Osmotically inactive skin Na$^+$ storage in rats

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**Titze, Jens, Rainer Lang, Christoph Ilies, Karl H. Schwind, Karl A. Kirsch, Peter Dietsch, Friedrich C. Luft, and Karl F. Hilgers.** Osmotically inactive skin Na$^+$ storage in rats. *Am J Physiol Renal Physiol* 285: F1108–F1117, 2003. First published July 29, 2003; 10.1152/ajprenal.00200.2003.—Compared with age-matched men, women are resistant to the hypertensive effects of dietary NaCl; however, after menopause, the incidence of salt-sensitive hypertension is similar in women and men. We recently suggested that osmotically inactive Na$^+$ storage contributes to the development of salt-sensitive hypertension. The connective tissues, including those immediately below the skin that may serve as a reservoir for osmotically inactive Na$^+$ storage, are affected by menopause. We tested the hypothesis that ovariectomy (OVX) might reduce osmotically inactive Na$^+$ storage capacity in the body, particularly in the skin. Male, female-fertile, and female OVX Sprague-Dawley (SD) rats were fed a high (8%)- or low (0.1%)-NaCl diet. The groups received the diet for 4 or 8 wk. At the end of the experiment, subgroups received 0.9% saline infusion and urinary Na$^+$ excretion was measured. Wet and dry weight (DW), water content in the body and skin, total body Na$^+$ (rSKNa$^+$) and skin Na$^+$ (rTBNa$^+$) content were measured relative to DW by desiccation and dry ashing. There were no gender differences in osmotically inactive Na$^+$ storage capacity between rats. All SD rats accumulated Na$^+$ if fed 8% NaCl, but rTBNa$^+$ was lower in OVX rats than in fertile rats on a low (P < 0.001) and a high (P < 0.05)-salt diet. OVX decreased rSKNa$^+$ (P < 0.01) in the rats. A high-salt diet led to Na$^+$ accumulation (∆SKNa$^+$) in the skin in all SD rats. Osmotically inactive skin Na$^+$ accumulation was ~66% of ∆SKNa$^+$ in female and 82% in male-fertile rats, but there was no osmotically inactive Na$^+$ accumulation in OVX rats fed 8% NaCl. We conclude that skin is an osmotically inactive Na$^+$ reservoir that accumulates Na$^+$ when dietary NaCl is excessive. OVX leads to an acquired reduction of osmotically inactive Na$^+$ storage in SD rats that predisposes the rats to volume excess despite a reduced Na$^+$ content relative to body weight.

ovariectomy; sodium reservoir; total body sodium; Dahl rats; hypertension

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and male SD rats to investigate gender differences in osmotically inactive Na⁺ storage. Our hypothesis was that OVX might lead to an acquired osmotically inactive Na⁺ storage incapacity and thus would predispose the rats to a volume-dependent blood pressure increase. Additionally, we reanalyzed the role of skin Na⁺ storage in male Dahl rats, presuming that a reduced skin Na⁺ storage capacity accounted for reduced osmotically inactive Na⁺ storage capacity in the rats.

MATERIALS AND METHODS

All animal experiments were done according to American Physiological Society guidelines and were duly approved by local government authorities.

Animals and Diets

Female rats. Forty female SD rats (Charles River) were divided into four groups with similar body weight (BW) (group 1: 193.3 ± 3.6 g; group 2: 198.5 ± 2.1 g; group 3: 198.0 ± 4.0 g; group 4: 197.1 ± 3.5 g) and fed a regular rat chow (0.61% NaCl) for 1 wk after arrival at our animal care facility. During week 1, the animals from groups 2 and 4 were ovariectomized (OVX). From week 2, the rats were fed different diets. Ten fertile (group 1) and 10 OVX (group 2) rats were fed a virtually Na⁺-free diet (<0.1% NaCl) for 8 consecutive wk. The other 10 fertile (group 3) and 10 OVX (group 4) rats were fed a high-Na⁺ diet (8% NaCl) for 8 wk. Both Na⁺ diets contained 0.95% Ca²⁺ and 0.70% K⁺.

Male rats. Six male SD rats (group 5, weight 246.8 ± 1.7 g) were fed a 0.1% NaCl diet and six male SD rats (group 6, weight 244.3 ± 1.9 g) were fed an 8% NaCl diet for 4 consecutive wk. Additionally, we reanalyzed the role of skin Na⁺ content in 20 salt-sensitive (SS) and 20 salt-resistant (SR) male Dahl rats (Dahl-JR-Strain, M&B, Ry, Denmark), which had been killed in a previously reported experiment (27). Ten SS (264.0 ± 5.9 g) and 10 SR (263.5 ± 10.2 g) rats had been given 0.1% NaCl. The remaining 10 SS (BW 264.0 ± 10.0 g) and 10 SR (263.1 ± 7.24 g) rats had been fed a high-NaCl diet (8% NaCl) for 4 wk.

Blood Pressure Measurement

At the end of the experiment, the animals received intraperitoneal anesthesia with 100 mg/kg BW methohexital and the left femoral arteries were catheterized. Arterial lines were connected to Statham transducers and a Gould polygraph, and mean arterial blood pressure (MAP) was measured in the completely conscious animals kept in a restrainer.

Volume Expansion Protocol

Together with the arterial line, the left femoral vein was catheterized and a catheter was implanted in the urinary bladder in five rats per subgroup. The rats were placed in a restrainer and received an intravenous background infusion of 3.75 ml/h 0.9% NaCl. Four hours after the operation, urine was sampled for a 30-min control period in completely conscious animals. Then, the rats received a volume expansion (VE) with intravenous saline (0.9% NaCl, 5% of BW) within 30 min. Urine was sampled during the VE period and for an additional 90 min thereafter (post-VE diuresis).

Bone and Carcass Ashing

The intestines were completely removed from all carcasses to exclude remains of chow. Small slices of femur cartilage were cut and shock-frozen in liquid nitrogen. We removed both femurs from all animals for histological and radiological investigations. The carcasses were dry ashed according to a protocol described in detail previously (27). Water content was determined by desiccation of the carcasses and skin at 90°C for 72 h from the difference between wet weight (WW) and dry weight (DW). After dry ashing at 190 and 450°C for 24 h at each temperature level, all cervical, thoracic, lumbar and sacral vertebrae, 12 caudal vertebrae, the forelimb bones (without forefeet), the tibias, and the pelvis were removed.

Table 1. Descriptive statistics on body weight, bone mass, and bone Na⁺ in fertile and OVX Sprague-Dawley rats

<table>
<thead>
<tr>
<th></th>
<th>Fertile</th>
<th>OVX</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass wet weight, g</td>
<td>238.93±5.53</td>
<td>300.30±8.44a</td>
<td>370.76±6.22</td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>239.39±4.79</td>
<td>294.46±7.79a</td>
<td>357.54±4.82</td>
</tr>
<tr>
<td>8% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcass DW, g</td>
<td>89.24±2.86</td>
<td>126.33±7.02a</td>
<td>124.92±3.67</td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>81.72±2.38</td>
<td>104.65±4.96a</td>
<td>115.60±1.48a</td>
</tr>
<tr>
<td>8% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>121±4</td>
<td>128±2</td>
<td>117±3</td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>132±5</td>
<td>133±6</td>
<td>132±8</td>
</tr>
<tr>
<td>8% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone ash mass, g</td>
<td>6.08±0.18</td>
<td>6.18±0.15</td>
<td>5.93±0.16</td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>5.90±0.10</td>
<td>6.74±0.163a</td>
<td>5.74±0.12</td>
</tr>
<tr>
<td>8% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative bone ash mass, %DW</td>
<td>6.85±0.24</td>
<td>5.01±0.26a</td>
<td>4.76±0.12</td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>7.27±0.20</td>
<td>6.58±0.40b</td>
<td>4.97±0.11</td>
</tr>
<tr>
<td>8% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative bone Na⁺, µmol/g DW</td>
<td>30.08±1.72</td>
<td>23.12±1.48d</td>
<td>23.60±0.52</td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>33.62±1.76</td>
<td>28.48±1.84c</td>
<td>25.64±0.43c</td>
</tr>
<tr>
<td>8% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Na⁺/TBNa⁺ ratio</td>
<td>0.182±0.007</td>
<td>0.181±0.003</td>
<td>0.155±0.005</td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>0.171±0.008</td>
<td>0.175±0.005</td>
<td>0.146±0.002</td>
</tr>
<tr>
<td>8% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. OVX, ovariectomized; DW, dry weight; TBNa⁺, total body Na⁺. *P (diet) < 0.05; bP (diet) < 0.01; cP (OVX) < 0.05; dP (OVX) < 0.01; eP (OVX) < 0.001.
and ashed separately at 700°C for an additional 24 h. Then, each sample was dissolved in 20 ml 10% HNO₃. End ashing of the carcasses after bone removal was done at 600°C for 48 h. Carcass ashes were dissolved in 100 g 5% HNO₃.

Skin Ashing

Female rats. Six of 10 carcasses from groups 1-4 were skinned completely to determine total skin Na⁺ and skin water content. In the remaining four carcasses per group, we removed a piece of back skin to investigate Na⁺ and water content relative to skin weight.

Male rats. All carcasses from male SD rats (groups 5 and 6) were skinned completely to determine total skin Na⁺ and skin water content in the animals. In Dahl rats, we removed a piece of skin from the back (average WW 5.82 ± 0.37 g) for isolated water and Na⁺ determination.

All skin samples were dessicated at 90°C for 72 h. Skin water content was calculated from the difference between WW and DW. After dry ashing at 190°C for 24 h and 600°C for an additional 48 h, the skin ashes were dissolved in 20 ml 10% HNO₃.

Electrolyte Measurements

Na⁺ concentrations in blood samples were measured with a flame photometer (model EFIX, Eppendorf, Hamburg, Germany). Na⁺ concentrations in the dissolved ashes were measured with flame photometry (model 3100, PerkinElmer, Rodgau, Germany).

Data Analysis

Data are expressed as means ± SE. MAP, weights, water contents, and electrolyte concentrations were analyzed with multivariate analysis (GLM, SPSS, version 10.0). Post hoc tests were performed with the Bonferroni algorithm. Scatter plots and curve fitting were done with Origin software (version 6.0).

Na⁺ and water relationship. In contrast to female-fertile rats, increased body weight and redistribution of carcass composition with decreases of fat-free DW and increase of carcass fat are well-known effects of OVX in rats (16). Our previous osmotically inactive Na⁺ calculations in male rats were based on the relationship between Na⁺ and water content relative to WW (27), assuming a rather constant fat content in male rats. Assuming that Na⁺ and water balance takes place in fat-free body compartments predominately, increased carcass fat in OVX rats would lead to erroneous osmotically active Na⁺ estimations after correction for BW in OVX rats, if compared with fertile rats. Thus we investigated the relationship between changes in Na⁺ content and alterations of water content in skin without normalizing the data for skin weight by calculating the ratio between total skin Na⁺ (SKNa⁺; mmol) and total skin water (SKW; ml) content in each rat

\[
R_{SKNa⁺/SKW} = \frac{SKNa⁺}{SKW} \quad (1)
\]

Fig. 1. A: total body Na⁺ [rTBNa⁺; mmol/g dry weight (DW)] relative to DW. B: relative skin Na⁺ (rSKNa⁺; mmol/g DW). C: total body water [rTBW; ml/g wet weight (WW)] relative to WW. D: relative skin water (rSKW; ml/g WW) in fertile female and male or ovariec-to-mized (OVX) Sprague-Dawley (SD) rats fed 0.1 or 8% NaCl.

*P<0.05; †P<0.01; #P<0.05; §P<0.01; °P<0.001. Data are averages ± SE.
Increased R(SKNa<sup>+</sup>/SKW) indicated osmotically inactive Na<sup>+</sup> storage in skin.

We then estimated osmotically inactive skin Na<sup>+</sup> storage without normalizing the data to body weight. On the basis of the assumption that water accumulation (SKW; ml) was due to osmotically active extracellular Na<sup>+</sup> accumulation, the fraction of osmotically active Na<sup>+</sup> [SKNa<sub>a</sub>; mmol] was estimated from the relative skin water accumulation (∆SKW; ml/g), skin wet weight (SWW; g), and the serum Na<sup>+</sup> [Na<sub>serum</sub>; mmol/ml] concentration

\[ \Delta \text{SKNa}_{a} = \text{∆SKW} \times \text{SWW} \times \text{Na}_{\text{serum}} \]  

Osmotically inactive Na<sup>+</sup> accumulation in skin was characterized by an increasing ratio between SKNa<sup>+</sup> and SKW (Eq. 1) on a high-NaCl diet compared with a low-NaCl diet [∆R(SKNa<sup>+</sup>/SKW); mmol/ml]. Thus the fraction of osmotically inactive Na<sup>+</sup> accumulation [SKNa<sub>i</sub>; mmol] was estimated from ∆R(SKNa<sup>+</sup>/SKW) with the help of the skin water content

\[ \Delta \text{SKNa}_{i} = \Delta R_{\text{SKNa}^{+}/\text{SKW}} \times \text{SKW} \]  

Internal Na<sup>+</sup> balance. In addition to the extracellular volume (ECV), skin and bone are other Na<sup>+</sup> compartments in the body that contain Na<sup>+</sup> in an osmotically inactive form. To determine changes in internal Na<sup>+</sup> balance in the rats, we calculated the ratio between bone Na<sup>+</sup> (mmol) in the removed bones and total body Na<sup>+</sup> (TBNa<sup>+</sup>; mmol) in each animal. Accordingly, the ratio between total skin Na<sup>+</sup> (SKNa<sup>+</sup>; mmol) and TBNa<sup>+</sup> in the completely skinned carcass was calculated

\[ R_{\text{SKNa}^{+} / \text{TBNa}^{+}} = \frac{\text{SKNa}^{+}}{\text{TBNa}^{+}} \]  

An increased R(SKNa<sup>+</sup>/TBNa<sup>+</sup>) indicated TBNa<sup>+</sup> redistribution in favor of the skin.

Fig. 2. A: ratio (R) between total skin Na<sup>+</sup> (SKNa<sup>+</sup>; mmol) and total body Na<sup>+</sup> (TBNa<sup>+</sup>; mmol). B: ratio between total skin water (SKW; ml) and total body water (TBW; ml). C: ratio between skinned carcass Na<sup>+</sup> (CNa<sup>+</sup>; mmol) and water content (CW; ml). D: ratio between SKNa<sup>+</sup> (mmol) and SKW (ml). Completely skinned female-fertile, OVX, and male-fertile SD rats fed 0.1 or 8% NaCl. *P<sub>diet</sub> < 0.05; †P<sub>diet</sub> < 0.01; ‡P<sub>diet</sub> < 0.001; #P<sub>O VX</sub> < 0.05. Data are averages ± SE.
Osmotically Inactive Na⁺ Storage in Skin

Initially focusing on the role of bone in osmotically inactive Na⁺ storage, we only removed a piece of back skin in Dahl rats. Thus the role of skin in internal Na⁺ balance could not be measured in Dahl rats by separating total skin Na⁺ and total body Na⁺ as indicated in equation 4. We thus investigated internal Na⁺ balance in Dahl rats by normalizing total body Na⁺ (rTBNa⁺; mmol/g DW) and skin Na⁺ content (rSKNa⁺; mmol/g DW) relative to tissue DW

\[ R_{\text{SKNa}⁺/\text{TBNa}⁺} = \frac{\text{rSKNa}⁺}{\text{rTBNa}⁺} \]  

An increased \( R_{\text{SKNa}⁺/\text{TBNa}⁺} \) would thus indicate TBNa⁺ redistribution in favor of the skin.

RESULTS

Body Weight, Bone Ash Mass, and Bone Na⁺

Gender differences. Body weight (Table 1) was higher in male than in female-fertile rats, whereas bone ash mass and bone Na⁺ content relative to DW (Table 1) were lower in male than in female SD rats. Relative bone Na⁺ content was unchanged in female rats fed 8% NaCl. In male rats, NaCl consumption increased relative bone Na⁺ content not because of inorganic Na⁺ storage in bone, but due to decreased carcass DW and stable bone mass in rats fed 8% NaCl.

OVX. OVX led to increased WW, DW, and bone ash mass in female rats (Table 1), whereas relative bone ash mass was decreased. OVX rats fed 8% NaCl increased bone ash mass, whereas carcass weight was decreased. Thus bone Na⁺ content relative to BW was increased in OVX rats fed 8% NaCl. The ratio between bone Na⁺ and TBNa⁺ was unchanged in fertile and OVX rats fed 8% NaCl, indicating that Na⁺ distribution between bone and the other Na⁺ compartments of the body was not altered on different diets.

Total Body Na⁺, Skin Na⁺, and Tissue Water Content

Gender differences. Both genders increased total body Na⁺ relative to body mass (rTBNa⁺; mmol/g DW) if fed a high-NaCl diet (Fig. 1A). In contrast to female rats, male rats did not accumulate water on a high-NaCl diet (Fig. 1C). Corresponding gender differences were apparent in relative skin Na⁺ (rSKNa⁺; mmol/g DW) in the rats (Fig. 1, B and D). Relative skin water content (rSKW; ml/g WW) was increased in female rats fed 8% NaCl (Fig. 1D) and was also higher in male rats fed a high-salt diet [GLM: \( P_{\text{diet}} = 0.06 \); nonparametric Mann-Whitney test: \( P_{\text{diet}} < 0.05 \)].

OVX. rTBNa⁺ and rTBW were lower in OVX rats than in fertile rats \( P_{\text{OVX}} < 0.01 \). Similar to fertile female rats, OVX rats increased relative body Na⁺ and water on a high-salt diet. rSKNa⁺ and rSKW were lower in OVX rats than in fertile rats \( P_{\text{OVX}} < 0.01 \). As observed in fertile SD rats, a high-salt diet increased Na⁺ and water content in the skin of OVX rats.

Na⁺ and Water Distribution in the Body

Gender differences. Na⁺ and water distribution in the body were not gender specific. In both fertile female and male SD rats, dietary NaCl loading predominantly

Table 2. SWW, SKW, and serum Na⁺ concentration

<table>
<thead>
<tr>
<th></th>
<th>SWW, g</th>
<th>SKW, ml</th>
<th>Na⁺ (serum) mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, fertile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>44.5 ± 2.0</td>
<td>23.2 ± 0.6</td>
<td>155.1 ± 0.9</td>
</tr>
<tr>
<td>8% NaCl</td>
<td>44.5 ± 0.5</td>
<td>25.6 ± 0.8</td>
<td>151.9 ± 1.0*</td>
</tr>
<tr>
<td>OVX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>67.2 ± 3.7</td>
<td>28.9 ± 1.1</td>
<td>158.7 ± 0.5</td>
</tr>
<tr>
<td>8% NaCl</td>
<td>58.5 ± 3.0</td>
<td>29.6 ± 0.0</td>
<td>154.8 ± 1.7*</td>
</tr>
<tr>
<td>Male, fertile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% NaCl</td>
<td>71.8 ± 1.2</td>
<td>41.0 ± 0.8</td>
<td>156.8 ± 1.6</td>
</tr>
<tr>
<td>8% NaCl</td>
<td>66.0 ± 1.0</td>
<td>39.1 ± 0.8</td>
<td>156.2 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. SWW, skin WW; SKW, skin water; ns, not significant. Data were obtained from totally skinned female-fertile and OVX and male SD rats. *\( P_{\text{diet}} < 0.05 \); **\( P_{\text{diet}} > 0.1 \).
increased the skin $Na^+$ content (Fig. 2A) as indicated by an increased ratio between $SKNa^+/H11001$ (mmol) and $TBNa^+ (mmol)$. Although body $Na^+$ had been preferably distributed into skin in fertile rats of both genders, the relationship between total SKW and total water content in the completely skinned rest carcasses [$R(SKW/TBW)$; Fig. 2B] was unchanged, indicating that $Na^+$ had been stored in skin in an osmotically inactive form.

OVX. $R(SKNa^+/TBNa^+)$ was higher in OVX rats than in fertile rats on a low-salt diet. In contrast to fertile rats, $R(SKNa^+/TBNa^+)$ was unchanged in OVX rats fed 8% NaCl (Fig. 2A). Similar to fertile rats, $R(SKW/TBW)$ was unchanged in OVX rats fed 8% NaCl (Fig. 2B).

Carcass and Skin $Na^+$ Content and Its Relationship to Water Content

Gender differences. As judged from the relationship between $Na^+$ and water in the carcasses, both genders fed 8% NaCl identically accumulated skin $Na^+$ (Fig. 2D) in an osmotically inactive form, as indicated by an increased ratio between absolute $Na^+$ (mmol) and water (ml) content. There was no gender difference in $Na^+$-induced water accumulation between the rats, neither in the skinned carcasses [$P(\text{gender}) > 0.1; \text{Fig. 2C}$] nor in the skin [$P(\text{gender}) > 0.1; \text{Fig. 2D}$].

OVX. The relationship between carcass $Na^+$ ($CNa^+$; mmol) and carcass water ($CW$; ml) was lower in OVX than in female-fertile rats, indicating reduced osmotically inactive $Na^+$ storage in OVX skinned carcasses irrespective from the diet (Fig. 2C). In contrast to fertile rats, high-NaCl consumption was not associated with osmotically inactive $Na^+$ storage in OVX rats, as indicated by an unchanged $R(SKNa^+/SKW)$ in OVX rats fed 8% NaCl (Fig. 2D).

Osmotically Inactive $Na^+$ Storage in Skin

In female-fertile rats fed 8% NaCl, the fraction of osmotically active skin $Na^+$ accumulation [$\Delta SKNa^a_{(a)}$, mmol], according to Eq. 2 (Fig. 1D, Table 2), was 0.053 ml/g $\times 44.5$ g $\times 0.152$ mmol/ml = 0.36 mmol in the average rat skin. According to Eq. 3 (Fig. 2D, Table 2), the fraction of osmotically inactive $Na^+$ accumulation [$\Delta SKNa^i_{(a)}$, mmol] was 0.027 mmol/ml $\times 25.6$ ml = 0.69 mmol in the average rat skin. In male-fertile rats, $\Delta SKNa^a_{(a)}$ was 0.020 ml/g $\times 66.0$ g $\times 0.156$ mmol/ml = 0.21 mmol in the average rat skin. $\Delta SKNa^i_{(a)}$ was 0.024 mmol/ml $\times 39.1$ ml = 0.94 mmol in the average rat skin. In OVX rats, where no osmotically inactive $Na^+$ accumulation was identified in skin [as indicated by an unchanged $R(SKNa^+/SKW)$ on a high-salt diet], $\Delta SKNa^a_{(a)}$ was 0.077 ml/g $\times 58.5$ g $\times 0.155$ mmol/ml = 0.70 mmol in rats fed 8% NaCl in the average rat skin. In sum-

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Fig. 5. Relationship between $R(SKNa^+/SKW)$ (mmol/ml) and urinary $Na^+$ concentration ($U_{Na}$; mmol/l) in fertile female and male SD rats (A) and OVX SD rats (B). Relationship between $R(SKNa^+/SKW)$ and urinary $K^+$ concentration ($U_{K}$; mmol/l) in fertile female and male SD rats (C) and OVX SD rats (D). $U_{Na}$ and $U_{K}$ measured during 30-min mild saline infusion (3.75 ml/h). The scatter points in brackets have not been taken into consideration for curve fitting.
mary, osmotically inactive skin Na\(^+\) accumulation was ~66% in female- and 82% in male-fertile rats, whereas there was no osmotically inactive Na\(^+\) accumulation in OVX rats fed 8% NaCl (Fig. 3).

**Total Body Na\(^+\) and Water and Its Relationship to Blood Pressure**

As indicated in Table 1, there was no dietary or OVX effect on average blood pressure in the rats. However, multivariate comparison of means of dietary or OVX effects on blood pressure does not consider the individual impact of increasing body Na\(^+\) content on total body water and blood pressure in the rats. Figure 4A shows the relationship between rTBNa\(^+\) and MAP (mmHg) in OVX and fertile rats. Increased rTBNa\(^+\) correlated directly with MAP in OVX rats, but not in fertile rats. Additionally, there was a direct correlation between rTBW and MAP in OVX rats (Fig. 4B), whereas there was no relationship between water retention and MAP in fertile rats. Similar to fertile female rats, there was no direct correlation between rTBNa\(^+\) or rTBW and MAP in male SD rats (data not shown).

**Skin Na\(^+\) Content and Natriuresis During Saline Infusion**

Table 3 shows natriuresis (UNa\(_V\); mmol/g WW) and Na\(^+\) accumulation in OVX and fertile female and male rats during saline infusion. The rats received a continuous 3.75-ml/h saline background infusion and a 30-min VE with 5% BW 0.9% NaCl. UNa\(_V\) was measured 30 min previous to VE (control), during 30 min VE, and 90 min after VE had been stopped (post). UNa\(_V\) in response to mild saline background infusion previous to VE was affected by the previous dietary regime in female- and male-fertile rats (Table 3), but not in OVX rats. Fertile rats fed a low-NaCl diet tended to accumulate Na\(^+\), whereas Na\(^+\) balance in fertile rats fed 8% NaCl was negative during background infusion (\(\Delta\)TBNa\(^+\) control). Within 90 min after acute VE had been stopped, there was no significant Na\(^+\) accumulation in all groups of rats.

In fertile rats of both genders, osmotically inactive skin Na\(^+\) content [as indicated by an increased R\(_{SKNa^+/SKW}\)] correlated positively with urinary Na\(^+\) concentration (UNa\(_V\); mmol/l; Fig. 5A) and negatively with urinary K\(^+\) concentration (Uk; mmol/l; Fig. 5C) during mild saline infusion previous to VE. In contrast, there was no correlation between R\(_{SKNa^+/SKW}\) and UNa (Fig. 5B) in OVX rats, whereas a negative correlation between R\(_{SKNa^+/SKW}\) and Uk was still evident in OVX rats (Fig. 5D). Correspondingly, during acute VE, R\(_{SKNa^+/SKW}\) and UNa correlated directly in both female (\(P < 0.05\))- and male (\(P < 0.01\))-fertile rats, whereas there was no correlation between R\(_{SKNa^+/SKW}\) and UNa in OVX rats (\(P > 0.1\); data not shown).

**Skin Na\(^+\) Storage in Dahl Rats**

Figure 6A shows the skin Na\(^+\) content (rSKNa\(^+\)) relative to skin DW in SS and SR Dahl rats fed a high- or low-NaCl diet. rSKNa\(^+\) was unchanged in SS and SR rats fed 8% NaCl. The relationship between rSKNa\(^+\) and total body Na\(^+\) [R\(_{SKNa^+/rTBNa^+}\); mmol/g skin DW and mmol/g total body DW] was decreased in SS rats and unchanged in SR rats fed 8% NaCl (Fig. 6B). In contrast to fertile SD rats (Fig. 2D), there was no significant osmotically inactive Na\(^+\) storage in skin in Dahl rats, as indicated by an unchanged R\(_{SKNa^+/SKW}\) (Fig. 6C). In contrast to fertile SD rats, there were neither positive correlations between R\(_{SKNa^+/SKW}\) and UNa (\(P > 0.1\)) nor negative correlations between R\(_{SKNa^+/SKW}\) and Uk (\(P > 0.1\)) in Dahl rats (data not shown).

**Fig. 6. A: rSKNa\(^+\) (mmol/g DW). B: ratio between rSKNa\(^+\) (mmol/g skin DW) and rTBNa\(^+\) (mmol/g total body DW). C: ratio between SKNa\(^+\) (mmol) and SKW (ml) in salt-resistant (SR) and salt-sensitive (SS) Dahl rats fed 0.1 or 8% NaCl. *P\(_{\text{intrastr}}\) < 0.05, intrastrain dietary effect.**
Our data indicate that skin Na\textsuperscript{+} storage plays a central role in osmotically inactive Na\textsuperscript{+} metabolism in rats. Fertile SD rats of both genders reacted to dietary Na\textsuperscript{+} loading with osmotically inactive Na\textsuperscript{+} storage in skin (Fig. 1). Total body Na\textsuperscript{+} was distributed in favor of the skin (Fig. 2A), whereas total body water distribution was unchanged (Fig. 2B) in SD rats fed 8\%, indicating a shift of internal Na\textsuperscript{+} balance in favor of skin as an osmotically inactive Na\textsuperscript{+} reservoir. The data support the notion that, in NaCl excess, extracellular volume homeostasis is not only maintained by osmotically active Na\textsuperscript{+} excretion through the kidneys but also by osmotically inactive Na\textsuperscript{+} storage in skin and internal Na\textsuperscript{+} balance redistribution in favor of osmotically inactive Na\textsuperscript{+} reservoirs.

We additionally investigated inherited and acquired osmotically inactive Na\textsuperscript{+} storage incapacity in rats. As a model of inherited Na\textsuperscript{+} storage incapacity, we recently reported that male Dahl rats display a reduced osmotically inactive Na\textsuperscript{+} storage capacity (27), but we could not precisely localize the compartment responsible for osmotically inactive Na\textsuperscript{+} storage. Reanalyzing our data, we thus focused on skin Na\textsuperscript{+} storage in Dahl rats. Na\textsuperscript{+} storage in skin was deficient in Dahl SS and SR rats compared with SD rats. In both Dahl strains, high NaCl did not increase skin Na\textsuperscript{+} content (Fig. 6A) and there was no osmotically inactive Na\textsuperscript{+} storage in reaction to a high-salt diet in Dahl rats (Fig. 6C). Thus Na\textsuperscript{+} excess in Dahl SS rats led to Na\textsuperscript{+} redistribution in favor of osmotically active Na\textsuperscript{+} compartments (the details have been outlined earlier; 27), and internal Na\textsuperscript{+} balance was redistributed in favor of Na\textsuperscript{+} compartments other than skin (Fig. 6B). This reduced osmotically inactive Na\textsuperscript{+} storage capacity predisposed the rats to a volume-sensitive blood pressure increase.

As a model of acquired osmotically inactive skin Na\textsuperscript{+} storage capacity, we ovariectomized female SD rats. In contrast to Dahl rats, both fertile and OVX rats accumulated Na\textsuperscript{+} in their skin if fed 8\% NaCl (Fig. 1).

However, as indicated by an unchanged SKNa\textsuperscript{+}/SKW ratio in rats fed a high-salt diet (Fig. 2D), there was less osmotically inactive skin Na\textsuperscript{+} accumulation on a high-NaCl diet in OVX rats compared with fertile rats. Correspondingly, the relationship between Na\textsuperscript{+} and water in the skinned carcasses was lower in OVX rats than in fertile rats (Fig. 2C). The reduced osmotically inactive Na\textsuperscript{+} storage capacity in OVX rats’ skin was associated with an unchanged SKNa\textsuperscript{+}/TBNa\textsuperscript{+} ratio in the body, whereas intact osmotically inactive skin Na\textsuperscript{+} storage in fertile rats fed a high-salt diet was associated with an internal Na\textsuperscript{+} balance shift into skin as indicated by an increased SKNa\textsuperscript{+}/TBNa\textsuperscript{+} ratio (Fig. 2A).

Increased natriuresis and decreased kaliuresis characterize a suppression of the renin-angiotensin-aldosterone system. In fertile SD rats, increased osmotically inactive Na\textsuperscript{+} storage in skin correlated directly with urinary Na\textsuperscript{+} concentration (Fig. 5A) and inversely with urinary K\textsuperscript{+} concentration (Fig. 5C) during controlled saline infusion experiments. We conclude that not only the circulating volume but also the Na\textsuperscript{+} content in osmotically inactive Na\textsuperscript{+} reservoirs may be operative in the regulation of body volume and total body Na\textsuperscript{+}. Furthermore, the Na\textsuperscript{+} content in osmotically inactive Na\textsuperscript{+} reservoirs influenced natriuresis and not only the volume of the isotonic saline infused. Thus natriuresis (as the effector of Na\textsuperscript{+} homeostasis) is not only regulated by the circulating volume but also by the Na\textsuperscript{+} content in osmotically inactive Na\textsuperscript{+} reservoirs. Na\textsuperscript{+} homeostasis is regarded to be regulated by changes in the extracellular fluid volume. In contrast to osmoregulation, where the system has a set point at a plasma osmolality, and the effector is vasopressin-mediated water excretion within minutes or hours, Na\textsuperscript{+}-regulatory mechanisms operate with a remarkable sluggishness (14, 29). The very existence of a set point for total body Na\textsuperscript{+} has been a matter of debate (1, 10). This debate was based on the assumption that changes in total body Na\textsuperscript{+} are always changes in extracellular volume. Our data indicate a dissociation of

Table 3. Natriuresis, Na\textsuperscript{+} accumulation, urinary Na\textsuperscript{+} concentration, and urinary K\textsuperscript{+} concentration during 3.75 ml/h 0.9% saline background infusion, acute VE, and sampled for 90 min after VE

<table>
<thead>
<tr>
<th></th>
<th>Female, Fertile</th>
<th>Male, Fertile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1% NaCl</td>
<td>8% NaCl</td>
</tr>
<tr>
<td>UNa,V control, mmol/g WW</td>
<td>0.87 ± 0.01</td>
<td>1.72 ± 0.26*</td>
</tr>
<tr>
<td>UNa,V VE, mmol/g WW</td>
<td>4.94 ± 0.39</td>
<td>5.78 ± 0.48</td>
</tr>
<tr>
<td>UNa,V post, mmol/g WW</td>
<td>7.07 ± 0.28</td>
<td>7.07 ± 0.10</td>
</tr>
<tr>
<td>rTBNa\textsuperscript{+} control, mmol/g WW</td>
<td>2.89 ± 0.49</td>
<td>2.89 ± 0.49</td>
</tr>
<tr>
<td>rTBNa\textsuperscript{+} VE, mmol/g WW</td>
<td>4.94 ± 0.39</td>
<td>4.39 ± 1.00</td>
</tr>
<tr>
<td>rTBNa\textsuperscript{+} post, mmol/g WW</td>
<td>7.07 ± 0.28</td>
<td>7.07 ± 0.10</td>
</tr>
<tr>
<td>UNa, control, mmol/l</td>
<td>83.6 ± 11.6</td>
<td>226.2 ± 36.8*</td>
</tr>
<tr>
<td>UNa, VE, mmol/l</td>
<td>126.9 ± 5.5</td>
<td>137.9 ± 5.3</td>
</tr>
<tr>
<td>UNa, post, mmol/l</td>
<td>146.7 ± 6.9</td>
<td>175.2 ± 6.8*</td>
</tr>
<tr>
<td>UK control, mmol/l</td>
<td>28.5 ± 11.3</td>
<td>39.5 ± 15.8*</td>
</tr>
<tr>
<td>UK VE, mmol/l</td>
<td>146.7 ± 6.9</td>
<td>175.2 ± 6.8*</td>
</tr>
<tr>
<td>UK post, mmol/l</td>
<td>28.5 ± 11.3</td>
<td>39.5 ± 15.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE of OVX and fertile female and male Sprague-Dawley (SD) rats. UNa,V, natriuresis; WW, wet weight; rTBNa\textsuperscript{+}, Na\textsuperscript{+} accumulation; UNa,V, urinary Na\textsuperscript{+} concentration; UK, urinary K\textsuperscript{+} concentration; VE, volume expansion; control, 30 min previous to VE; post, 90 min after VE. Acute VE involved 5% BW 0.9% saline within 30 min. *P < 0.05, intrastrain dietary effect.

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Na\(^+\) homeostasis and volume homeostasis. Na\(^+\) and volume excess in rats is characterized by renal Na\(^+\) and water excretion and “water-free” Na\(^+\) accumulation in the skin. Thus Na\(^+\) homeostasis is only partially characterized by volume homeostasis. We conclude that discussions on the existence of a set point for total body Na\(^+\) should include the potential role of osmotically inactive Na\(^+\) reservoirs and discriminate between volume regulation and Na\(^+\) homeostasis.

In contrast to fertile rats, there was no direct correlation between skin Na\(^+\) storage and urinary Na\(^+\) excretion in OVX, although an increased SKNa\(^+\)/SKW ratio and urinary K\(^+\) concentration still correlated negatively (Fig. 5). We conclude that the close functional relationship between volume regulation and skin Na\(^+\) storage was disturbed in OVX rats. The reduced osmotically inactive Na\(^+\) storage capacity in OVX rats was characterized by a TBNa\(^+\)-associated total body water increase. This total body water increase correlated directly with blood pressure in OVX rats (Fig. 4). Blood pressure is sensitive to alterations of female sex hormones (23). Estradiol substitution tends to lower blood pressure in postmenopausal women (7), whereas the extent of postmenopausal osteoporosis coincides with hypertension in women (2). OVX deteriorates or induces salt-sensitive hypertension in Dahl SS and spontaneously hypertensive rats (9, 20), and estradiol substitution was reported to attenuate hypertension in OVX SS Dahl rats (24). The pathophysiological approach toward the effect of estradiol on blood pressure has focused on the effects of the sex hormone on vascular resistance (7), vascular tone (3–5), vascular growth (6, 13), the sympathetic nerve system (9, 11, 26), and estradiol-induced renoprotection (18, 19). Our data suggest that a reduced osmotically inactive Na\(^+\) storage capacity in skin was present in OVX rats, raising still another possible estradiol-related effect. Although there was no significant dietary effect on blood pressure in OVX rats (Table 1), we speculate that the occurrence of a volume-sensitive blood pressure reaction might be one factor contributing to the multifactorial pathophysiological puzzle leading to hypertension. Of interest, the direct correlation between total body water and blood pressure occurred in OVX SD rats with the lowest rTBNa\(^+\) and thus lowest relative total body water. We conclude that the ability to partially osmotically inactivate TBNa\(^+\), and not the absolute TBNa\(^+\) value itself, might play an important role in the relationship between fluid volume and blood pressure.

In summary, our data demonstrate an inherited incapacity in (male) Dahl rats to transfer Na\(^+\) into skin in salt excess and an acquired incapacity to osmotically inactivate Na\(^+\) in OVX rats. Osmotically inactive Na\(^+\) storage incapacity in skin predisposed the rats to a volume-sensitive blood pressure increase. Whether this concept is transferable to OVX experiments in female salt-sensitive models (such as Dahl or spontaneously hypertensive rats) remains to be investigated. In conclusion, we suggest that impaired capacity for osmotically inactive Na\(^+\) storage may be an alternative or additional factor contributing to the salt-sensitive increase in blood pressure after menopause.

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**DISCLOSURES**

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