Microvascular Permeability after an Acute and Chronic Salt Load in Healthy Subjects

A Randomized Open-label Crossover Intervention Study

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ABSTRACT

Background: Sodium-induced microcirculatory changes, endothelial surface layer alterations in particular, may play an important role in sodium-mediated blood pressure elevation. However, effects of acute and chronic sodium loading on the endothelial surface layer and microcirculation in humans have not been established. The objective of this study was to assess sodium-induced changes in blood pressure and body weight as primary outcomes and also in microvascular permeability, sublingual microcirculatory dimensions, and urinary glycosaminoglycan excretion in healthy subjects.

Methods: Twelve normotensive males followed both a low-sodium diet (less than 50 mmol/day) and a high-sodium diet (more than 200 mmol/day) for eight days in randomized order, separated by a crossover period. After the low-sodium diet, hypertonic saline (5 mmol sodium/liter body water) was administered intravenously in 30 min.

Results: Both sodium interventions did not change blood pressure. Body weight increased with 2.5 (95% CI, 1.7 to 3.2) kg ($P < 0.001$) after dietary sodium loading. Acute intravenous sodium loading resulted in increased transcapillary escape rate of ^{125}I-labeled albumin (2.7 [0.1 to 5.3] % cpm $\cdot$ g^{-1} $\cdot$ h^{-1}; $P = 0.04$), whereas chronic dietary sodium loading did not affect transcapillary escape rate of ^{125}I-labeled albumin (–0.03 [–3.3 to 3.2] % cpm $\cdot$ g^{-1} $\cdot$ h^{-1}; $P = 1.00$), despite similar increases of plasma sodium and osmolality. Acute intravenous sodium loading coincided with significantly increased plasma volume, as assessed by the distribution volume of albumin, and significantly decreased urinary excretion of heparan sulfate and chondroitin sulfate. These changes were not observed after dietary sodium loading.

Conclusions: Our results suggest that intravenous sodium loading has direct adverse effects on the endothelial surface layer, independent of blood pressure. **(Anesthesiology 2018; 128:352-60)**

HIGH dietary sodium intake is associated with increased blood pressure,[1] a major cause of premature death worldwide. The adverse effects of sodium are partly explained by expansion of extracellular volume and direct effects of sodium on the vessel wall,[2] but the underlying mechanisms are not fully clarified yet. Microvascular alterations, such as rarefaction, remodeling, and endothelial dysfunction, are known to be associated with high blood pressure and end organ disease.[3] Because sodium is known to induce these microcirculatory changes, this might therefore explain, at least in part, the relation between sodium and high blood pressure. In humans, high sodium intake leads to a reversible decrease of skin capillary density in hypertensive subjects,[4,5] and in normotensive subjects, high-sodium diet results in impaired endothelial function.[6–8] Other studies have shown improvement of endothelial function after dietary sodium restriction, both in normotensive and hypertensive subjects.[9,10]

What We Already Know about This Topic

- Tissue glycosaminoglycans influence sodium homeostasis *via* nonosmotic storage of sodium
- Impairment of the microcirculatory endothelial surface layer, which consists of different glycosaminoglycans, decreases its vascular barrier function

What This Article Tells Us That Is New

- Twelve healthy males followed both a low-sodium diet and a high-sodium diet for eight days each in a randomized crossover study and received intravenous hypertonic saline infusion over the course of 30 min after the low-sodium diet
- Despite similar increases in plasma sodium, chloride, and osmolality, chronic dietary sodium loading did not affect microvascular permeability, but hypertonic saline infusion increased it
- Increased microvascular permeability after saline infusion coincided with decreased urinary glycosaminoglycan excretion, indicating damage to the endothelial surface layer

This article is featured in "This Month in Anesthesiology," page 1A. The results have been presented in abstract form at the 52nd ERA-EDTA Congress in London, United Kingdom (May 28–31, 2015) and at the 25th European Meeting on Hypertension and Cardiovascular Protection in Milan, Italy (June 12–15, 2015).

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Several studies have demonstrated that glycosaminoglycans in tissues such as skin and cartilage influence sodium homeostasis *via* nonosmotic storage of sodium.[11,12] This could indicate an important role for the microcirculatory endothelial surface layer in sodium handling because the endothelial surface layer is known to consist of different glycosaminoglycans. It is conceivable that nonosmotic sodium storage within these endothelial surface layer glycosaminoglycans may affect extracellular volume as represented by body weight and blood pressure changes after sodium loading.[13] In addition, impairment of the endothelial surface layer decreases its vascular barrier function and induces protein extravasation and tissue edema, loss of nutritional blood flow, and an increase in platelet and leukocyte adhesion.[14] A perturbed endothelial surface layer has been demonstrated in different disease states that are characterized by blood pressure alterations and increased vascular permeability,[15] including kidney disease,[16,17] diabetes,[18,19] atherosclerosis,[20] inflammation,[21] and hypervolemia.[22] Several intravenous fluids, both colloids and crystalloids, have been shown to increase shedding of endothelial surface layer constituents and impact microvascular permeability.[22,23] Moreover, *in vitro* studies have shown that sodium overloading increases endothelial surface layer stiffness and decreases endothelial surface layer height,[24] yet the effects of sodium loading on the endothelial surface layer in humans, either by chronic dietary sodium or acute sodium loading by means of intravenous administration of saline, have not been established. Therefore we hypothesized that an acute intravenous sodium load and a chronic dietary sodium load differently affect blood pressure, the endothelial surface layer, and microcirculation.

Materials and Methods

Study Population

In this experimental nonblinded crossover intervention study, we included healthy, nonsmoking, male volunteers between 18 and 40 yr old of age. Exclusion criteria were hypertension (greater than or equal to 140/90 mmHg), obesity (body mass index greater than or equal to 30 kg/m^2), and history of primary hyperlipoproteinemia, coagulation disorders, and renal or cardiovascular diseases. The study was performed at the Academic Medical Center in Amsterdam, The Netherlands, between September 2013 and October 2014 and was conducted according to the principles of the Declaration of Helsinki (originally adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, with last amendment in Fortaleza, Brazil, October 2013). All participants provided written informed consent, and approval was obtained from the local ethics committee. The study was registered in the Netherlands Trial Register (NTR4095; http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4095; accessed April 23, 2017). Recently our group has published a manuscript that answered an ancillary research question regarding quantification of nonosmotic sodium storage capacity, using data that were also obtained during this study.[25]

Study Design

All subjects were enrolled by one of the research physicians and randomized to both a low-sodium diet (less than 50 mmol daily) and a high-sodium diet (more than 200 mmol daily) for eight days, separated by a crossover period of at least 1 week. Block randomization was performed after the screening visit and informed consent by the research physician (block size: n = 6); six subjects started with the low-sodium diet, and six others commenced the study with high-sodium diet. Dietary compliance was verified at days 3, 6, and 8 with collection of 24-h urine samples. After an overnight fast, subjects visited our research department at day 8 of both diets for blood and urine sampling, hemodynamic and microcirculatory measurements. Subjects were instructed to refrain from alcohol intake and heavy physical exercise 24 h before the study visit and to avoid caffeine intake 12 h in advance. At the study visit, two intravenous catheters were placed in the left and right antecubital veins.

In the afternoon of day 8 of the low-sodium diet, hypertonic saline (≈2.4% NaCl) was administered intravenously during 30 min. We corrected the infused amount of sodium for total body water (5 mmol sodium/liter body water) by adding a calculated volume of 20% NaCl solution to 500 ml of 0.9% NaCl. Before infusion, subjects were requested to empty their bladder. Blood and urine sampling and hemodynamic and microcirculatory measurements were carried out at fixed time intervals up to 4 h after infusion.

The primary outcome of the study was the extracellular volume, as represented by body weight and blood pressure. Hemodynamic measurements were carried out with semiautomated devices for blood pressure measurement and finger arterial pulse contour analysis (see "Hemodynamic Measurements"). Secondary outcomes consisted of microcirculatory and endothelial surface layer measurements, including the transcapillary escape rate of ^{125}I-labeled albumin (TERalb), representing microvascular permeability,[19] and sublingual sidestream darkfield (SDF) imaging, measuring perfused boundary region, reflecting endothelial surface layer thickness. In addition, endothelial surface layer constituents in the urine were measured. Urinary radioactivity after ^{125}I-labeled albumin administration was indicative for the endothelial surface layer glomerular barrier function. Finally, SDF imaging was also used to quantify red blood cell (RBC) filling and microvascular density, *i.e.*, measures of microvascular perfusion.

Hemodynamic Measurements

Systolic blood pressure, diastolic blood pressure, and heart rate were measured at the right upper arm with an appropriate adjusted cuff size in both seated and supine positions with a semiautomated oscillometric device (Omron 705 IT, OMRON Healthcare, The Netherlands) after resting for at least 10 min in a quiet and temperature-controlled room. The mean of the last two measurements was used for analysis. Mean arterial pressure was calculated as 1/3 × (systolic blood pressure) + 2/3 × (diastolic blood pressure). Pulse

pressure was expressed as the difference between systolic blood pressure and diastolic blood pressure. Blood pressure, heart rate, mean arterial pressure, cardiac output, systemic vascular resistance, stroke volume, and an index of left ventricular contractility (dP/dt) were also measured in supine position at the intermediate phalanx of the left middle finger with finger arterial pulse contour analysis (Nexfin, BMEYE, The Netherlands). These hemodynamic parameters were calculated from the average of a 30-s stable recording period after at least 15 min of supine rest.[26]

Transcapillary Escape Rate of ^{125}I-labeled Albumin

Microvascular permeability was determined after both diets and 1 h after intravenous saline infusion by TERalb. We administered an intravenous bolus of saline solution with 100 kBq ^{125}I-labeled albumin.[19] Blood samples were drawn from the contralateral arm at baseline and after 3, 4, 5, 10, 15, 20, 30, 45, and 60 min. Urine was collected before and after the measurement. Radioactivity in plasma and urine was measured in duplicate with a Wizard^2 2480 Automatic Gamma Counter (PerkinElmer, USA). TERalb was calculated with regression analysis and expressed as percentage decline in plasma radioactivity per hour (% cpm $\cdot$ g^{-1} $\cdot$ h^{-1}). Plasma volume was determined by calculating the *y*-intercept of the disappearance curve of ^{125}I-albumin, corrected for the injected dose of tracer.

Sublingual Microvascular Imaging

Imaging of sublingual microvasculature was performed with a SDF MicroScan Video Microscope (MicroVision Medical, The Netherlands). The SDF camera captures the hemoglobin of passing red blood cells with green light emitting diodes (540 nm), using a 5× objective resulting in a field of view of 0.95 mm × 0.70 mm (0.665 mm^2) at a resolution of 720 × 576 pixels. The images were automatically captured with integrated Glycocheck software (version 1.2.7.7394, Glycocheck, The Netherlands) that identified all visible microvessels with a diameter between 5 and 25 μm. At every 10 μm along each microvessel, measurement sites were selected. Vessels with sufficient contrast in more than 60% of measurement sites were considered as valid vascular segments. Data acquisition automatically started when image quality was within acceptable range and automatically stopped when a data of minimum number of 3,000 measurement sites had been reached. Measurements were performed after low-sodium diet and high-sodium diet and 120 min after saline infusion. Three sequential measurement cycles were carried out in each participant, and the average values of these measurements were used for analysis. Glycocheck software assessed perfused boundary region, RBC filling, and microvascular density. The methods for the automatic analysis of endothelial surface layer dimensions have been described elsewhere.[27] Because the perfused boundary region is dependent on erythrocyte,[27,28] we corrected the perfused boundary region measurements for erythrocyte.

Urinary Glycosaminoglycan Measurement

Glycosaminoglycans were enzymatically digested into disaccharides, and the results were reported as previously described.[29] Disaccharide concentrations were adjusted for creatinine concentration of the urine samples. A validated high performance liquid chromatography with mass spectrometry/mass spectrometry method was used to quantify urinary excretion of heparan sulfate, dermatan sulfate, and chondroitin sulfate. Values below the lower limit of quantification were assigned values of half the lower limit of quantification.

Other Laboratory Testing

Laboratory testing included plasma hematocrit, sodium, potassium, chloride, osmolality, creatinine, glucose, and N-terminal pro b-type natriuretic peptide (NTproBNP). Urine analysis consisted of sodium, potassium, chloride, creatinine, and osmolality. All biochemical tests (plasma and urine) were performed on a COBAS C8000 modular analyzer (Roche Diagnostics, Germany) except for osmolality, which was performed on the Osmo station OM-6060 (Menarini Diagnostics, Italy). We used ion-selective electrode methods to measure sodium, potassium, and chloride in plasma and urine. Plasma and urinary osmolality were quantified by freezing point depression. Creatinine in plasma was measured with an enzymatic spectrophotometric method and creatinine in urine with a spectrophotometric method. We measured plasma glucose with an enzymatic (ultraviolet) spectrophotometric method and NTproBNP with a two–monoclonal antibody sandwich method. Plasma hematocrit was calculated with the formula: hematocrit (l/l) = (mean corpuscular volume (fl) × erythrocyte (10^12/l))/1,000. We calculated estimated glomerular filtration rate with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation.

Statistical Analysis

Continuous variables are presented as means and 95% CI and as median plus interquartile range when data were not normally distributed. Characteristics were compared using a paired *t* test or Wilcoxon signed rank test where appropriate. To compare the results of the three conditions, a general linear model with repeated measurements was used. For variables with a skewed distribution, log transformed values were used for repeated measurement analysis. We performed Bonferroni *post hoc* adjustment for multiple comparisons. Data was analyzed with IBM SPSS Statistics (version 22.0, 2013, IBM, USA). *P* values less than 0.05 were considered statistically significant. Sample size was calculated using primary endpoints. Our pilot data showed a 1.7 (SD 1.0) kg body weight difference between subjects on a low-sodium diet and a high-sodium diet. Thus, at least six subjects were needed for each group (based on a two-sided *t* test; power 80%, α-error 5%). We demonstrated a 5 mmHg (SD 5) systolic blood pressure difference between subjects on low-sodium diet and high-sodium diet, indicating that at least ten subjects were needed (two-sided *t* test; power 80%, α-error 5%). To provide enough power for both primary

outcomes and taking into account possible drop-out of subjects once they were included in the study, we decided to include at least 12 subjects.

Results

After screening of 19 healthy males, three subjects were excluded: one due to high systolic blood pressure and two others because of difficulties with blood drawing. Four subjects withdrew their consent after inclusion before randomization and did not participate in the sodium interventions. Therefore there are no data available on these subjects regarding the primary and secondary outcomes of this study. The trial was conducted in accordance to the original protocol. Twelve subjects with a mean age of 23 (range, 18 to 31) yr completed the study. All subjects successfully adhered to both diets, resulting in urinary sodium excretion of 19 (95% CI, 13 to 25) mmol/24 h after low-sodium diet, and 341 (275 to 407) mmol/24 h after high-sodium diet ($P < 0.001$), and urinary chloride excretion of 23 (21 to 26) mmol/24 h after low-sodium diet and 342 (282 to 402) mmol/24 h after high-sodium diet ($P < 0.001$). Urinary potassium excretion was comparable ($P = 0.93$) after low-sodium diet (88 [71 to 104] mmol/24 h) and high-sodium diet (92 [79 to 105] mmol/24 h). Saline infusion contained 542 (539 to 546) ml of 2.4 (2.3 to 2.5) % NaCl. In comparison with low-sodium diet, the body weight increased to 76.5 (72.2 to 80.7) kg (+ 2.5 [1.7 to 3.2] kg, $P < 0.001$) after high-sodium diet. There were no adverse events reported during the study—not during the diets but also not after infusion.

Increase of Plasma Volume after Saline Infusion without Hemodynamic Changes

In comparison to the low-sodium diet, acute saline infusion induced a significant increase ($P = 0.01$) in plasma volume of 287 (113 to 462) ml, resulting in a volume of 3,727 (3,377 to 4,077) ml. Plasma volume after high-sodium diet (3,568 [3,166 to 3,971] ml) and after low-sodium diet (3,440 [3,030 to 3,850] ml) were comparable ($P = 0.99$), also when corrected for body weight (low-sodium diet: 46 [42 to 51] ml/kg; high-sodium diet: 47 [42 to 51] ml/kg, $P = 1.00$). We detected no distinct peripheral and central hemodynamic changes, as measured with the semiautomatic oscillometric device and finger arterial pulse contour analysis respectively, despite a small increase of cardiac output (0.6 [0.2 to 1.0] l/min; $P = 0.03$) after high-sodium diet compared to saline infusion. Table 1 summarizes the hemodynamic characteristics of the different sodium conditions.

Endothelial Surface Layer Changes after Saline Infusion, but Not after High-sodium Diet

TERalb did not differ between low-sodium diet (7.0 [5.5 to 8.6] %) and high-sodium diet (7.0 [4.5 to 9.5] %) but increased significantly after saline infusion to 9.7 (7.8 to 11.6) % ($P = 0.04$). Correction for hematocrit, to eliminate the influence of plasma volume changes during the sampling period, demonstrated similar results of TERalb (data not shown). Urinary radioactivity significantly increased after saline infusion in comparison to both the low-sodium diet and high-sodium diet ($P = 0.006$ and $P = 0.006$, respectively). Figure 1 gives an overview of TERalb and changes in urinary radioactivity.

Perfused boundary region as measured by SDF imaging did not change after high-sodium diet (1.95 [1.88 to 2.03] μm) or saline infusion (1.92 [1.81 to 2.03] μm) in comparison to low-sodium diet (2.02 [1.93 to 2.12] μm). The correction of the perfused boundary region for erythrocyte showed no changes in the levels of erythrocyte-corrected

Table 1. Hemodynamic Characteristics

Characteristics (N = 12)	Low-sodium Diet, Mean (95% CI)	High-sodium Diet, Mean (95% CI)	Saline infusion, Mean (95% CI)	*P* Value
Plasma volume, ml	3,440 (3,030–3,850)	3,568 (3,166–3,971)	3,727 (3,377–4,077)	0.03*
Plasma volume/body weight, ml/kg	46 (42–51)	46 (42–51)	50 (47–54)	0.004†
Blood pressure, Omron				
Systolic blood pressure, mmHg	117 (112–122)	118 (115–122)	116 (111–120)	0.25
Diastolic blood pressure, mmHg	58 (55–62)	58 (54–61)	58 (56–61)	0.76
Heart rate, beats/min	56 (51–61)	57 (50–63)	54 (49–60)	0.45
Mean arterial pressure (mmHg)	78 (75–81)	78 (75–81)	78 (75–80)	0.84
Pulse pressure (mmHg)	59 (54–64)	61 (57–65)	57 (54–61)	0.18
Finger arterial pulse contour analysis (Nexfin)				
Systolic blood pressure, mmHg	127 (122–133)	130 (125–136)	127 (121–134)	0.45
Diastolic blood pressure, mmHg	72 (69–75)	73 (70–77)	73 (69–77)	0.83
Heart rate, beats/min	56 (51–61)	59 (52–66)	55 (50–60)	0.09
Mean arterial pressure, mmHg	91 (87–94)	93 (88–98)	91 (86–96)	0.41
Cardiac output, l/min	6.6 (5.9–7.3)	7.2 (6.3–8.0)	6.5 (5.9–7.2)	0.02‡
Systemic vascular resistance, dyn s/cm^5*	1,134 (986–1,282)	1,072 (944–1,200)	1,137 (991–1,283)	0.25
Stroke Volume (ml)	118 (112–125)	122 (116–128)	119 (112–125)	0.21

Bonferroni *post hoc* test results:

*Low-sodium diet *versus* saline infusion $P < 0.05$. †Low-sodium diet *versus* saline infusion $P < 0.05$; high-sodium diet *versus* saline infusion $P < 0.005$. ‡High-sodium diet *versus* saline infusion $P < 0.05$.

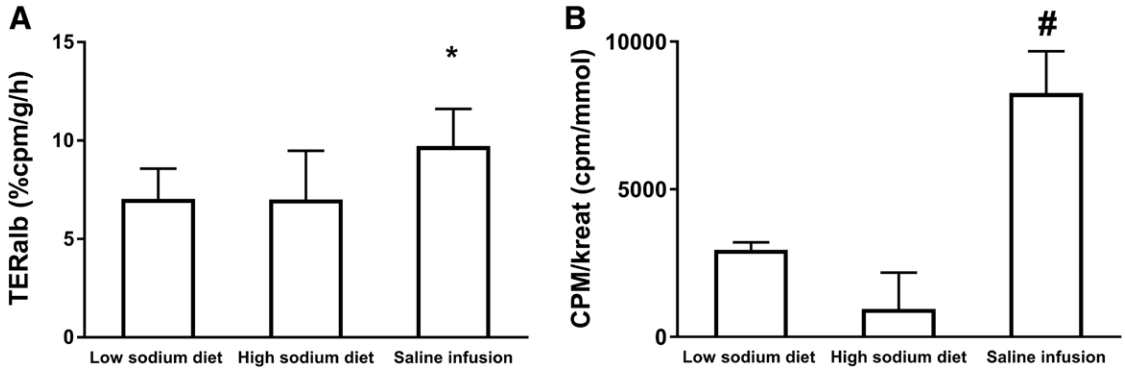

Fig. 1. Transcapillary escape rate (TERalb) and urinary radioactivity after the three sodium interventions. (*A*) TERalb did not differ between low-sodium diet (7.0 [5.5 to 8.6]%) and high-sodium diet (7.0 [4.5 to 9.5]%) but increased significantly after saline infusion to 9.7 (7.8 to 11.6)%. **P* = 0.04 compared to low-sodium diet. (*B*) Urinary radioactivity was also significantly increased after saline infusion. #*P* = 0.006 compared to low-sodium diet. **P* = 0.006 compared to high-sodium diet. The data are represented as means and 95% CI (n = 12). The *P* values were derived from Bonferroni *post hoc* tests. CPM = counts per minute; kreat = creatinine in urine.

perfused boundary region after saline infusion (*P* = 0.14) (fig. 2). After saline infusion, decreases in heparan sulfate and chondroitin sulfate disaccharide concentrations were detected in urine, whereas the urinary amount of dermatan sulfate disaccharides did not change after saline infusion. Figure 3A shows the percentage change of concentrations of the urinary glycosaminoglycans (corrected for urinary creatinine) in comparison to low-sodium diet, after high-sodium diet and saline infusion. The decrease of total heparan sulfates disaccharides was mainly caused by a decline of the nonsulfated and monosulfated heparan sulfate disaccharides (fig. 3B1). Relative to total heparan sulfates, only the monosulfated fraction altered significantly (fig. 3B2).

No Changes in Sublingual Microvascular Perfusion after Sodium Interventions

Sublingual microvascular density was similar after low-sodium diet (2,526 [2,341 to 2,712] μm/mm^2), high-sodium diet (2,612 [2,244 to 2,980] μm/mm^2) and after saline infusion (2,724 [2,513 to 2,935] μm/mm^2). RBC filling showed no differences between diets (low-sodium diet: 74.4 [72.7 to 76.2]%; high-sodium diet: 75.1 [73.5 to 76.7]%) or after saline infusion (75.5 [73.8 to 77.3]%).

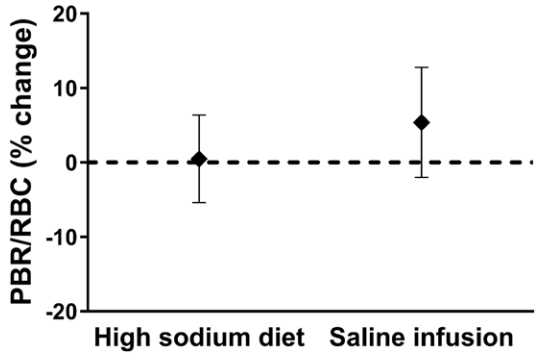

Fig. 2. Sublingual perfused boundary region corrected for erythrocyte (red blood cell [RBC]) increases after saline infusion. The correction of perfused boundary region (PBR) for erythrocyte showed no changes in erythrocyte-corrected perfused boundary region values after saline infusion (*P* = 0.14) compared to the low-sodium diet. The data are represented as the means and 95% CI (n = 12).

Change in Plasma Electrolytes and Fractional Sodium Excretion

Both sodium loading experiments induced a significant increase of plasma sodium, chloride, and osmolality, whereas plasma potassium remained stable. Hematocrit decreased after saline infusion. The sodium/creatinine ratio and fractional excretion of sodium were both increased after the high-sodium diet and the saline infusion compared to the low-sodium diet, with the highest levels after high-sodium diet. Table 2 gives an overview of the plasma and urine characteristics.

Discussion

We demonstrate that acute intravenous sodium loading causes an increase of microvascular permeability, whereas chronic dietary sodium loading does not affect microvascular permeability, despite similar increases in plasma sodium, chloride, and osmolality. Both interventions have no significant impact on blood pressure, whereas chronic dietary sodium loading increases body weight substantially. The increase of microvascular permeability after saline infusion coincides with increased plasma volume, decreased hematocrit reflecting hemodilution, and decreased urinary glycosaminoglycan excretion, which were not observed after a high-sodium diet. Both sodium loading interventions had no significant impact on sublingual microcirculatory density and RBC filling. These results suggest that an acute intravenous sodium load has direct adverse effects on the endothelial surface layer, compared to chronic high sodium intake, independent of blood pressure.

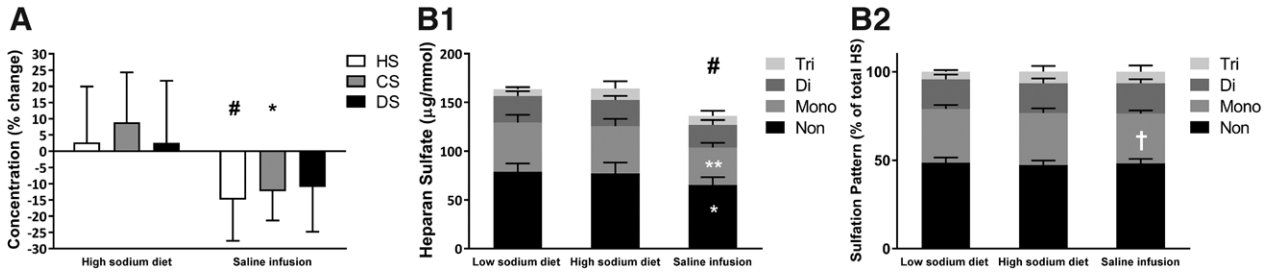

Fig. 3. Urinary glycosaminoglycan disaccharide concentrations decrease after saline infusion. (*A*) Saline infusion induced a decrease of urinary heparan sulfate (HS) and chondroitin sulfate (CS) glycosaminoglycans. #*P* = 0.03 compared to low-sodium diet; **P* = 0.01 compared to low-sodium diet. The urinary amount of dermatan sulfate (DS) disaccharides did not change after saline infusion. (*B1*) The decrease of total heparan sulfates disaccharides was mainly caused by a decline of the nonsulfated and monosulfated heparan sulfates. (*B2*) Only the monosulfated fraction (% of total heparan sulfates) altered significantly. #Total heparan sulfate concentration: *versus* low-sodium diet, *P* = 0.02; *Non: *versus* low-sodium diet, *P* = 0.01; **Mono: *versus* low-sodium diet, *P* = 0.04, and *versus* high-sodium diet, *P* = 0.03. †Mono: *versus* low-sodium diet *P* = 0.03. The data are represented as the means and 95% CI (n = 12). The *P* values derived from Bonferroni *post hoc* tests. Di = disulfated disaccharides (D0S6 and D2S00); Mono = monosulfated disaccharides (D0S0, D0A6, and D2A0); Non = nonsulfated disaccharides (D0A0); Tri = trisulfated disaccharides (D2S6).

Table 2. Plasma and Urine Characteristics

Characteristics (N = 12)	Low-sodium Diet, Mean (95% CI)	High-sodium Diet, Mean (95% CI)	Saline Infusion, Mean (95% CI)	*P* Value
Plasma				
Hematocrit, l/l	0.43 (0.41–0.44)	0.42 (0.41–0.43)	0.39 (0.38–0.40)	< 0.001*
Sodium, mmol/l	138 (137–139)	140 (139–141)	141 (140–141)	< 0.001†
Potassium, mmol/l	3.9 (3.7–4.1)	3.9 (3.9–4.0)	4.1 (3.8–4.4)	0.11
Chloride, mmol/l	100 (99–101)	103 (102–105)	105 (104–106)	< 0.001*
Glucose, mmol/l	4.9 (4.7–5.1)	5.0 (4.7–5.2)	4.9 (4.6–5.2)	0.73
Osmolality, mOsm/kg	285 (283–287)	290 (287–292)	290 (288–292)	0.005‡
Creatinine, µmol/l	84 (78–90)	77 (71–83)	—	< 0.001
Estimated glomerular filtration rate, ml · min^{-1} · 1.73 m^{-2}	112 (103–121)	120 (112–127)	—	0.001
NTproBNP, median (IQR), ng/l	8.5 (5.2–11.9)	16.4 (9.3 – 37.2)	9.1 (5.3–12.3)	0.11
Urine				
Sodium/creatinine ratio	1.3 (0.9–1.7)	24.6 (20.2–29.0)	6.3 (3.3–9.4)	< 0.001§
Fractional excretion of sodium (%)	0.08 (0.06–0.1)	1.4 (1.1–1.7)	0.4 (0.2–0.6)	< 0.001‖

Bonferroni *post hoc* test results:

*Low-sodium diet *versus* saline infusion, *P* < 0.001; low-sodium diet *versus* high-sodium diet, *P* < 0.001. †Low-sodium diet *versus* saline infusion, *P* < 0.005; low-sodium diet *versus* high-sodium diet, *P* < 0.005. ‡Low-sodium diet *versus* saline infusion, *P* < 0.001; low-sodium diet *versus* high-sodium diet, *P* < 0.05. §Low-sodium diet *versus* saline infusion, *P* < 0.01; low-sodium diet *versus* high-sodium diet, *P* < 0.001; high-sodium diet *versus* saline infusion, *P* < 0.001. ‖Low-sodium diet *versus* saline infusion, *P* < 0.01; low-sodium diet *versus* high-sodium diet, *P* < 0.001; high-sodium diet *versus* saline infusion, *P* < 0.001.

IQR = interquartile range; NTproBNP = N-terminal pro b-type natriuretic peptide.

To our knowledge, this is the first *in vivo* study in humans that demonstrates increased microvascular permeability after saline infusion. Our observation is in agreement with earlier studies that studied the effects of plasma volume expansion. Parving *et al.*[30] have demonstrated that infusion of both 25% human serum albumin and 6% dextran in healthy volunteers resulted in increased plasma volume and increased permeability measured with TERalb. Furthermore, volume loading with colloids (5% albumin or 6% hydroxyethyl starch 130/0.4) containing 0.9% NaCl is associated with a decrease in endothelial surface layer volume[31] and increased shedding of endothelial surface layer constituents into the circulation,[22] indicating the damaging effects of these infusions on the barrier function of the endothelial surface layer. However, the microvascular effects of hypertonic saline infusion are mainly studied in the context of shock and the subsequent phase of fluid resuscitation. A recent study in a rat model of hemorrhagic shock and fluid resuscitation demonstrated that changes in microvascular permeability were correlated with alterations in both glycocalyx thickness, as well as with changes in plasma endothelial surface layer components.[32]

Apart from the increase in microvascular permeability, we observed an increase in urinary radioactivity after hypertonic saline infusion. Because there are no reasons to assume that hypertonic saline results in a dissociation of the ^{125}I-labeled albumin complex (*i.e.*, leading to higher "free" fraction ^{125}I), the increased urinary radioactivity after saline

infusion indicates either increased transglomerular passage or decreased tubular reabsorption of ^{125}I-labeled albumin. For now, we cannot make differentiation between these two possibilities. However, given the fractional sodium excretion of less than 1%, reflecting normal tubular function, increased glomerular permeability to albumin may represent the best explanation. Of note, because urinary albumin was in all subjects below lower limit of quantification at all interventions, we were not able to quantify total albuminuria in this group of healthy young men.

We also demonstrated a decrease of the fractions of urinary heparan sulfate (low sulfated disaccharides in particular) and chondroitin sulfate, which are important constituents of the endothelial surface layer. A possible explanation might be that due to the increased vascular permeability, heparan sulfate and chondroitin sulfate have already leaked into the extravascular space. This seems to be corroborated by a study demonstrating that urinary excretion of heparan sulfate was decreased in diabetic patients, who are characterized with reduced endothelial surface layer, compared with nondiabetic controls, and that a more distinct decrease was present in patients with diabetic nephropathy (*i.e.*, subjects with more severe endothelial surface layer loss as compared to diabetic patients without nephropathy).[33] One might have expected increased excretion of urinary glycosaminoglycans, because increased shedding is thought to be a marker of endothelial surface layer damage. However, data regarding shedding of glycosaminoglycans after fluid challenges are limited. Chappell *et al.*[22] measured serum and urine heparan sulfate after infusion of 6% hydroxyethyl starch 130/0.4 and were not able to demonstrate differences before and after infusion in heparan sulfate concentrations, neither in urine nor in serum. These observations indicate that it remains difficult to interpret changes of endothelial surface layer components in the context of endothelial surface layer damage, especially after fluid resuscitation.

Although the dietary sodium load caused a comparable increase in plasma sodium and osmolality as the intravenous sodium load and induced weight gain indicating (extracellular) volume expansion, this intervention was not associated with microvascular or hemodynamic changes. This may indicate that our young and healthy cohort may be capable of compensating for endothelial surface layer and microvascular damage (*i.e.*, increased permeability of the microcirculation) in response to a dietary sodium load. These results correspond with our findings that in healthy male subjects sublingual detected changes of the endothelial surface layer and microcirculation are only manifest in subjects that demonstrate a sodium-sensitive blood pressure response (personal communication, Liffert Vogt, M.D., Ph.D., Division of Nephrology, Department of Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands, 2016).

Mechanistically, the increase of microvascular permeability after saline infusion can be the result of endothelial surface layer damage due to either the sodium contents of the infusion or the increment of plasma volume. The endothelial surface layer consists of the endothelial glycocalyx and the adsorbed plasma proteins. It has been shown that hemodilution caused by infusion with artificial fluids can lead to reduced blood flow resistance, indicating reduction of the endothelial surface layer, as demonstrated in a meta-analysis of experimental animal studies.[34] Saline-based infusions increased vascular permeability to a greater extent than infusates containing plasma products.[34,35] Infusions containing sodium might cause more washout of the adsorbed plasma proteins, whereas infusions containing proteins may be able to restore the endothelial surface layer and its vascular barrier properties. This observation can further be supported by the concurrent increase of plasma volume after the saline infusion, because this may also suggest compaction of the endothelial surface layer volume[36] indicative of reduced endothelial surface layer thickness.[31]

Another possible contributor to the observed increased microvascular permeability might be atrial natriuretic peptide (ANP). In addition to the well known diuretic and natriuretic effects of ANP, this hormone is also associated with increased microvascular permeability and increased shedding of endothelial surface layer constituents[22,37,38] and rapid shifts of fluids from the intravascular to interstitial space.[39] Volume loading with 6% hydroxyethyl starch 130/0.4 before elective surgery resulted in increased plasma concentrations of ANP.[22] Furthermore infusion of 2 l of 0.9% NaCl (308 mmol Na^+) is known to cause an increase in plasma ANP levels.[40] Unfortunately we were not able to measure ANP. We did measure NTproBNP, another member of the natriuretic peptide family. We did not detect changes in NTproBNP. Our observations of NTproBNP are corresponding with data from two previous studies,[41,42] that show that b-type natriuretic peptide but not ANP remains unaffected after acute sodium loading. ANP could therefore explain the observed effects on the endothelial surface layer after saline infusion. However, this remains merely speculative.

A limitation of this study is that TERalb, urinary shedding products, and the perfused boundary region are indirect measurements of the endothelial surface layer. Therefore, we are not able to provide results about the structure, volume, and thickness of this layer. Moreover, it remains unclear whether urinary glycosaminoglycan shedding concentrations are a reflection of endothelial surface layer damage and are related to shedding of plasma glycosaminoglycans. We did attempt to measure plasma glycosaminoglycans with high performance liquid chromatography with mass spectrometry/mass spectrometry, but the plasma levels of glycosaminoglycan disaccharides were not quantifiable. Second, the other microcirculatory parameters were visualized sublingually, and it remains uncertain whether our findings also apply to other microvascular beds. Furthermore, we cannot differentiate whether the plasma volume expansion or the hypertonic sodium contents of the infusion, causing an acute

increase in plasma sodium and osmolality, are responsible for the observed increase of TERalb. We also cannot distinguish whether the increase in TERalb is explained to some extent by an increase of paracellular hyperfiltration or diffusion *via* the transcellular pathway. Future studies comparing different infusion fluids, *e.g.*, 0.9% NaCl, other crystalloids and colloid infusions, should be carried out to determine whether our results can be explained either by the increased plasma volume or by the sodium contents of the infusion.

In conclusion, our study shows that a dietary sodium load and an acute intravenous sodium load in healthy male subjects have different effects on the endothelial surface layer and microcirculation despite comparable osmolar changes. An acute intravenous sodium load induced increased microvascular permeability and plasma volume, which was accompanied by a decrease in urinary fractions of heparan sulfate and chondroitin sulfate, indicating damage to the endothelial surface layer. Although we found no hemodynamic changes, our study demonstrates deleterious microvascular effects of saline infusion in healthy subjects. This might indicate that fluid therapy with hypertonic saline, often used in hospital settings and in critically ill patients, can be harmful in these patient groups. At the same time, we were not able to demonstrate effects of a dietary sodium load on the endothelial surface layer, microcirculation, and blood pressure. This seems to indicate that healthy subjects are able to adjust to the impact of a dietary sodium load. Further research regarding potentially regenerative effects of the endothelial surface layer in the context of a chronic high sodium load is needed to provide more insight into underlying mechanisms of sodium intake and sodium-mediated increase of blood pressure.

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Competing Interests

The authors declare no competing interests.

Reproducible Science

Full protocol available at: n.m.rorije@amc.uva.nl. Raw data available at: n.m.rorije@amc.uva.nl.

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