLT1, the enzyme initiating glycosaminoglycan synthesis in arteries and muscle, and could show increased $[Na]_T$ in biopsies with higher XYLT1 expression. This leaves the question, which mechanisms trigger glycosaminoglycan synthesis, namely XYLT1 expression. In our clinical database, arterial XYLT1 expression was correlated with calculated serum osmolality and serum phosphate. Whereas extracellular osmolality has been shown in chondrocytes to induce glycosaminoglycan synthesis (17), we could not reproduce this in our in vitro experiment with human dermal fibroblasts exposed to similar concentrations of extracellular sodium. Likewise, we could not induce XYLT1 expression with increased extracellular phosphate concentrations. Because most of our patients had a CRP within the normal range and were normotensive, we have too little information to study the impact of systemic inflammation or hyperaldosteronism on $[Na]_T$. However, addition of TGF-β to the culture medium resulted in a 12-fold increase in XYLT1 expression. XYLT1 expression has been stimulated by TGF-β in cardiac fibroblasts, and increased XYLT1 expression has also been reported in cardiac tissue (3). Because increased TGF-β expression has been reported in renal failure, such TGF-β expression may represent the link between altered renal phosphate handling and increased $[Na]_T$ and cutaneous inflammation and also form a pathophysiological basis for renocardial syndrome (22).

Our observations support the relevance of glycosaminoglycan expression. Glycosaminoglycans are known to serve as scaffolds that bind lipoproteins and cytokines. Furthermore, glycosaminoglycan overproduction has experimentally been linked to increased aortic calcification (8, 12, 13). Hence a number of potential sequelae of such increased glycosaminoglycan synthesis may be suspected in the long-term follow-up of the patients included in our study.

In contrast to previous studies, we could not find any difference between patients with impaired sodium excretion and healthy humans. This observation is supported by the work of Dahlmann et al. in which there was no significant difference in noninvasively measured $[Na]_T$ in skin and muscle between dialysis patients and healthy controls (4). Possibly because of the rather narrow age range of our patients and a preponderance of postmenopausal women, we were not able to detect any effect of age or gender on sodium tissue concentrations (4). Instead, we detected a strong intraindividual correlation of $[Na]_T$ throughout tissues examined. This suggests that sodium storage may reflect rather an individual physiological response than necessarily a consequence of disease.

Our observations of highly variable arterial $[Na]_T$ offer another interesting explanation to most recent work in which noninvasively measured skin sodium concentrations were found to predict left ventricular hypertrophy in patients with mild to moderate chronic kidney disease (15). Assuming a similar correlation of $[Na]_T$ measured in skin with arteries for that cohort, one might assume that patients with high skin sodium concentrations also have increased arteriolar glycosaminoglycan synthesis and sodium storage, resulting in vascular stiffening, arterial hypertension, and left ventricular hypertrophy.

Conclusion. In summary, we provide human data to support a pathophysiological role of glycosaminoglycan synthesis in water-free sodium storage. $[Na]_T$ are highly variable in humans; vary between muscle, skin, and arterial tissue; and correlate with glycosaminoglycan as visualized on Alcian staining. In vivo, expression of XYLT1, the enzyme initiating glycosaminoglycan synthesis, correlates to calculated osmolality and serum-phosphate levels while in vitro only TGF-β induced XYLT1 expression. Further analysis of these mechanisms may enhance the understanding of sodium handling and complications associated therewith.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.F. and M.S. conceived and designed research; M.F., B.M., E.S., and P.N. performed experiments; M.F., B.M., E.S., A.H., R.K., I.P., B.S., U.S., P.N., and M.S. analyzed data; M.F., B.M., E.S., A.H., R.K., I.P., U.S., P.N., and M.S. interpreted results of experiments; M.F., E.S., and I.P. prepared figures; M.F. and M.S. drafted manuscript; M.F., B.M., E.S., A.H., R.K., I.P., B.S., U.S., P.N., and M.S. edited and revised manuscript; M.F., B.M., E.S., A.H., R.K., I.P., B.S., U.S., P.N., and M.S. approved final version of manuscript.

REFERENCES

1. **Adrogué HJ, Madias NE.** Sodium and potassium in the pathogenesis of hypertension. *N Engl J Med* 356: 1966–1978, 2007. doi:10.1056/NEJMra064486.
2. **Cohen CD, Frach K, Schlöndorff D, Kretzler M.** Quantitative gene expression analysis in renal biopsies: a novel protocol for a high-throughput multicenter application. *Kidney Int* 61: 133–140, 2002. doi:10.1046/j.1523-1755.2002.00113.x.
3. **Cuellar K, Chuong H, Hubbell SM, Hinsdale ME.** Biosynthesis of chondroitin and heparan sulfate in chinese hamster ovary cells depends on xylosyltransferase II. *J Biol Chem* 282: 5195–5200, 2007. doi:10.1074/jbc.M611048200.
4. **Dahlmann A, Dörfelt K, Eicher F, Linz P, Kopp C, Mössinger I, Horn S, Büschges-Seraphin B, Wabel P, Hammon M, Cavallaro A, Eckardt KU, Kotanko P, Levin NW, Johannes B, Uder M, Luft FC, Müller DN, Titze JM.** Magnetic resonance-determined sodium removal from tissue stores in hemodialysis patients. *Kidney Int* 87: 434–441, 2015. doi:10.1038/ki.2014.269.
5. **Haas C, Aicher WK, Dinkel A, Peter HH, Eibel H.** Characterization of SV40T antigen immortalized human synovial fibroblasts: maintained expression patterns of EGR-1, HLA-DR and some surface receptors. *Rheumatol Int* 16: 241–247, 1997. doi:10.1007/BF01375656.
6. **Ivanova LN, Archibasova VK, Shterental' IS.** Sodium-depositing function of the skin in white rats. *Fiziol Zh SSSR Im I M Sechenova* 64: 358–363, 1978.
7. **Jablonski KL, Fedorova OV, Racine ML, Geolfos CJ, Gates PE, Chonchol M, Fleenor BS, Lakatta EG, Bagrov AY, Seals DR.** Dietary sodium restriction and association with urinary marinobufagenin, blood

pressure, and aortic stiffness. *Clin J Am Soc Nephrol* 8: 1952–1959, 2013. doi:10.2215/CJN.00900113.

8. **Kaplan M, Aviram M.** Retention of oxidized LDL by extracellular matrix proteoglycans leads to its uptake by macrophages: an alternative approach to study lipoproteins cellular uptake. *Arterioscler Thromb Vasc Biol* 21: 386–393, 2001. doi:10.1161/01.ATV.21.3.386.
9. **Kopp C, Linz P, Wachsmuth L, Dahlmann A, Horbach T, Schöfl C, Renz W, Santoro D, Niendorf T, Müller DN, Neininger M, Cavallaro A, Eckardt KU, Schmieder RE, Luft FC, Uder M, Titze J.** (23)Na magnetic resonance imaging of tissue sodium. *Hypertension* 59: 167–172, 2012. doi:10.1161/HYPERTENSIONAHA.111.183517.
10. **Kotchen TA, Cowley AW Jr, Frohlich ED.** Salt in health and disease--a delicate balance. *N Engl J Med* 368: 2531–2532, 2013. doi:10.1056/NEJMra1212606.
11. **Mobasheri A.** Correlation between [Na+], [glycosaminoglycan] and Na+/K+ pump density in the extracellular matrix of bovine articular cartilage. *Physiol Res* 47: 47–52, 1998.
12. **Mortier A, Van Damme J, Proost P.** Overview of the mechanisms regulating chemokine activity and availability. *Immunol Lett* 145: 2–9, 2012. doi:10.1016/j.imlet.2012.04.015.
13. **Purnomo E, Emoto N, Nugrahaningsih DA, Nakayama K, Yagi K, Heiden S, Nadanaka S, Kitagawa H, Hirata K.** Glycosaminoglycan overproduction in the aorta increases aortic calcification in murine chronic kidney disease. *J Am Heart Assoc* 2: e000405, 2013. doi:10.1161/JAHA.113.000405.
14. **Rakova N, Jüttner K, Dahlmann A, Schröder A, Linz P, Kopp C, Rauh M, Goller U, Beck L, Agureev A, Vassilieva G, Lenkova L, Johannes B, Wabel P, Moissl U, Vienken J, Gerzer R, Eckardt KU, Müller DN, Kirsch K, Morukov B, Luft FC, Titze J.** Long-term space flight simulation reveals infradian rhythmicity in human Na(+) balance. *Cell Metab* 17: 125–131, 2013. doi:10.1016/j.cmet.2012.11.013.
15. **Schneider MP, Raff U, Kopp C, Scheppach JB, Toncar S, Wanner C, Schlieper G, Saritas T, Floege J, Schmid M, Birukov A, Dahlmann A, Linz P, Janka R, Uder M, Schmieder RE, Titze JM, Eckardt KU.** Skin Sodium Concentration Correlates with Left Ventricular Hypertrophy in CKD. *J Am Soc Nephrol* ASN.2016060662, 2017. doi:10.1681/ASN.2016060662.
16. **Streeten DH, Rapoport A, Conn JW.** Existence of a slowly exchangeable pool of body sodium in normal subjects and its diminuition in patients with primary aldosteronism. *J Clin Endocrinol Metab* 23: 928–937, 1963. doi:10.1210/jcem-23-9-928.
17. **Takeno K, Kobayashi S, Negoro K, Uchida K, Miyazaki T, Yayama T, Shimada S, Baba H.** Physical limitations to tissue engineering of intervertebral disc cells: effect of extracellular osmotic change on glycosaminoglycan production and cell metabolism. Laboratory investigation. *J Neurosurg Spine* 7: 637–644, 2007. doi:10.3171/SPI-07/12/637.
18. **Titze J, Bauer K, Schafflhuber M, Dietsch P, Lang R, Schwind KH, Luft FC, Eckardt KU, Hilgers KF.** Internal sodium balance in DOCA-salt rats: a body composition study. *Am J Physiol Renal Physiol* 289: F793–F802, 2005. doi:10.1152/ajprenal.00096.2005.
19. **Titze J, Shakibaei M, Schafflhuber M, Schulze-Tanzil G, Porst M, Schwind KH, Dietsch P, Hilgers KF.** Glycosaminoglycan polymerization may enable osmotically inactive Na+ storage in the skin. *Am J Physiol Heart Circ Physiol* 287: H203–H208, 2004. doi:10.1152/ajpheart.01237.2003.
20. **von Toerne C, Schmidt C, Adams J, Kiss E, Bedke J, Porubsky S, Gretz N, Lindenmeyer MT, Cohen CD, Gröne HJ, Nelson PJ.** Wnt pathway regulation in chronic renal allograft damage. *Am J Transplant* 9: 2223–2239, 2009. doi:10.1111/j.1600-6143.2009.02762.x.
21. **Winter WT, Smith PJC, Arnott S.** Hyaluronic acid: structure of a fully extended 3-fold helical sodium salt and comparison with the less extended 4-fold helical forms. *J Mol Biol* 99: 219–235, 1975. doi:10.1016/S0022-2836(75)80142-2.
22. **Wong MG, Perkovic V, Woodward M, Chalmers J, Li Q, Hillis GS, Yaghobian Azari D, Jun M, Poulter N, Hamet P, Williams B, Neal B, Mancia G, Cooper M, Pollock CA.** Circulating bone morphogenetic protein-7 and transforming growth factor-β1 are better predictors of renal end points in patients with type 2 diabetes mellitus. *Kidney Int* 83: 278–284, 2013. doi:10.1038/ki.2012.383.