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Sodium Storage in Human Tissues is Mediated by Glycosaminoglycan Expression

Michael Fischereder[1], Bernhard Michalke[2], Elisa Schmöckel[3], Antje Habicht[4], Raphael Kunisch[1], Ivana Pavelic[1], Bernadette Szabados[5], Ulf Schönermarck[1], Peter J. Nelson[1], Manfred Stangl[6]

[1]Medizinische Klinik und Poliklinik IV, Renal Division, Klinikum der LMU Munich, Germany

[2]Research Unit Analytical Biochemistry, Helmholtz Zentrum München-German research center for Environmental Health GmbH, Neuherberg, Germany

[3]Institut für Pathologie, Klinikum der LMU Munich, Germany

[4]Transplantationszentrum, Klinikum der LMU Munich, Germany

[5]Urologische Klinik, Klinikum der LMU Munich, Germany

[6]Chirurgische Klinik - Klinikum der LMU Munich, Germany

Contribution: M.F., P.J.N and M.S. participated in research design, B.M. performed sodium sprectroscopv, E.S. performed blinded pathological tissue analysis, A.H., R.K., I.P. B.S. and U.S. performed patient management, data extraction and specimen processing, P.J.N. performed gene expression analysis. All authors contributed to data analysis and writing of the manuscript.

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Corresponding author:

Prof. Dr. med. Michael Fischereder

Klinikum der LMU, Medizinische Klinik und Poliklinik IV, Renal Division

Marchioninistrasse 15, D-81377 Munich, Germany

Phone: ++ 49 89 4400 72212; Fax: ++ 49 89 4400 72363

Michael.fischereder@med.uni-muenchen.de

Abstract:

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. 27 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, XYLT-1 expression by real-time PCR. $[Na]_T$ of arteries were ranging between 0.86 and 9.83 g/kg wet weight and were significantly higher in arteries (4.52 $\pm$ 1.82 g/kg) than in muscle (2.03 $\pm$ 1.41 g/kg; $p<0.001$) or skin (3.24 $\pm$ 2.26 g/kg wet weight; $p=0.038$). 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.

Introduction

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 pathophysiologic understanding, sodium is thought to be closely linked to body water, primarily the extracellular compartment. While 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 into 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, scattered over a wide range from approximately 40 to 110 mmol / kg wet weight (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. As 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 tissue sodium concentrations ($[Na]_T$) of skin, muscle and arteries in dialysis patients and healthy kidney donors. In order 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 prior to surgery except for iPTH and serum phosphate for which the most recent value from the preceding six months was used. Serum osmolality was calculated from serum concentrations of sodium, glucose and urea. Blood pressure was measured in clinic prior to hospital admission. Kidney donors were also routinely examined by 24 hour ambulatory blood pressure monitoring.

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 (approximately 2 x 3 x 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, one part was frozen at -20°C without further additives for measurement of tissue sodium concentration.

Measurement of tissue sodium concentrations

$[Na]_T$ was determined from frozen specimens by inductively coupled plasma-optical emission spectroscopy. The samples (100 mg) were properly weighed into quartz vessels. Subsequently, 1 mL

HNO_3, suprapure, subboiling distilled (Merck, Darmstadt) was added. The vessels were closed and introduced into a pressure digestion system (Seif, Unterschleissheim, Germany) for 10 h at 170 ^{0}C. The resulting clear solution was filled up exactly to 5 mL with Milli-Q H_2O and was then ready for element determination.

An inductively coupled plasma atomic emission spectrometer (ICP-AES) „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: Na: 589.592 nm. The RF power was set to 1350 W, the plasma gas was 15 L Ar /min, the auxiliary gas was 0.2 L Ar/min, whereas the nebulizer gas was 600 mL Ar/min. Every ten measurements three 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 -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 minutes, rinsing in distilled water, staining with 0.5% Periodic acid for 5 minutes, rinsing in distilled water, optimized Schiff’s solution for 5 minutes, rinsing in tap water for 5 minutes, staining with haemalaun for 5 minutes, rinsing in running water for 5 minutes, 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 tissue sodium concentrations.

XYLT1 expression

In order 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. Dulbeccos Modified Eagle Medium (Gibco, Darmstadt, Germany) with 10% fetal calf serum (Invitrogen, Darmstadt, Germany) and antibiotic additive (pencicillin/streptomycin, Invitrogen, Darmstadt, Germany). Then, 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-ß (10 mg/ml; Sigma, St. Louis, USA) for 48 hours. Then RNA was extracted with lysis buffer (Novex, Invitrogen, Carlsbad, USA). 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 mean $\pm$ standard deviation. $[Na]_T$

between various tissues were compared with a two-sided t-test. Pearson's correlation was used to determine relationship between tissue sodium concentrations in various tissues, glycosaminoglycan staining and XYLT-expression and to determine relationship between clinical parameters and arterial XYLT-1 expression. For in vitro experiments, unpaired *t* test was used to analyze data between 2 groups. All analyses were performed with IBM SPSS Statistical Software Version 22. $p < 0.05$ was considered significant.

Study approval

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

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 BMI. As expected, dialysis patients were more likely to be male and exhibited significantly higher systolic blood pressure on both office and 24-hour measurements as well as higher pulse pressure. Dialysis patients also received significantly more antihypertensive medications.

Serum-creatinine, urea, potassium, phosphate, calculated serum-osmolality and iPTH were significantly higher in dialysis patients whereas hemoglobin was significantly lower. Serum concentrations for sodium, glucose, CRP 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 weight for arteries, 0.6 and 7.1 g/kg wet weight for muscle and 1.0 and 14 g/kg wet weight for skin. There was an 11- fold to 14-fold increase in $[Na]_T$ between lowest and highest measurements. Also, mean measured $[Na]_T$ were significantly lower in muscle with 2.0 ($\pm$ 1.4) g/kg than in skin biopsies with 3.2 ($\pm$ 2.3) g/kg ($p<0.001$). Highest mean $[Na]_T$ of 4.5 ($\pm$ 1.8) g/kg wet weight were measured in arterial tissue (fig. 1; $p<0.001$ vs. muscle; $p=0.038$ vs. skin).

$[Na]_T$ 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).

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

Tissue specific potassium concentrations

Tissue specific potassium concentrations $[K]_T$ in mmol/g dry weight were for skin 0.045 + 0.028 mmol / g DW (donors: 0.039 + 0.031 mmol/ g DW; dialysis patients 0.049 + 0.025 mmol/ g DW, $p=0.821$), for arteries 0.119 + 0.081 mmol / g DW (donors: 0.107 + 0.063 mmol/ g DW; dialysis patients 0.125 +

0.089 mmol/ g DW, p=0.997), for muscle 0.194 + 0.251 mmol / g DW (donors: 0.136 + 0.167 mmol/ g DW; dialysis patients 0.241 + 0.297 mmol/ g DW, p=0.415).

Intraindividually, $[K]_T$ exhibited a strong positive correlation with $[Na]_T$ for arteries (r=0.730, p<0.001; fig. 3a) and skin (r=0.877, p<0.001; fig. 3b). In contrast, for muscle $[K]_T$ and $[Na]_T$ were inversely correlated (r=-0.492, p<0.001; fig. 3c).

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 tissue sodium concentration of 2.1 g/kg wet weight (fig. 4a) and an artery with a high $[Na]_T$ of 6.2 g/kg wet weight (fig. 4a).

As shown in figure 4c, 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 (fig. 5, r=0.392; p=0.003).

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 to baseline by incubation with TGF-ß ($6.1 \times 10^{-6} \pm 4.8 \times 10^{-6}$ vs. $7.2 \times$

209 10^{-5} + 4.0 x 10^{-5}; p=0.030; fig. 6). Medium with hypertonic sodium or elevated extracellular phosphate
210 concentration was without significant effect on XYLT1 expression.

211

212 **Discussion**

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

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

231 Aside from water-free sodium storage osmotically neutral sodium potassium exchange also has to be
232 considered as an explanation for our observations. However, in our patients $[K]_T$ and $[Na]_T$ showed a
233 highly significant positive correlation in skin and artery. As glycosaminoglycans may incorporate both

234 cations, sodium and potassium, into their tertiary structure, this observation adds further to the
235 assumption of intraindividually different glycosaminoglycan expression.

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

258 Our observations support the relevance of glycosaminoglycan expression. Glycosaminoglycans are
259 known to serve as scaffolds which bind lipoproteins, cytokines and 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 non-invasively measured tissue sodium concentrations in skin and muscle between dialysis patients and healthy controls (4). Possibly due to the rather narrow age range of our patients and a preponderance of post-menopausal 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 tissue sodium concentrations offer another interesting explanation to most recent work in which non-invasively 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 tissue sodium concentrations 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-ß induced XYLT1 expression. Further analysis of these mechanisms may enhance the understanding of sodium handling and complications associated therewith.

Disclosures

Non conflicts of interest to disclose.

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362 Captations:

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

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

368 Fig. 3: Tissue potassium concentrations and tissue sodium concentrations in the respective tissue are
369 positively correlated when measured in arteries (n= 25; $r=0.877$, $p<0.001$; fig. 3a) and inversely
370 correlated when measured in muscle (n=31; $r=-0.492$, $p<0.001$; fig. 3c). The measured tissue
371 concentrations are reported in g/kg wet weight.

372 Fig. 4: Glycosaminoglycan staining of one representative arterial biopsy specimens for patients
373 with low (a) or high (b) tissue sodium concentrations. The measured tissue sodium concentrations
374 are reported in g/kg wet weight. (c) Results of measured tissue sodium concentrations according
375 to the intensity of Alcian-PAS staining in skin (n=6), muscle (n=10) and arteries (n=6) per blinded
376 scoring of biopsy specimens (n=22; $r=0.588$; $p=0.004$).

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

379 Figure 6: Relative induction in XYLT1 expression compared to control. XYLT1 expression was
380 determined by RT-PCR from human skin fibroblasts, incubated with medium, or medium with
381 either 200 mmol sodium, addition of 8 mmol phosphate or 10 ng/ml TGF-ß. The data for control
382 (n=16) and incubation with TGF-ß (n=14) represent results from four sets of independent
383 experiments. Exposure to hyperosmolar medium (n=7) and increased phosphate concentration
384 (n=7) represent results from two independent experiments. (Data shown as mean $\pm$ standard
385 deviation)

386

387 Table 1: Demographic data of dialysis patients and healthy kidney donors. Data are reported as mean

388 ± standard deviation, units are reported in [].

Parameter **Mean (Std. Dev.)**	**Donors** **(n=21)**	**Dialysis Patients** **(n=27)**	**p value**
Sex [% male]	45	74	0.042
Age [years]	55.1 (10.9)	51.9 (14.7)	0.416
Weight [kg]	74.0 (16.4)	72.7 (10.0)	0.744
BMI [kg/m²]	24.9 (4.2)	24.3 (3.1)	0.569
Dialysis vintage [years]	-	1.5 (1.7)	-
Diuresis [ml/d]	full	1625 (1372)	-
Blood pressure systolic/ diastolic [mmHg, office]	124 (10.3) / 76 (5.3)	136 (16.4) / 79 (9.4)	0.010 0.241
Blood pressure systolic/ diastolic [mmHg, 24hr]	123 (8.7) / 74 (7.5)	138 (11.7) / 78 (7.6)	0.001 0.210
Pulse pressure [mmHg, office]	49 (8.4)	58 (16)	0.030
Number 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 calc. [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

Parameter **Mean (Std. Dev.)**	**Donors** **(n=21)**	**Dialysis Patients** **(n=27)**	**p value**
Tissue concentration skin [mmol / g dry weight]	0.287 ± 0.176	0.308 ± 0.146	0.526
Tissue concentration artery [mmol / g dry weight]	0.378 ± 0.213	0.412 + 0.269	0.723
Tissue concentration muscle [mmol / g dry weight]	0.195 ± 0.127	0.204 ± 0.92	0.790

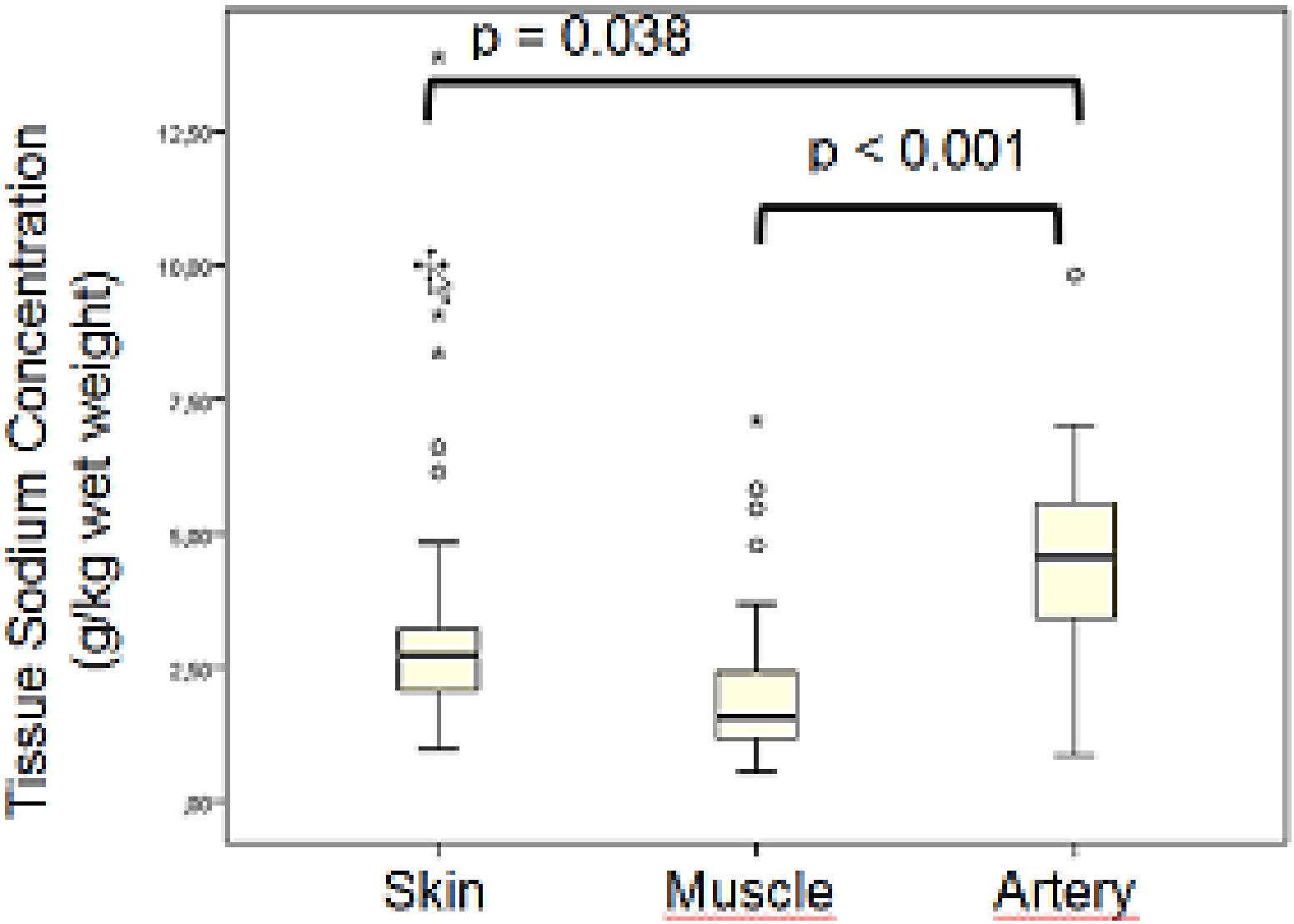
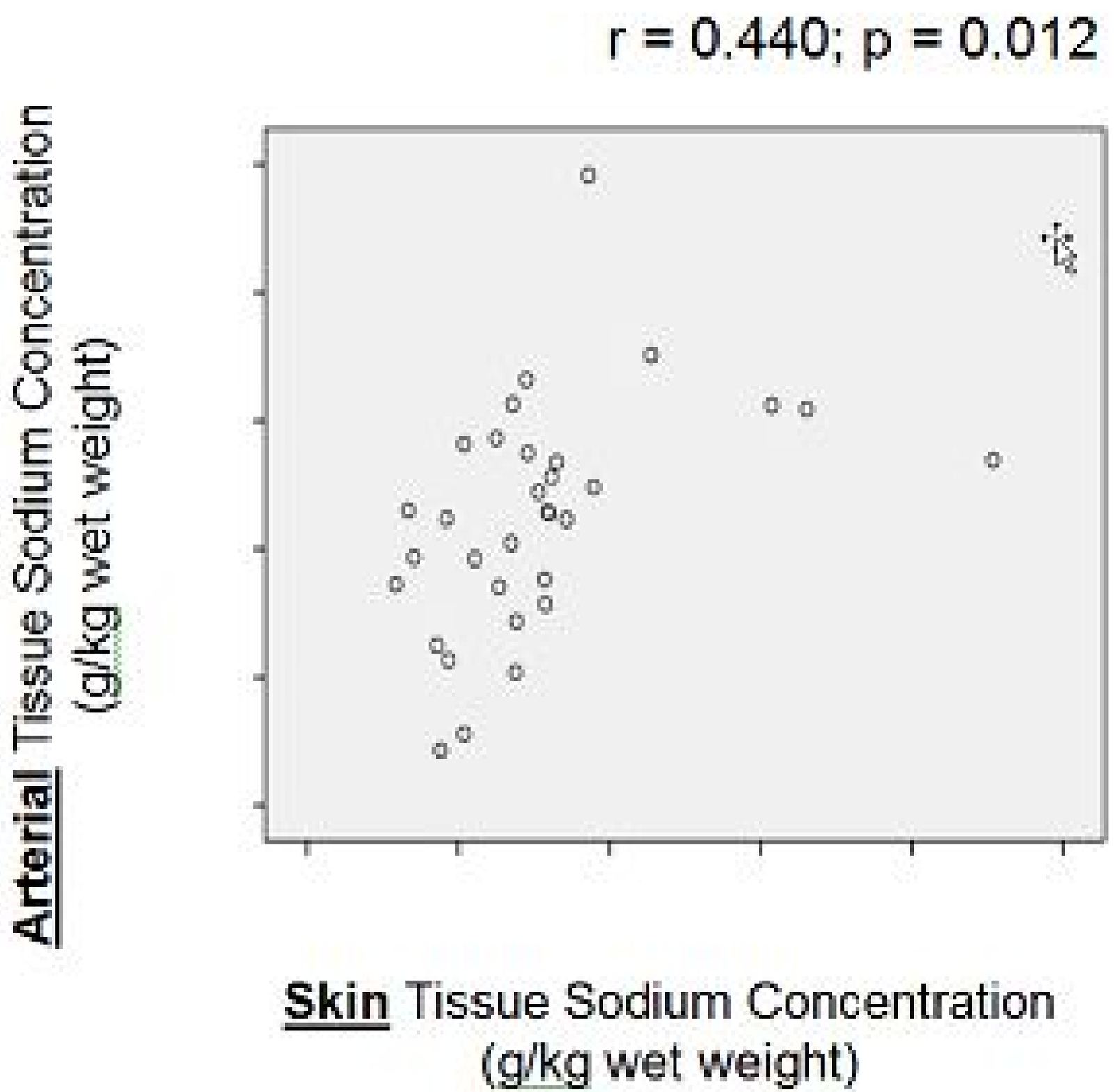
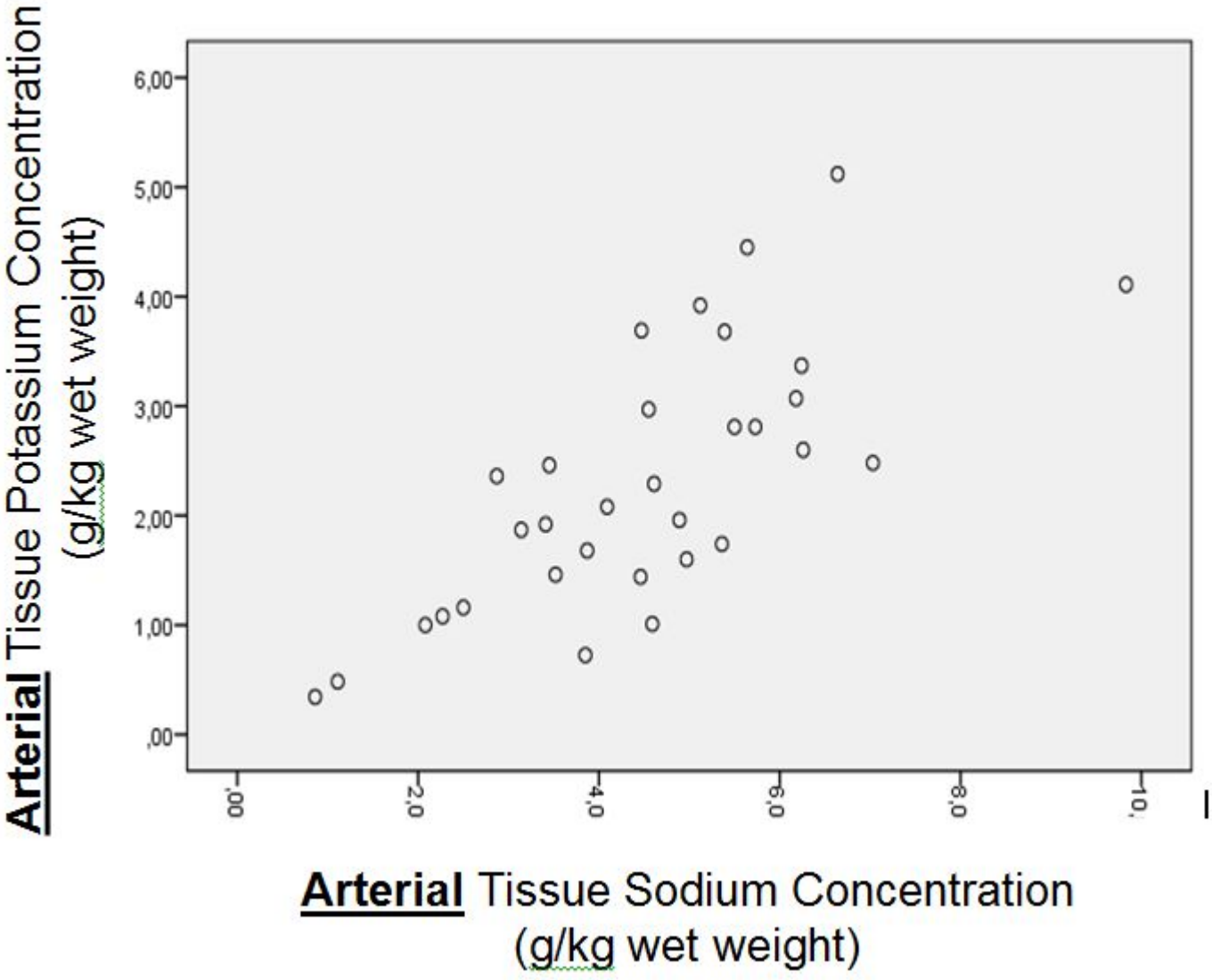

p = 0.038
p < 0.001
Tissue Sodium Concentration
(g/kg wet weight)
12,50
10,00
7,50
5,00
2,50
,00
Skin
Muscle
Artery

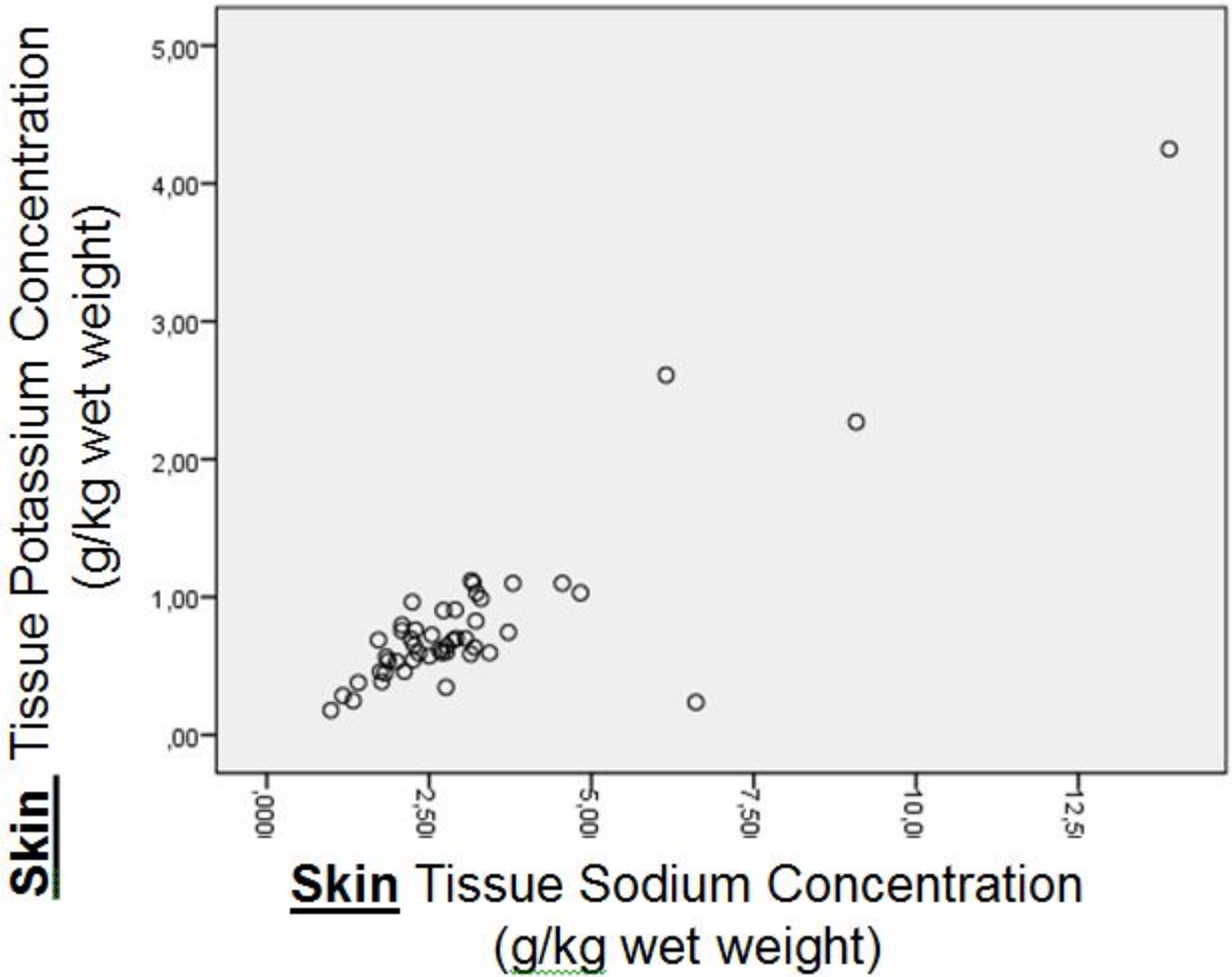
Figure 2


r = 0.440; p = 0.012
Arterial Tissue Sodium Concentration (g/kg wet weight)
Skin Tissue Sodium Concentration (g/kg wet weight)

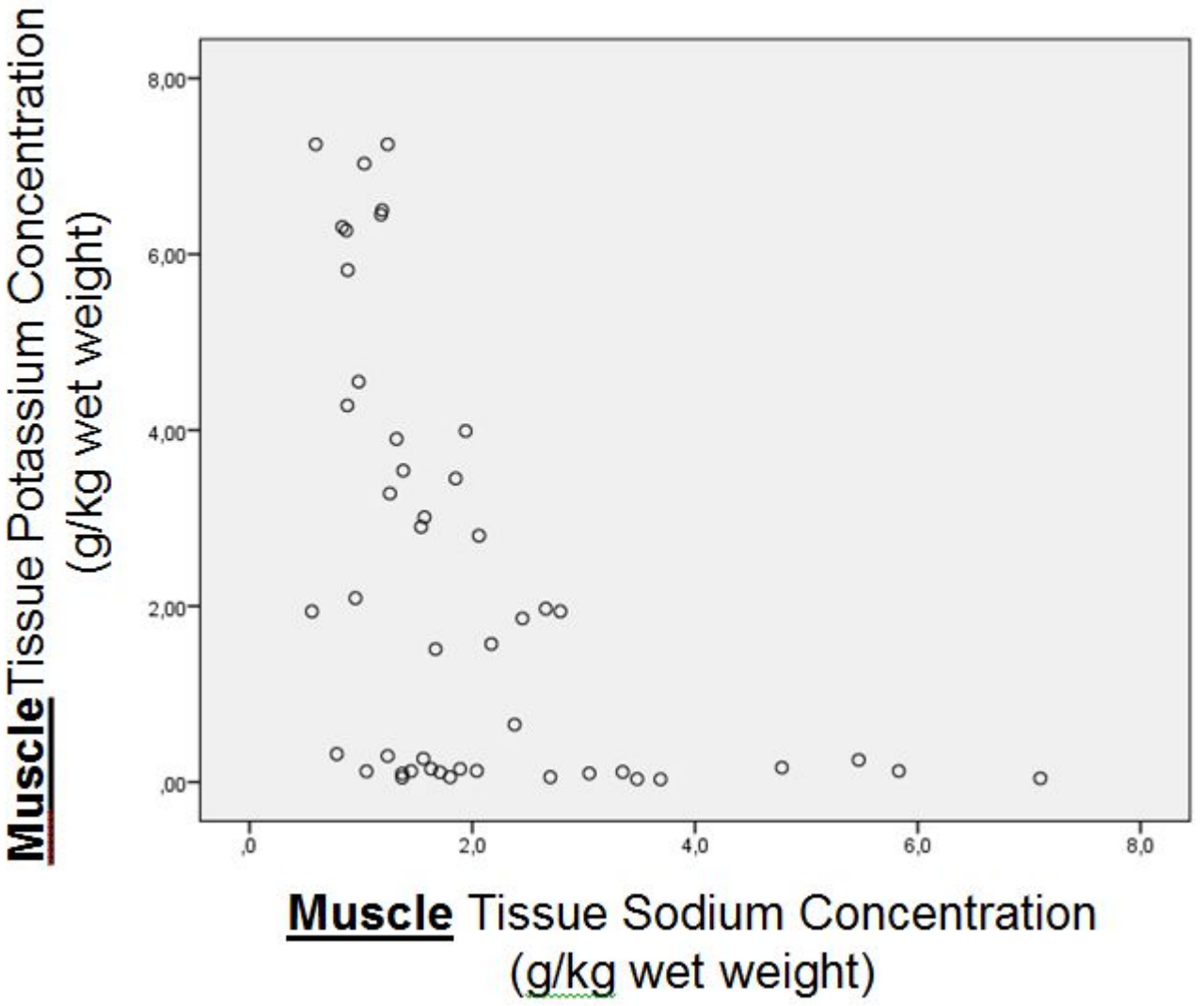
r = 0.730; p < 0.001

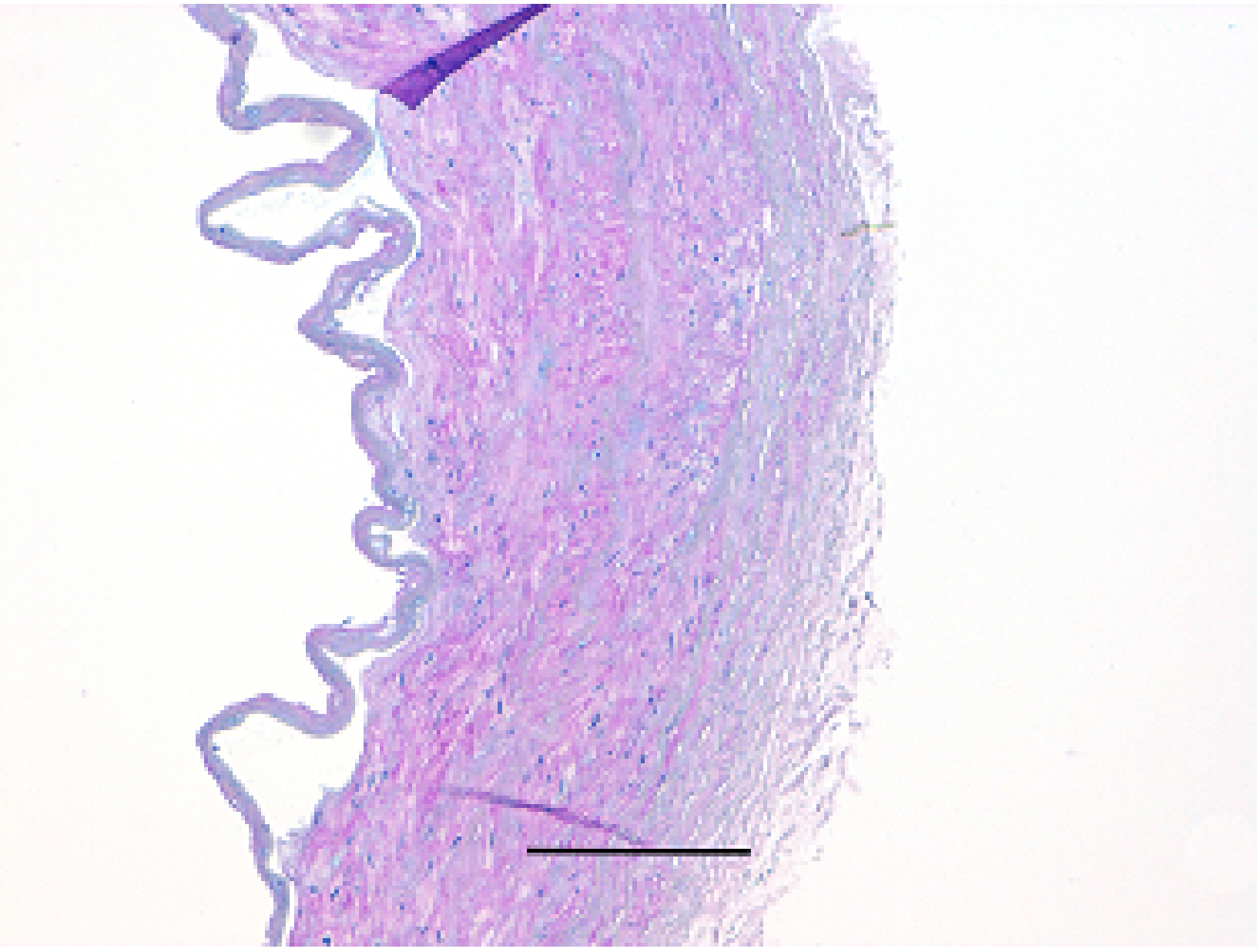

Arterial Tissue Potassium Concentration
(g/kg wet weight)
6,00
5,00
4,00
3,00
2,00
1,00
,00
Arterial Tissue Sodium Concentration
(g/kg wet weight)

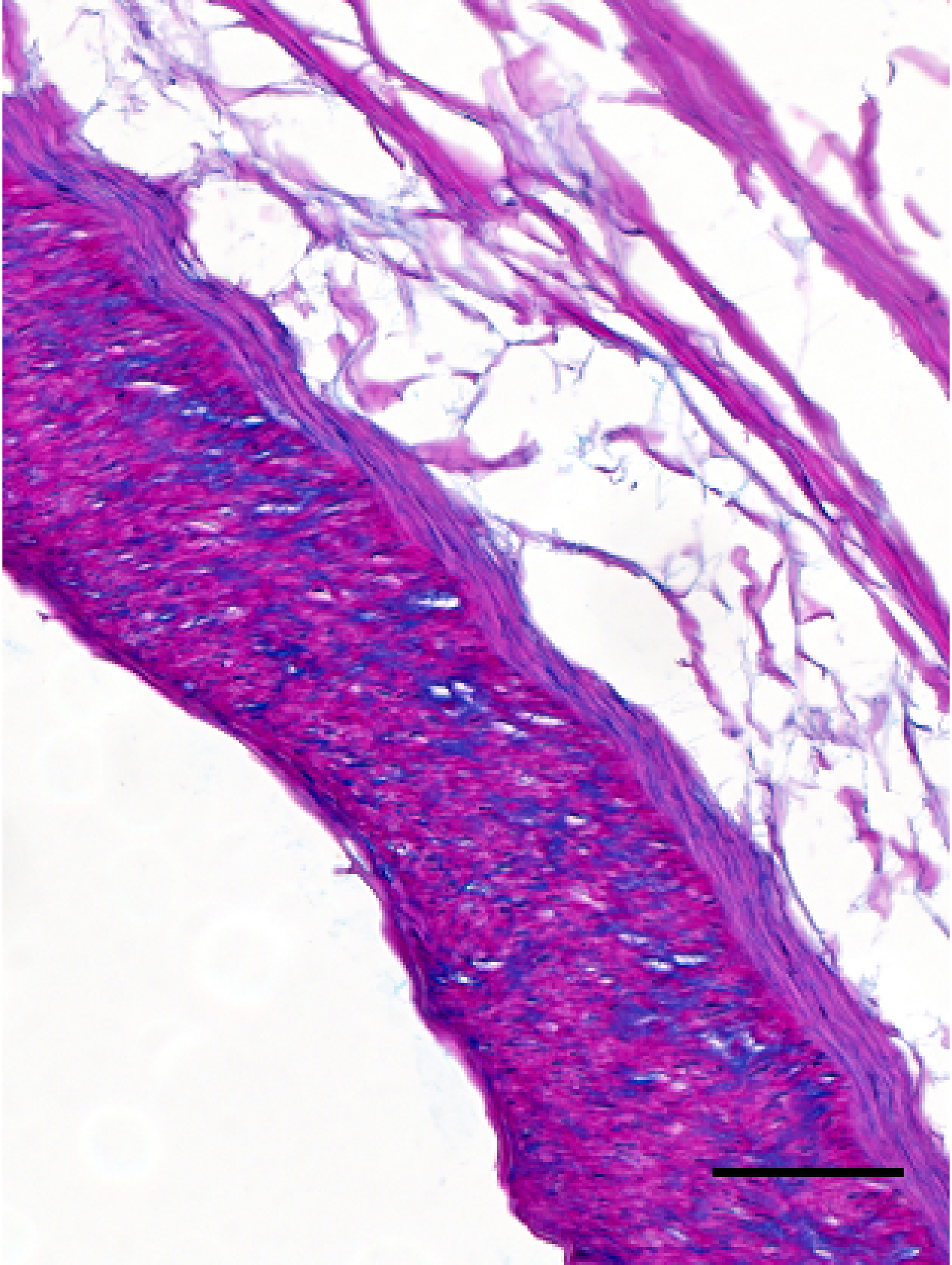
$r = 0.877$; $p < 0.001$

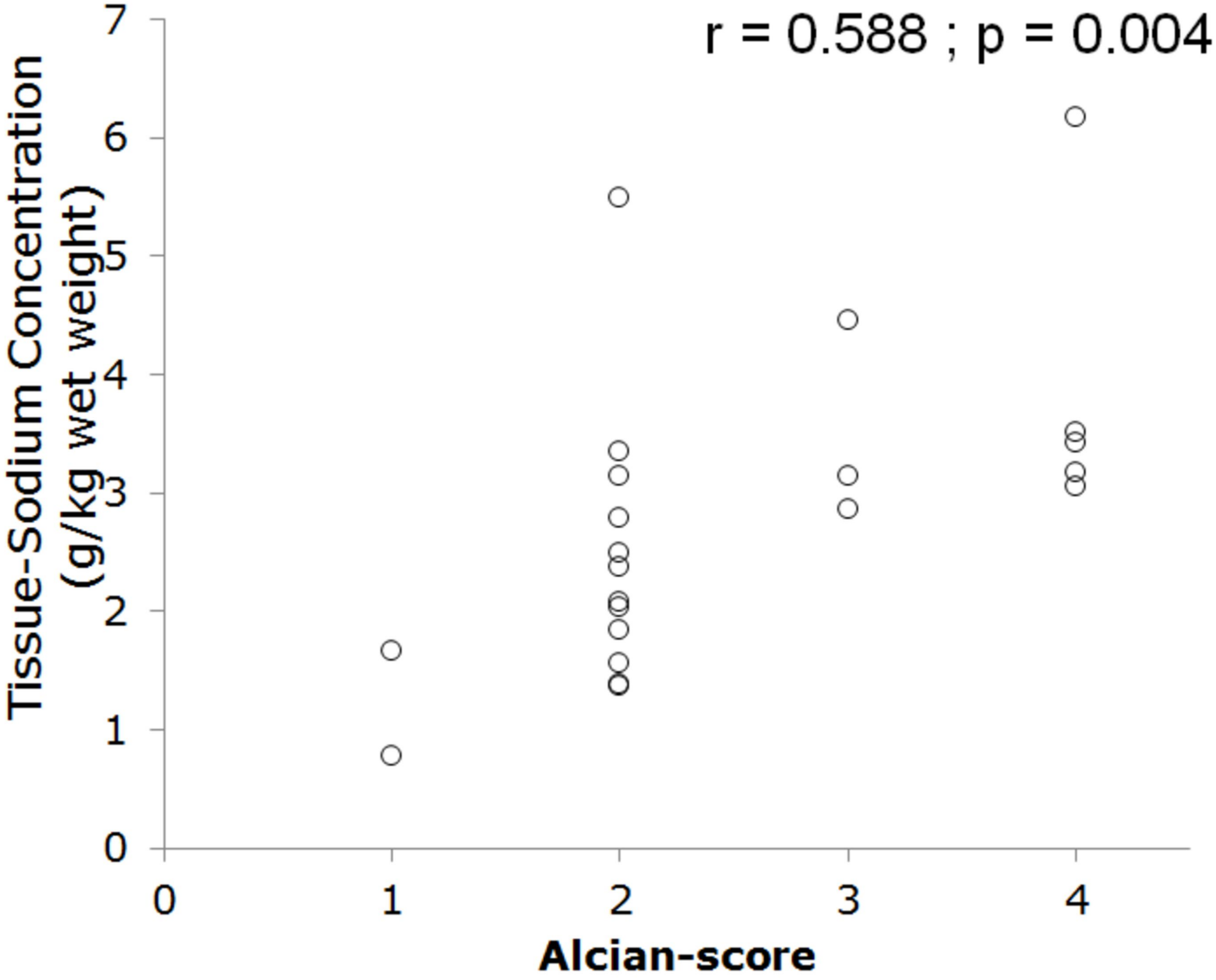

Skin Tissue Potassium Concentration (g/kg wet weight)
5,00
4,00
3,00
2,00
1,00
,00
2,50
5,00
7,50
10,0
12,5
Skin Tissue Sodium Concentration (g/kg wet weight)

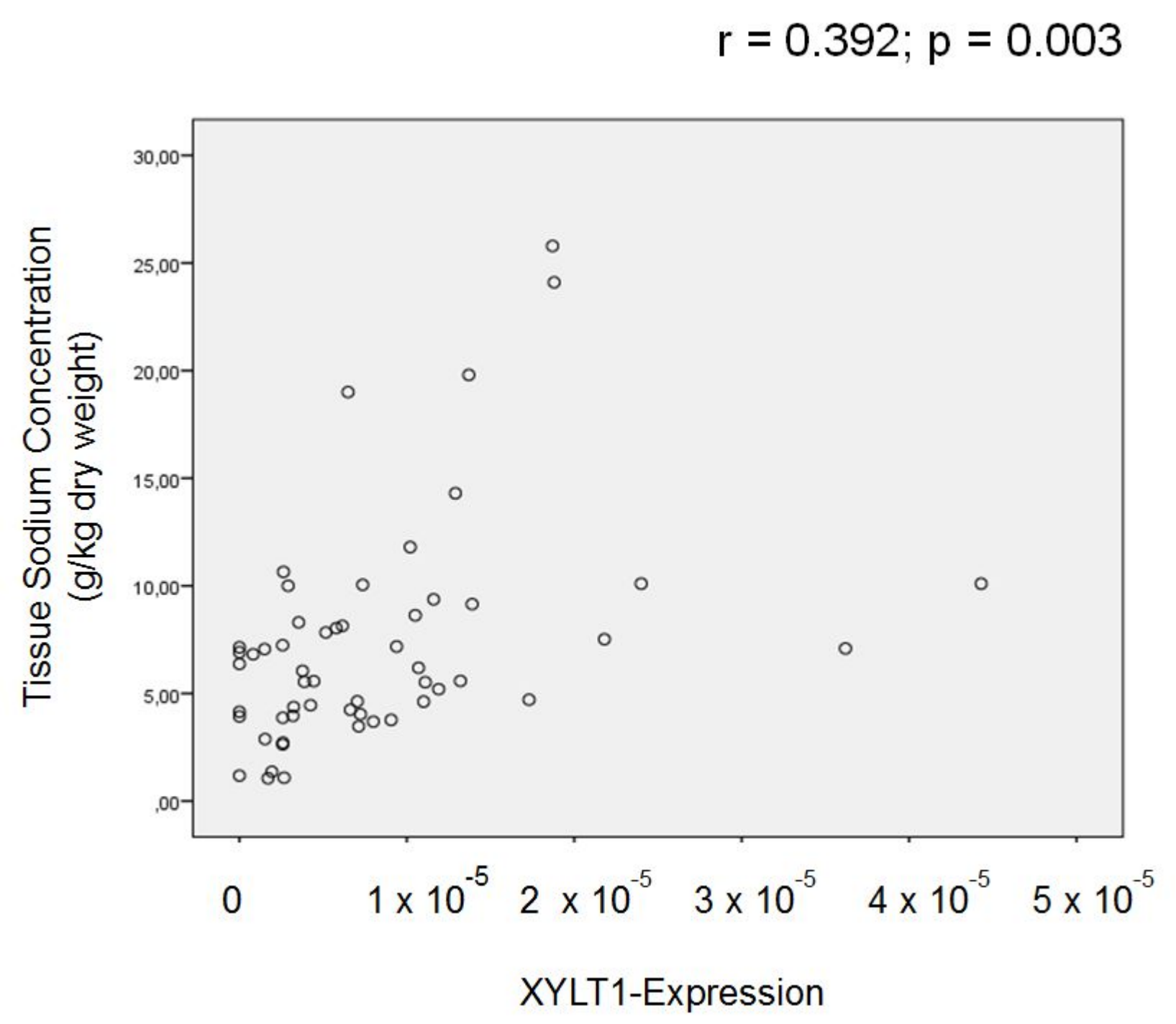
r = -0.492; p < 0.001

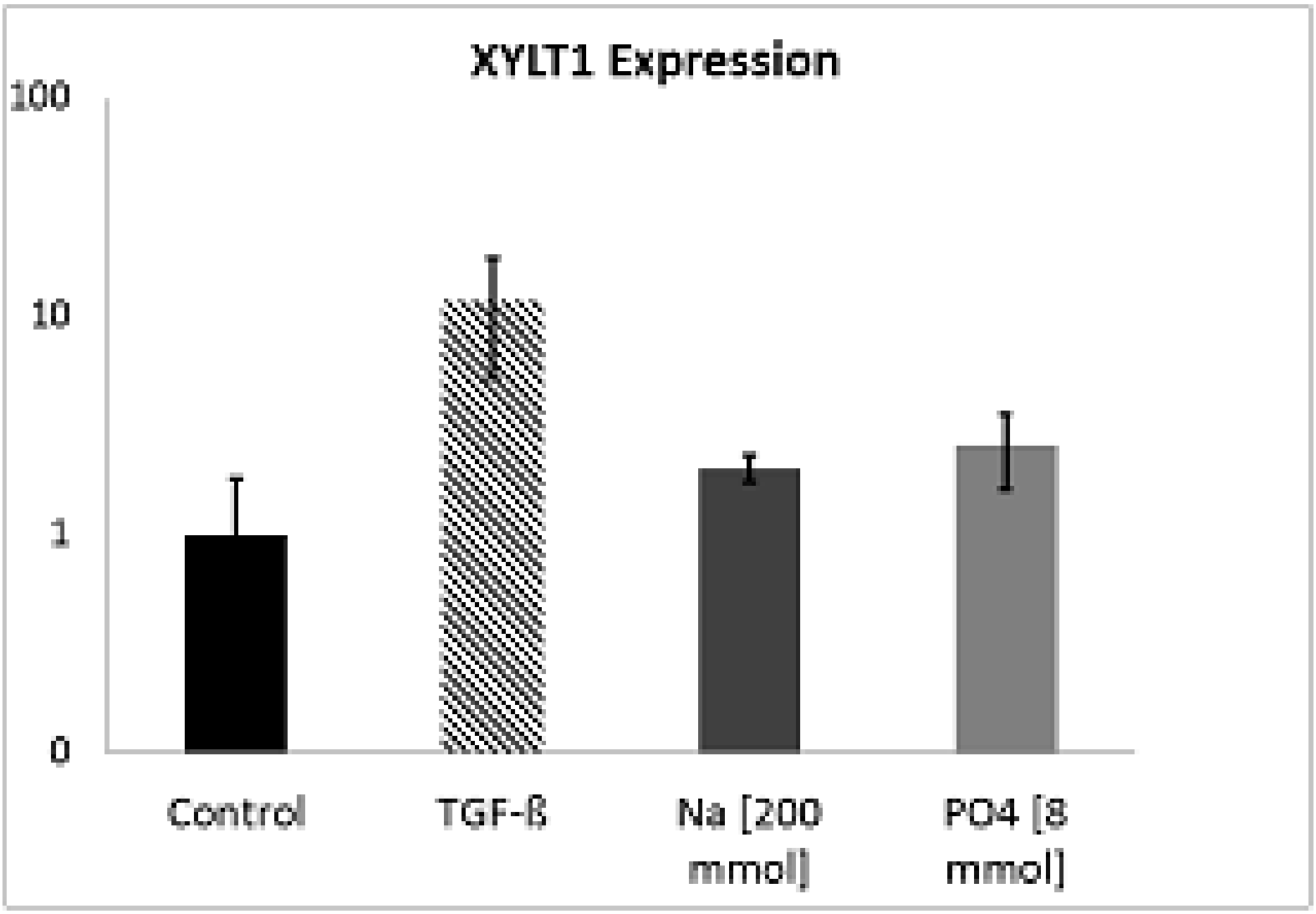

Muscle Tissue Potassium Concentration (g/kg wet weight)
8,00
6,00
4,00
2,00
,00
,0
2,0
4,0
6,0
8,0
Muscle Tissue Sodium Concentration (g/kg wet weight)






r = 0.588 ; p = 0.004
Tissue-Sodium Concentration (g/kg wet weight)
0
1
2
3
4
5
6
7
0
1
2
3
4
Alcian-score


r = 0.392; p = 0.003
Tissue Sodium Concentration (g/kg dry weight)
30,00
25,00
20,00
15,00
10,00
5,00
,00
0
1 x 10-5
2 x 10-5
3 x 10-5
4 x 10-5
5 x 10-5
XYLT1-Expression


XYLT1 Expression
100
10
1
0
Control
TGF-ß
Na [200 mmol]
PO4 [8 mmol]